Grafting of PEO to Glass, Nitinol, and Pyrolytic Carbon Surfaces by γ Irradiation

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Abstract: Glass, nitinol, and pyrolytic carbon surfaces were grafted with poly(ethylene oxide) (PEO) and PEO-containing Pluronic® surfactants by γ irradiation. These substrates were coated with a primer layer of trichlorovinylsilane (TCVS), which allows grafting of organic polymers. The TCVS-coated substrates were adsorbed with PEO or Pluronics® and exposed to 0.3 Mrad of γ radiation to graft the polymer to the surface. PEO-grafted substrates were characterized by contact angle measurement, X-ray photoelectron spectroscopy, fibrinogen adsorption, and platelet adhesion and activation. Surface modification with PEO reduced fibrinogen adsorption by as much as 99%. Platelet adhesion was significantly reduced or prevented on the modified surfaces. Protein- and platelet-resistance effects were independent of hydrophilicity of the PEO-grafted surfaces. Polymer grafting by γ radiation to TCVS-coated substrates provides a facile process to improve thromboresistance of inorganic biomaterials. © 1997 John Wiley & Sons, Inc. J Biomed Mater Res (Appl Biomater) 38: 289–302, 1997

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INTRODUCTION

Biomaterials are used to restore, augment, or replace natural bodily functions and may contact tissue fluid, blood, and other biological fluids for prolonged durations. Many different kinds of materials, including polymers, ceramics, metals, carbon, and composites, have been used for biomedical applications.[1] One of the major problems in the use of blood-contacting biomaterials has been surface-induced thrombosis, which is initiated by plasma protein adsorption and platelet activation.[2,3]

Several processes occur when artificial materials are placed in contact with blood. The first event is the adsorption of plasma proteins. The adsorbed protein molecules may serve as sites for platelet adhesion and activation by interaction with specific receptors on the platelet membrane.[4–6] Recruitment and activation of more platelets follows, resulting in thrombus formation. Interaction of plasma proteins with foreign surfaces may also initiate the immune complement and coagulation pathways.[7–14] Fouling and failure of the implanted device are the ultimate

consequences. Surface modification with various polymers, such as poly(ethylene oxide) (PEO), heparin, and albumin, has been shown to drastically reduce protein adsorption and platelet adhesion to biomaterials.[15–24] Hydrophilic polymers form a diffuse layer when bound to surfaces,[25] which exerts steric repulsion to prevent protein adsorption and platelet adhesion. Surface modification of biomaterials with these surface-passivating molecules, however, has been rather difficult because most biomaterials do not have chemically active functional groups on their surfaces.

Biomaterial surfaces have been modified by various means, including simple polymer adsorption and covalent grafting. To provide effective steric repulsion, and thus resist protein adsorption and platelet adhesion, polymers should be tightly bound to the surface, preferably by covalent bonding. Simple adsorption may not be effective because the adsorbed polymer molecules may be displaced by molecules with greater affinity for the surface. Covalent attachment of the hydrophilic polymer to the surface is required to ensure adequate and predictable results of surface modification.

Covalent grafting approaches may be divided into two main groups: graft polymerization and graft coupling. In graft polymerization polymer chains are synthesized *in situ* on the reactive surface, whereas graft coupling binds preformed polymer molecules to the surface. Plasma polymerization is commonly used to polymerize monomers onto

surfaces, resulting in highly crosslinked polymer layers. Metal surfaces have reportedly been modified this way.[26] Chemical graft coupling methods have been widely used to modify polymer surfaces.[15–18,27–32] These methods employ chemical reactions between polymer molecules and reactive surface sites. Chemical grafting therefore relies on the presence of complementary reactive groups on the polymer and surface, necessitating a different approach to each specific system. Metal substrates pose a special challenge in the design of such polymer–surface coupling schemes.

We used trichlorovinylsilane (TCVS) as a ''primer'' to modify the surfaces of glass, metal, and pyrolytic carbon substrates. The vinyl group modified surface allows covalent grafting of unmodified PEO or any other water-soluble polymers to the primed surfaces by γ radiation in an aqueous environment. The PEO-grafted surfaces were resistant to protein adsorption and platelet adhesion. These beneficial effects are demonstrated on clinically relevant model materials.

MATERIALS AND METHODS

Surface and Polymer Preparation

Glass capillary tubes (1.5-mm i.d. $\times$ 100 mm long, Kimble Products, Vineland, NJ) or microscope coverslips (9 $\times$ 22 mm, #1 thickness, Bellco, Vineland, NJ) were cleaned by immersion in chromic-sulfuric acid solution overnight. Clean substrates were rinsed with copious deionized distilled water (DDW) and dried at 60°C. Nitinol wires (0.005 in. nominal diameter, Progressive Angioplasty Systems, Inc., Menlo Park, CA) and pyrolytic carbon disks (Medtronic Inc., Irvine, CA) were cleaned by soaking in 1% sodium dodecylsulfate (SDS) solution with vortexing for 5 min followed by rinsing in DDW and sonicating for 10 min in fresh DDW. They were then dried at 60°C overnight.

Clean substrates were silanized by immersing in a solution of 5% TCVS (Aldrich, Milwaukee, WI) or dimethyldichlorosilane (DDS, Aldrich) in chloroform (analytical grade, Mallinkrodt, Paris, KY) at room temperature. After 3 h they were rinsed sequentially in fresh chloroform, absolute ethanol, and DDW. Finally, the silanized substrates were dried and cured at 60°C overnight. They were covered and stored at room temperature until use.

Pluronic® surfactants (BASF Corp., Parsippany, NJ), which are triblock copolymers of the general formula (ethylene oxide)$_n$-(propylene oxide)$_m$-(ethylene oxide)$_n$, were dissolved in DDW to concentrations ranging from 0.01 to 25 mg/mL. PEO (MW 5,000, Aldrich) was dissolved in DDW to 1.0 mg/mL.

Contact Angle Measurement

TCVS- and DDS-modified glass coverslips were used for contact angle measurements. Silanized glass coverslips were immersed in either DDW or a 1 mg/mL solution of Pluronic® F127 (PF127) for 1 h. The DDW or Pluronic® solution containing glass coverslips was exposed to γ radiation for 0.3 Mrad by placing in a GR-12 irradiator (US Nuclear Corp., Burbank, CA) equipped with a cobalt-60 γ energy source. The glass coverslips were then washed in 1% SDS overnight, thoroughly rinsed with running DDW, and dried at 60°C. Dry samples were kept covered at room temperature until use. Untreated TCVS- and DDS-glass coverslips were used as another control in addition to the samples γ irradiated in DDW. Advancing and receding contact angles were determined by a goniometer (Rame–Hart, Mountain Lakes, NJ) using DDW filtered through a 0.22-μm filter (Millipore, Bedford, MA). Samples were held in a humidity chamber equilibrated with DDW at room temperature prior to taking measurements.

X-Ray Photoelectron Spectroscopy (XPS) Analysis

TCVS-glass coverslips were immersed in DDW for 1 h then exposed to 0.3 Mrad γ radiation. They were rinsed with running DDW and dried at 60 °C. Untreated TCVS-glass coverslips were also used. Samples for XPS were secured on 45° sample mounts and analyzed on a Physical Electronics model 548 instrument employing MgKα radiation. Survey spectra were collected with 100-eV pass energy and a 1-eV step at 20 ms/step. Ten sweeps were collected per spectrum.

Protein Adsorption to Polymer-Grafted Surfaces

For the protein adsorption study, glass tubes (5-mm diameter) were used instead of glass coverslips because of the convenience of introducing fibrinogen solution to the surface without exposing the surface to the air. TCVS-coated tubes were exposed to a variety of Pluronic® surfactants or PEO homopolymer at the concentration of 1 mg/mL. After 1 h they were exposed to 0.3 Mrad γ radiation in the presence of bulk polymer solution. They were then washed in 1% SDS overnight and used for the fibrinogen adsorption study. TCVS-nitinol and TCVS-pyrolytic carbon (TCVS-PC) were similarly grafted with PF127 in the presence of bulk polymer solution at the concentration of 1 mg/mL.

In another study examining the effect of the surface Pluronic® concentration on the reduction in protein adsorption, silanized tubes were exposed to PF127 or Pluronic® P105 (PP105) ranging in concentration from 0.01 to 25 mg/mL. After 1 h they were rinsed of excess polymer solution with DDW and exposed to 0.3 Mrad γ radiation in the presence of DDW. All grafted samples were washed in 1% SDS overnight and rinsed thoroughly with DDW. They were dried at 60 °C and stored covered at room temperature until use.

Human fibrinogen (Sigma, St. Louis, MO) was radiolabeled with ^{125}I using Enzymobead® reagent (Bio-Rad, Richmond, CA). Radiolabeled fibrinogen was purified from the reaction mixture by gel filtration over a BioGel

P6-DG (Bio-Rad) column equilibrated with phosphate buffered saline (PBS, pH 7.2). Radiolabeled fibrinogen was mixed with native protein in a 1:39 mass ratio to produce the adsorption solution containing 0.1 mg/mL of fibrinogen.

The interior surfaces of modified tubes were hydrated in PBS for 1 h. The buffer solution was replaced with a fibrinogen solution without exposing the surface to air. After 1 h at room temperature they were rinsed with PBS to displace nonadsorbed protein. Samples were assayed on a γ counter (Gamma 5500B, Beckman, Arlington Heights, IL) to determine the amount of protein adsorbed to the surface. The raw data were divided by the sample surface area and protein specific radioactivity to yield data in units of micrograms per square centimeter.

Platelet Adhesion and Activation

TCVS- and DDS-glass coverslips and TCVS-PC were grafted with PF127 in the presence of bulk polymer solution at the concentration of 1 mg/mL, as were the samples for protein adsorption experiments. TCVS-nitinol was similarly grafted with PF108.

Human blood from healthy volunteers was collected in 5-mL tubes containing 72 U heparin (Vacutainer®, Becton–Dickinson, Rutherford, NJ) and centrifuged at 100 × g for 10 min. The supernatant platelet rich plasma (PRP) fraction was collected and incubated in a water bath at 37 °C for 30 min. The red cell fraction was discarded. Modified glass coverslips were exposed to PRP in flow chambers, as previously described.[6] PC disks and nitinol wire samples were exposed to PRP in 3.5-mL plastic tubes (Falcon®, Becton–Dickinson) on a rotator at 14 rpm. After 1 h all samples were rinsed with PBS. Adherent platelets were fixed with 2% glutaraldehyde in PBS for 30 min and rinsed with PBS. Fixed platelets were stained with 0.1% Coomassie brilliant blue for glass samples or labeled with rhodamine-phalloidan, a fluorescent probe, for nitinol wire samples. Glass and nitinol samples were examined on an inverted optical microscope (Nikon, Garden City, NY) in transmission and epifluorescence modes, respectively. Fixed PC samples were prepared for scanning electron microscopy by dehydration in a graded ethanol series, critical point drying in carbon dioxide, and mounting on aluminum sample stubs. Dried samples were kept in a desiccator over Drierite® until examination. They were sputtered with an Au/Pd target immediately prior to observation on a Jeol JSM-840 scanning microscope.

RESULTS

Surface Characterization

Advancing and receding contact angles of water droplets on DDS- and TCVS-glass samples are shown in Table I. Untreated DDS-glass exhibited a large advancing contact

**TABLE I. Water Contact Angles on Untreated and Treated DDS-Glass and TCVS-Glass**

Contact Angle	Untreated	γ Irradiated in	
		DDW	PF127
DDS-glass			
Advancing	89.9 ± 1.8	90.5 ± 2.5	88.3 ± 2.5
Receding	85.4 ± 3.0	77.7 ± 6.4	62.4 ± 7.7
TCVS-glass			
Advancing	68.8 ± 3.2	0	64.0 ± 4.3
Receding	61.6 ± 3.1	0	39.5 ± 4.4

The concentration of PF127 was 1 mg/mL. Values are mean ± SEM. $n = 4$.

angle of 89.9° and a receding angle of 85.4°. DDS-glass exposed to γ radiation showed advancing and receding angles that were not any different from those of control DDS-glass ($p = 0.57$). DDS-glass γ irradiated in PF127 solution exhibited the advancing angle of 88.3°. This value was essentially identical with that of the untreated DDS ($p = 0.17$). The receding angle, however, was reduced to 62.4°. These data indicate that there was a slight increase in hydrophilicity imparted by γ irradiation in PF127. The large standard errors for the receding angles indicate heterogeneity in the γ-irradiated surfaces.

TCVS-glass exhibited an advancing contact angle of 68.8° and a receding angle of 61.6°. TCVS is apparently more hydrophilic than DDS, as reflected in the approximately 20° difference in advancing contact angles. When γ irradiated in DDW, TCVS-glass became very hydrophilic. Water did not form droplets on the surface but spread evenly over a wide area. The γ-irradiation process transforms the TCVS-glass surface from moderately hydrophobic to very hydrophilic. TCVS-glass γ irradiated in PF127 solution had an advancing contact angle of 64.0° and receding angle of 39.5°. These values represent only a slight decrease in advancing angle, but more than a 20° decrease in receding angle compared to the control. The γ irradiation of TCVS-glass in DDW or PF127 apparently alters its surface chemistry such that it becomes significantly more hydrophilic.

XPS spectra of TCVS-glass before and after γ irradiation in DDW for 0.3 Mrad are shown in Figure 1(A) and 1(B), respectively. The spectrum of the untreated TCVS [Fig. 1(A)] appears as expected, showing C, O, and Si as the main constituents at the surface. A strong O1s peak at 534 eV dominates the spectrum. Also present are a C1s peak at 284 eV and the Si2s and Si2p peaks at approximately 156 and 104 eV, respectively. The C1s peak is large relative to the Si peaks, indicating the presence of hydrocarbon groups on the surface. Si is present both from the silane applied and the underlying glass. After being γ irradiated in DDW, TCVS-glass qualitatively shows the same surface atomic composition, with changes in the relative intensities [Fig. 1(B)]. The same peaks are present with no chemical shifts evident. This is intuitive, because the surface chemistry was expected to change with respect
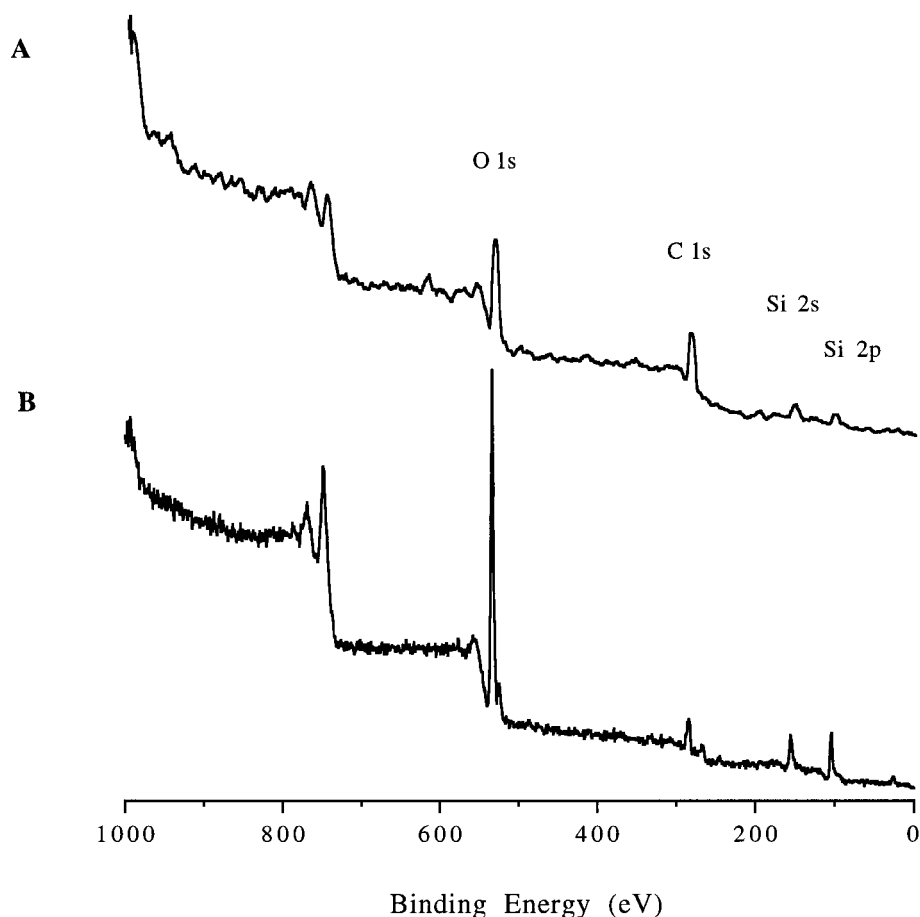
Figure 1. XPS spectra of (A) untreated TCVS-glass and (B) TCVS-glass after exposure to 0.3 Mrad γ radiation in water.

to the relative amounts of C, O, and Si, rather than the addition of other types of atoms. The ratios of peak heights were calculated among O1s, C1s, and Si2s (or average of Si2s and Si2p) peaks. The peak ratio of O1s/Si2s was 6.4 for both untreated and γ-irradiated TCVS-glass. On the other hand, the peak ratios of O1s/C1s were 1.8 and 5.2 for untreated and γ-irradiated TCVS-glasses, respectively. The increase in the O1s/C1s ratio with no change in the O1s/Si2s ratio should be noted. It suggests that a portion of the vinyl groups were removed from the surface, leaving behind bulk glass with residual vinyl groups. This may have resulted in free spreading of water on the surface as shown in Table I. The partial removal of carbons from the TCVS-glass by γ irradiation is understandable because γ irradiation is known to cleave chemical bonds as well as crosslinking by the produced radicals. In the presence of either Pluronics® or PEO on the surface, such a process may result in either covalent grafting of polymers to the surface or mechanical entrapment of the adsorbed polymers during the cleavage and crosslinking process by γ irradiation. Attempts to quantify this change by precise atomic concentration measurements were confounded by instrument limitations.

Protein Adsorption

Figure 2 shows fibrinogen adsorption to TCVS-glass grafted with various Pluronic® surfactants and PEO homopolymer. The number of repeating ethylene oxide (EO) units in the Pluronic® surfactants ranged from 2 (L31) to 128 (PF108). TCVS-glass was also grafted with PEO homopolymer, which has approximately 110 EO units. Untreated TCVS-glass and TCVS-glass γ irradiated in DDW exhibited fibrinogen adsorptions of 0.47 and 0.35 $\mu g/cm^2$, respectively. All grafted surfaces, irrespective of the PEO block length, showed substantially reduced fibrinogen adsorption. The surface fibrinogen concentrations were below 0.02 $\mu g/cm^2$ on those surfaces, except on the Pluronic® L31-coated surface. It is rather surprising to observe that the PEO chain with only 3 EO units in Pluronic® L61 is as effective as the PEO chain with 128 EO units in Pluronic® F108. However, this was not totally unexpected because protein adsorption can be effectively prevented as long as the surface coverage is complete, regardless of the chain length.[33] PEO homopolymer was also highly effective at decreasing fibrinogen adsorption to less than 0.01 $\mu g/cm^2$. Note that polymers were grafted to the TCVS-
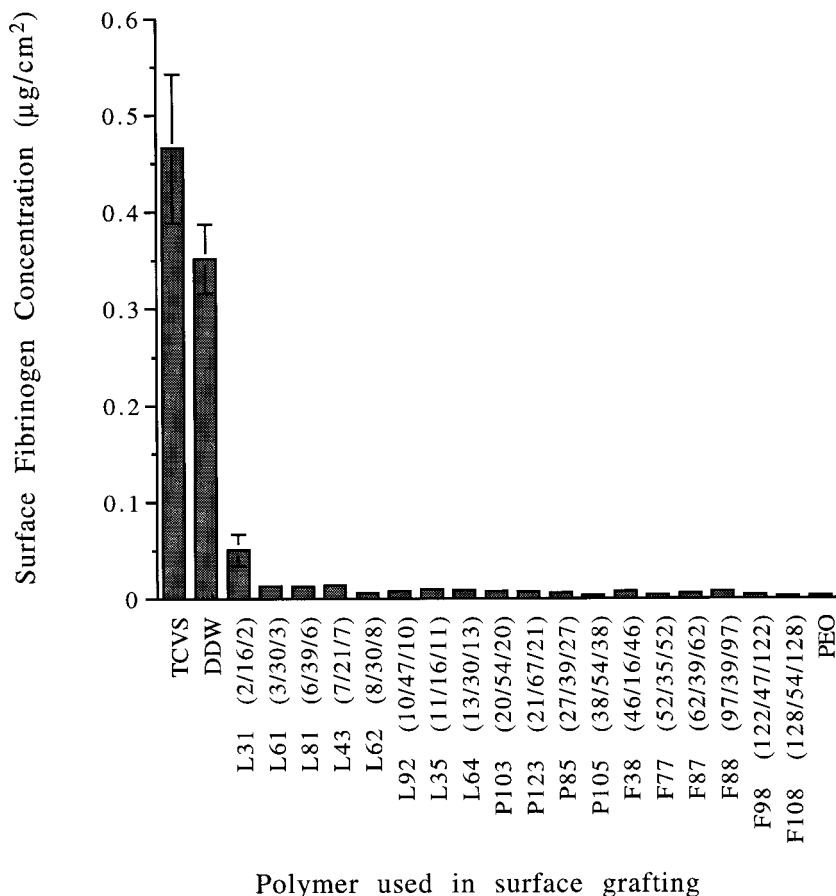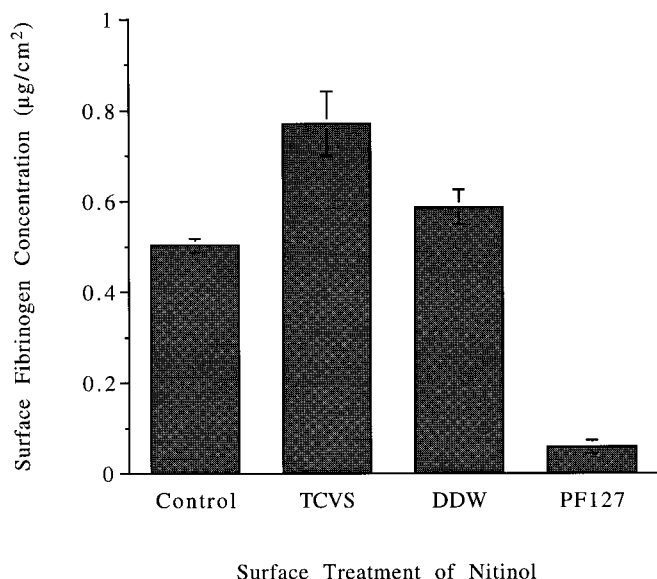
Figure 2. Fibrinogen adsorption to TCVS-glass (TCVS), TCVS-glass γ irradiated in DDW (DDW), and TCVS-glass grafted with various Pluronic® surfactants (L, P, and F series) or PEO homopolymer (PEO) by exposing to 0.3 Mrad γ radiation in the presence of 1 mg/mL polymer solution. The three numbers in parentheses indicate the numbers of repeating units of ethylene oxide (EO) and propylene oxide (PO) in the PEO/poly(PO)/PEO triblock copolymer of Pluronics®. The fibrinogen concentration used for adsorption was 0.1 mg/mL. Values are mean ± SEM; $n = 3$ except for two controls that have $n = 6$.

glass by exposing them to γ irradiation while immersed in the polymer solution. This always resulted in better grafting than exposure of the surface to γ irradiation in DDW after rinsing the surface (see below). These data suggest that any Pluronic® surfactant grafted in high density is capable of preventing protein adsorption to surfaces, regardless of the length of the grafted PEO chains.

Fibrinogen adsorption to PF127-grafted nitinol wire is shown in Figure 3. Fibrinogen adsorbed to the untreated nitinol to the extent of 0.51 μg/cm^2. TCVS-nitinol exhibited greater fibrinogen adsorption of 0.77 μg/cm^2, while γ irradiation in DDW reduced this value to 0.59 μg/cm^2. Fibrinogen adsorption to PF127-grafted nitinol was reduced to 0.06 μg/cm^2, which corresponds to an 88% reduction relative to the untreated nitinol. The reduction in fibrinogen adsorption by PF127 grafting was less dramatic (only 34%) on PC. The surface fibrinogen concentrations on untreated PC and PF127-grafted PC were 0.86 ± 0.19 and 0.57 ± 0.07 μg/cm^2, respectively ($n = 3$). These surface fibrinogen concentrations should be taken with caution, be-

cause they were calculated based on the apparent surface area of PC, which has a highly irregular surface. Although the true magnitude of fibrinogen adsorption is thus substantially less, the relative decrease in fibrinogen adsorption after PF127 grafting should remain at the same level.

To examine the effect of the surface Pluronic® concentration on the prevention of fibrinogen adsorption, the grafting of two Pluronics® to TCVS-glass was done with a slight modification. As described earlier, after 1 h of polymer adsorption, the surfaces were washed with DDW and exposed to γ irradiation in DDW. Fibrinogen adsorption to TCVS- or DDS-glass treated with varying concentrations of PF127 and PP105 was determined to compare polymer grafting efficiency of the surfaces. PF127 and PP105 were chosen based on their PEO block lengths. PF127 has 98 repeating units per PEO block, while PP105 has 38. The polymer solution concentration used in the surface grafting step was varied to provide a range of PEO surface densities. Thus, the relative effects PEO chain length and surface grafting density on protein adsorption could be examined.

Figure 3. Fibrinogen adsorption to control nitinol (Control), TCVS-nitinol (TCVS), TCVS-nitinol γ irradiated in DDW (DDW), and nitinol grafted with PF127 (PF127). The fibrinogen concentration used for adsorption was 0.1 mg/mL. Values are mean $\pm$ SEM; $n = 3$.

Figure 4 shows fibrinogen adsorption to PF127 treated surfaces. Untreated TCVS-glass and TCVS-glass γ irradiated in DDW exhibited fibrinogen adsorption of 0.45 ± 0.06 and $0.35 \pm 0.06 \ \mu g/cm^2$, respectively. Analysis of

variance (ANOVA) indicated that fibrinogen adsorption decreased significantly ($p < 0.05$) after γ irradiation. The significant decrease was observed when the concentration of the bulk PF127 solution was 0.1 mg/mL and higher. Fibrinogen adsorption decreased rapidly to 0.10 $\mu g/cm^2$ when the concentration of PF127 in the adsorption solution was 0.1 mg/mL. As the PF127 bulk concentration increased to 1 mg/mL, fibrinogen adsorption decreased to a minimum value of approximately 0.08 $\mu g/cm^2$. Above 0.1 mg/mL, fibrinogen adsorption remained essentially constant, irrespective of bulk PF127 concentration in the adsorption solution. PF127 grafting was responsible for decreasing fibrinogen adsorption to TCVS-glass by more than 80%. Untreated DDS-glass and DDS-glass γ irradiated in DDW exhibited fibrinogen adsorptions of 0.51 ± 0.05 and $0.45 \pm 0.05 \ \mu g/cm^2$, respectively. Fibrinogen adsorption was the same, irrespective of increasing PF127 concentration in the treatment of the DDS-glass, however. ANOVA showed that there was no significant difference ($p = 0.17$) in fibrinogen adsorption after γ irradiation of DDS-glass. At all PF127 concentrations used, the surface fibrinogen concentration ranged from 0.32 to 0.37 $\mu g/cm^2$. This represents a maximal reduction in protein adsorption to DDS glass of 40%.

Figure 5 shows fibrinogen adsorption to TCVS- and DDS-glass treated with PP105. Fibrinogen adsorption to TCVS-glass decreased to 0.27 $\mu g/cm^2$ when the PP105 concentration used to prepare the samples was 0.01 mg/mL. The γ irradiation of TCVS-glass in the presence of
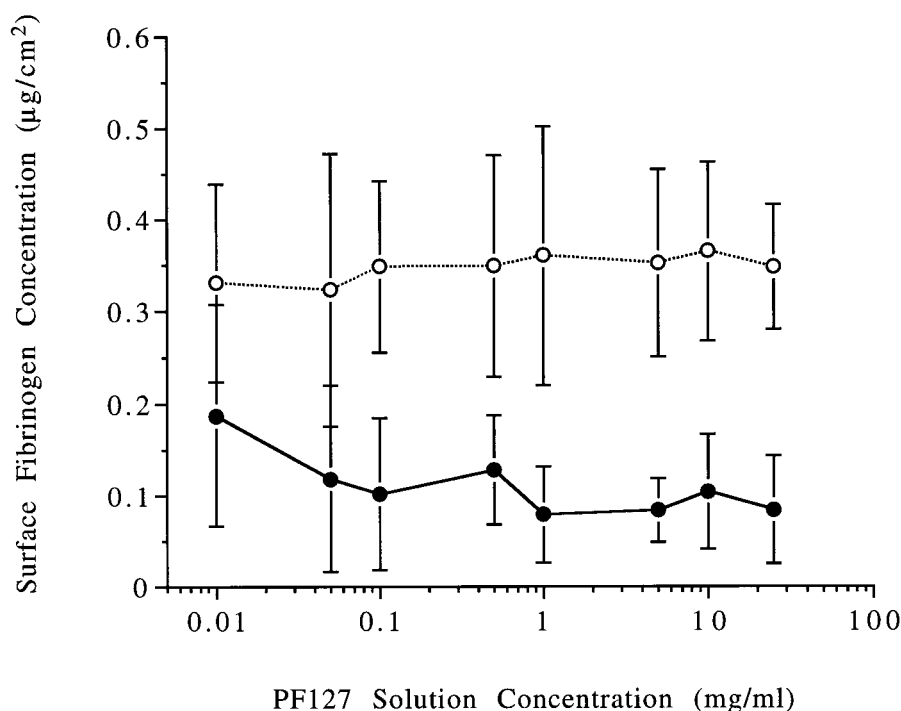
Figure 4. Fibrinogen adsorption to (●) TCVS-glass and (○) DDS-glass adsorbed with PF127 for 1 h, rinsed of polymer solution with water, and exposed to 0.3 Mrad of γ irradiation in water. The fibrinogen concentration used for adsorption was 0.1 mg/mL. Values are mean $\pm$ SEM; $n = 3$.
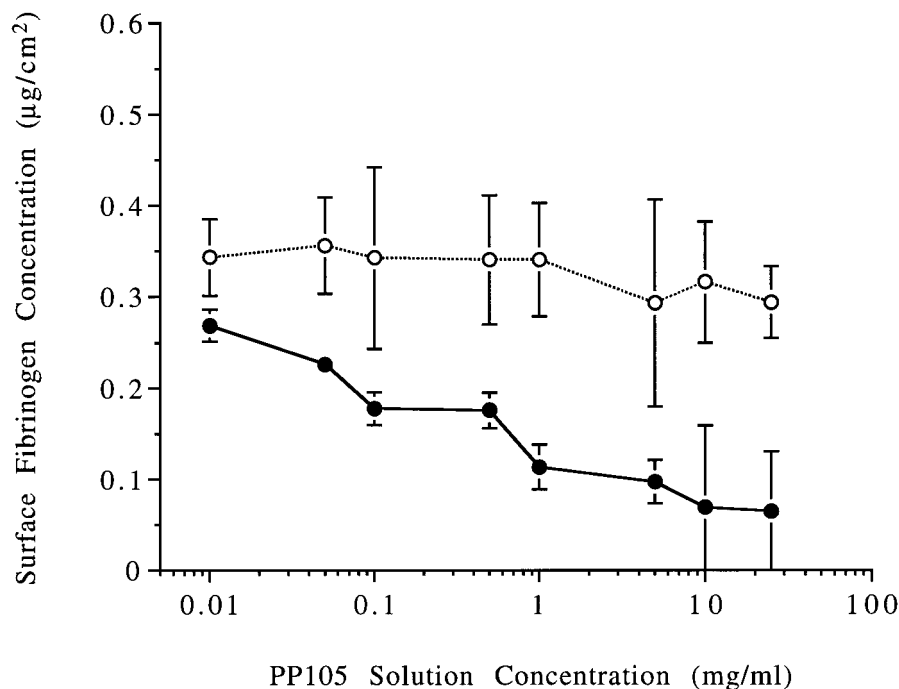
Figure 5. Fibrinogen adsorption to (●) TCVS-glass and (○) DDS-glass adsorbed with PP105 for 1 h, rinsed of polymer solution with water, and exposed to 0.3 Mrad of γ irradiation in water. The fibrinogen concentration used for adsorption was 0.1 mg/mL. Values are mean ± SEM; $n = 3$.

PP105 significantly reduced fibrinogen adsorption ($p < 0.05$). The difference was observed even when the concentration of the bulk PP105 solution was 0.01 mg/mL. Fibrinogen adsorption continued to decrease with increases in PP105 concentration up to 10 mg/mL. Above 10 mg/mL, fibrinogen adsorption remained at approximately 0.07 μg/cm². Fibrinogen adsorption to TCVS-glass was decreased by 84% as a result of PP105 grafting. DDS-glass treated with PP105 at the bulk concentration ranging from 0.01 to 10 mg/mL exhibited fibrinogen adsorption in the range of 0.30 and 0.36 μg/cm². The decrease in fibrinogen adsorption to DDS by PP105 was maximally 45%. ANOVA, however, showed that there was no significant reduction ($p = 0.15$) in fibrinogen adsorption by γ irradiation.

Platelet Adhesion and Activation

Figure 6(A) and 6(B) show platelet adhesion from PRP onto TCVS- and DDS-glass samples, respectively. The untreated control surfaces showed extensive platelet adhesion and activation. The entire surface was covered by activated platelets in multiple layers. As expected, the hydrophobic nature of TCVS- and DDS-glass rendered them highly thrombogenic. TCVS- and DDS-glass γ irradiated in DDW elicited the same platelet response as the control surfaces, respectively shown in Figure 6(C) and 6(D). Fully activated platelets covered the entire surface in multiple layers. There was no apparent difference between untreated and γ-irradiated surfaces of either TCVS or DDS treatment,

nor between the two treatments. This indicates that γ irradiation in the aqueous environment had no effect on thrombogenicity, irrespective of surface hydrophilicity. Platelet interactions with DDS- and TCVS-glass γ irradiated in the presence of PF127 (1 mg/mL) are shown in Figure 6(E) and 6(F), respectively. The difference in platelet response to DDS and TCVS surface treatments is obvious. The DDS sample was similar to the samples γ irradiated in DDW and untreated control samples. A virtually complete layer of platelets covered the entire surface. The platelets were fully spread and activated and present in multiple layers. This indicates that PF127 was not effectively grafted to DDS with a γ-radiation dose of 0.3 Mrad. The TCVS-glass samples, however, was entirely different. No adherent platelets were found on these samples. Grafting with PF127 was highly effective at preventing platelet adhesion. This suggests that PF127 is efficiently grafted to TCVS-glass with 0.3 Mrad of γ radiation.

Platelet interactions with nitinol wire are shown in Figure 7(A–D). In these epifluorescence micrographs, platelets appear white against the black metallic background. Blurring at the outer edges of the images is due to the curved geometry of the samples and fixed depth of focus of the microscope. The untreated nitinol surface showed nearly complete coverage by platelets [Fig. 7(A,B)]. The adherent platelets were spread and activated, as in the DDS- and TCVS-glass, and generally present in multiple layers. Despite its hydrophilic surface, untreated nitinol proved to be thrombogenic. PF108-grafted nitinol exhibited much
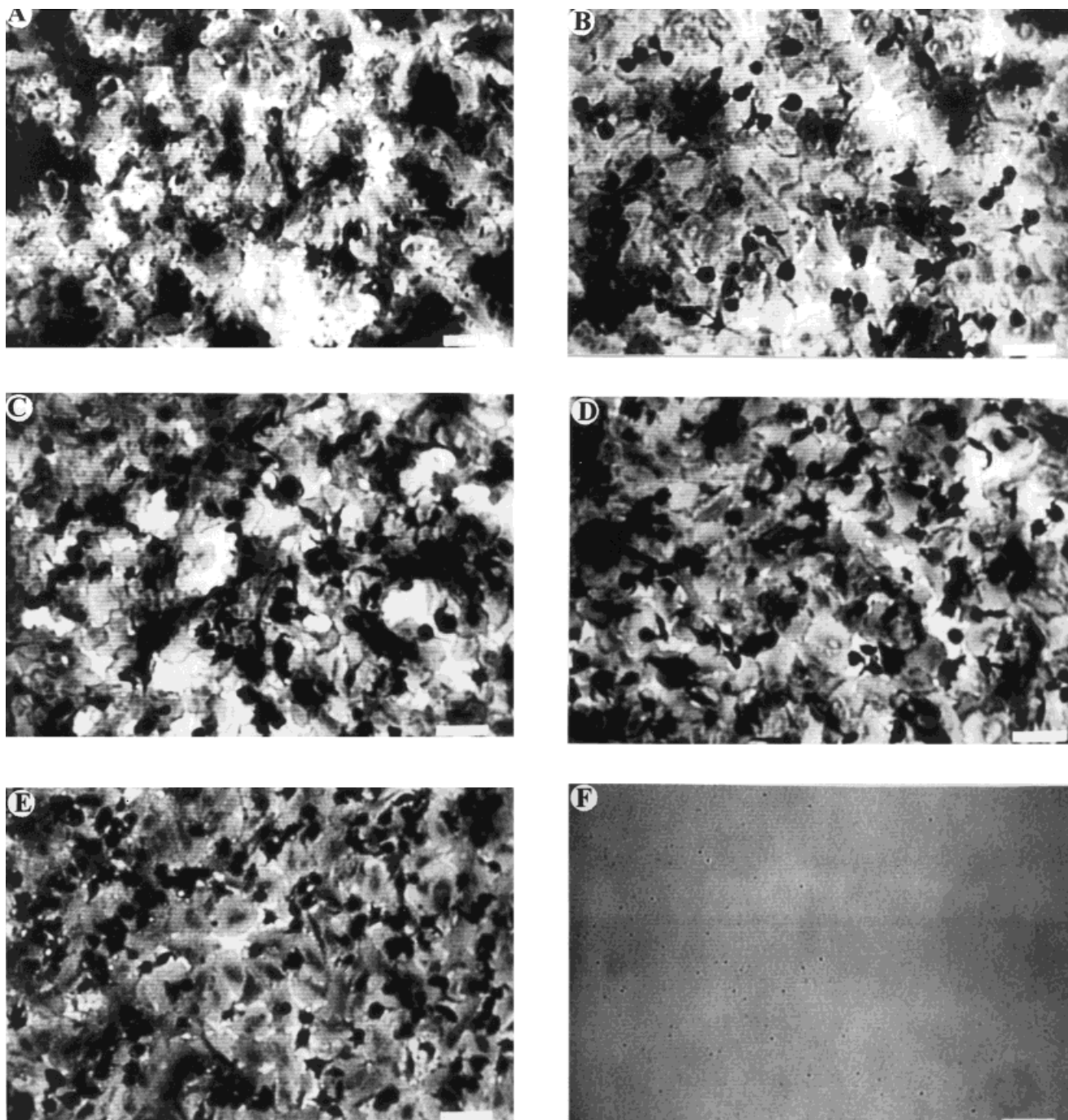
Figure 6. Brightfield video micrographs of glass surfaces exposed to PRP for 1 h. Surfaces tested were (A) control DDS-glass, (B) control TCVS-glass, (C) DDS-glass γ irradiated in water, (D) TCVS-glass γ irradiated in water, (E) DDS-glass γ irradiated in PF127 (1 mg/mL) solution, and (F) TCVS-glass γ irradiated in PF127 (1 mg/mL) solution. Platelets were stained black in these figures. Scale bar = 10 μm.

reduced platelet adhesion. Most fields of view at 20× magnification showed no platelets. Those platelets that were present did not appear to be surface activated, but simply contact adherent. PF108 grafting effectively prevented platelet adhesion and activation on the nitinol surface.

Scanning electron micrographs were taken of PRP-exposed PC samples. Figure 8(A,B) shows platelet adhesion

to control PC samples. Only a single layer of fully activated platelets are seen. Under 2500× magnification no fields of view were free of adherent platelets. Multilayer coverage was only seen in small, isolated areas. Unlike glass and nitinol, the control PC surface exhibited low intrinsic platelet activation. PC grafted with PF127 nonetheless showed a decrease in platelet adhesion, as seen in Figure 8(C,D).
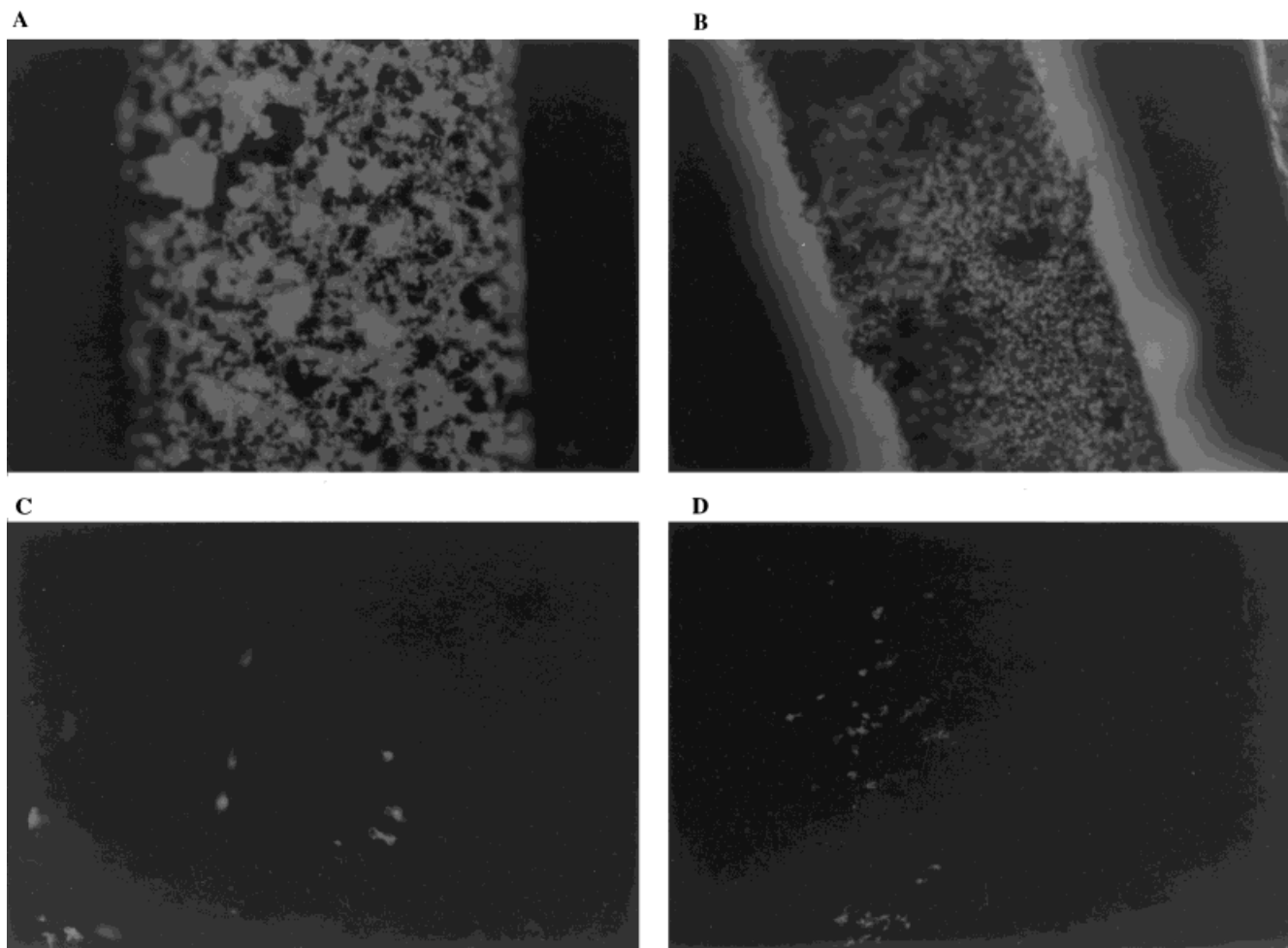
Figure 7. Epifluorescence micrographs of nitinol surfaces exposed to PRP for 1 h. Surfaces tested were (A, B) control nitinol and (C, D) nitinol grafted with PF108. Platelets appear white in these epifluorescence figures and black background indicates no platelet adhesion. Original magnification = (A) ×40 and (B–D) ×20.

PF127-grafted PC was nearly devoid of platelets. Only small areas of contact adherent platelets were present, but the vast majority of the 2500× fields of view showed no platelets. This indicates that modification of the PC surface, which is already only slightly thrombogenic, with PF127 further improves its thromboresistance, virtually eliminating platelet adhesion.

DISCUSSION

Mechanism of Silanization and Polymer Grafting

The TCVS coating process is described schematically in Figure 9(A). First, TCVS molecules are hydrolyzed by water adsorbed on the glass surface and yield silanetriols. Silanetriol molecules interact with each other and the hydroxyl- or oxide-rich surface by hydrogen bonding.[34–39] Finally, when dried and allowed to cure, the silanes and the surface condense and form a covalently bound silane layer. ''Curing'' refers to the formation of the covalent bonds between the silane molecules and the substrate surface.[40,41] The long-term stability of the bonded TCVS layer is important in clinical application of the approach. These covalent bonds are known to be stable in solutions at pH 4 to 7.[42] In addition, three leaving groups in TCVS are known to result in a polymeric network by horizontal polymerization,[36,43] which appears to be more resistant to hydrolysis.[44] The *in vivo* long-term stability, however, needs to be investigated. The proposed mechanism of polymer grafting on the vinyl surface by γ radiation is shown in Figure 9(B). First, a vinyl group on the surface is ionized, either by absorption of γ photons or interaction with water-derived radicals, forming surface bound vinyl free radicals. These radical species are expected to attack adsorbed polymer molecules, forming new covalent bonds between the surface and polymer. The long chains represent PEO blocks in the molecule. Any vinyl radical that does not react with a polymer molecule may react with the solvent water molecules, enriching the surface in oxygen content and hydro-
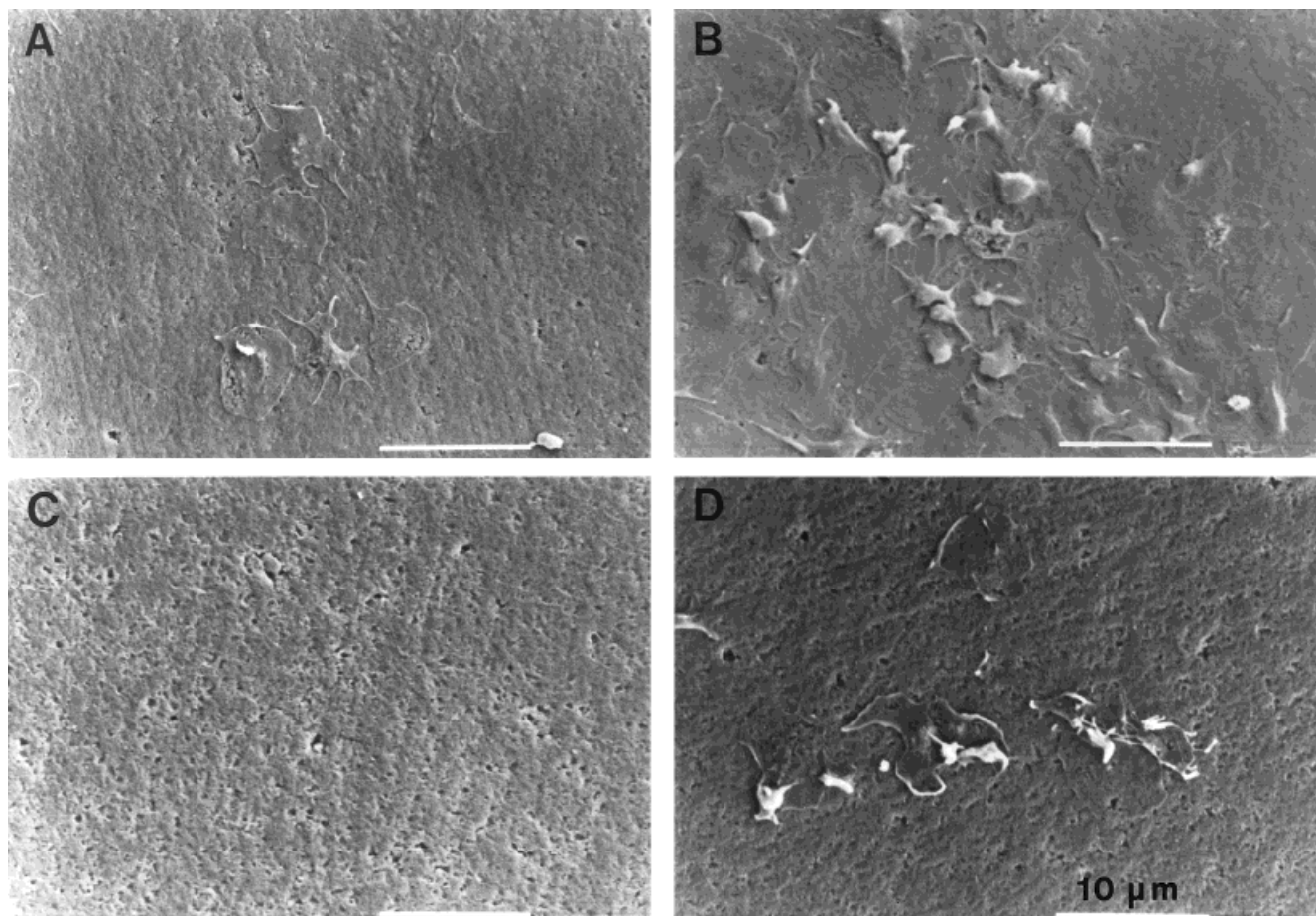
Figure 8. Scanning electron micrographs of (A, B) control PC and (C, D) PC grafted with PF127 after exposure to PRP for 1 h. Scale bar = 10 μm.

philicity. By comparison the saturated hydrocarbon nature of DDS-glass is not expected to significantly ionize with small γ-radiation doses. This translates into less efficient polymer grafting than with TCVS at equivalent γ-radiation doses, as indicated by results in Figures 4 and 5, showing no reduction in fibrinogen adsorption, and in Figure 6, showing no reduction in platelet adhesion on DDS-glass after γ irradiation. It is also possible that the adsorbed polymers may be mechanically entrapped on the TCVS-glass during the cleavage and crosslinking process by γ irradiation. Such mechanical entrapment, if it occurs, should be far less efficient on DDS glass that provides no vinyl groups.

The proposed grafting mechanism is supported by surface analysis and fibrinogen adsorption data. Advancing and receding water contact angles on control TCVS-glass were moderately high, which was expected for an intact hydrocarbonlike surface. The γ irradiation in the presence of DDW converted this into a very hydrophilic surface as shown by the zero contact angle in Table I. This hydrophilic surface exhibited a predictable decrease in fibrinogen adsorption. DDS-glass did not exhibit significant changes in hydrophilicity or fibrinogen adsorption following γ irradia-

tion in water. Because the structure of DDS-glass consists of methyl groups bound to the surface through silyl ether bonds, it is intuitive that small γ-radiation doses induce only minor changes in the surface chemistry. It contains no functional groups that are particularly susceptible to activation with low doses of γ radiation. Thus, any changes that occur are inefficient and result in heterogeneous surface chemistry. The disparate surface chemistries between TCVS- and DDS-glass, and their intrinsic propensities toward γ irradiation, are apparently responsible for the differences.

Protein adsorption onto TCVS- and DDS-glass treated with Pluronic® surfactants illuminates the functional difference between the TCVS and DDS surface treatments. As seen in Figures 4 and 5, fibrinogen adsorption to TCVS surfaces was dependent on the polymer solution concentration used during the adsorption step of the surface preparation. As the solution concentration increased for PF127 and PP105, there was a corresponding decrease in fibrinogen adsorption up to a point. Above a certain bulk concentration of Pluronic® surfactants no further reduction in protein adsorption was observed, irrespective of increasing solution concentration. Reductions in fibrinogen adsorption by

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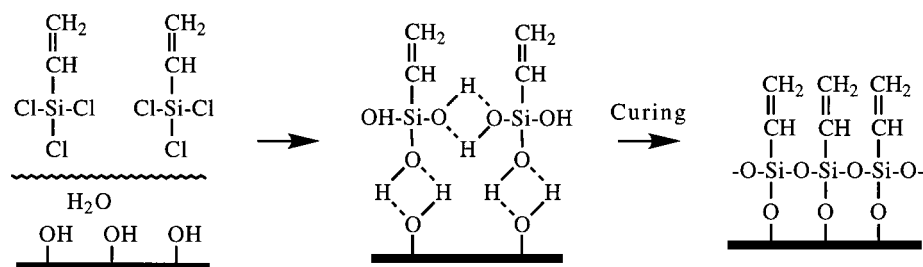
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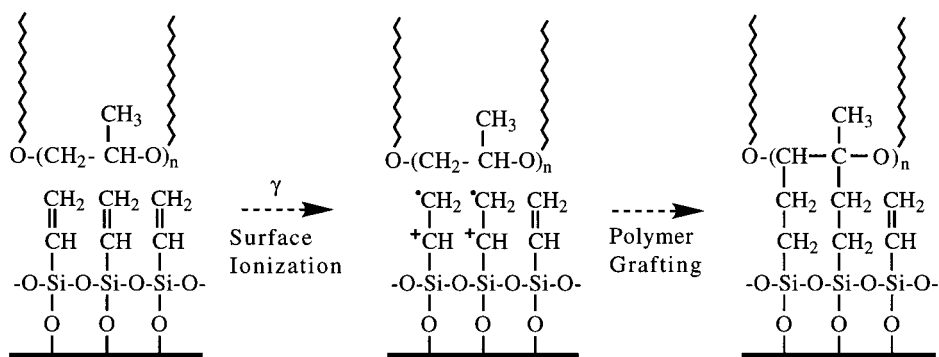
Figure 9. Schematic representation of (A) surface silanization by TCVS and (B) proposed mechanism of covalent grafting of Pluronics® to the TCVS-primed surface.

more than 80% were achieved on TCVS-glass grafted with PF127 or PP105. Similarly treated DDS-glass, on the other hand, exhibited fibrinogen adsorption that was essentially independent of polymer concentration. The relatively inert DDS surface was not activated by 0.3 Mrad of γ radiation, so the adsorbed polymer was not grafted with a density significant enough to prevent fibrinogen adsorption more than by physical adsorption. SDS washing after γ irradiation removed the adsorbed polymer, rendering the finished samples virtually identical for both PF127 and PP105.

Fibrinogen adsorption was always lower on TCVS-coated substrates after γ irradiation in DDW, compared with the nonirradiated surface. The 20% reduction in fibrinogen adsorption on the γ-irradiated surface is an example. Similarly treated DDS-glass samples exhibited only a 12% decrease in fibrinogen adsorption. These data are representative of our routine experience in the laboratory with fibrinogen adsorption. The fibrinogen adsorption on γ-irradiated TCVS-glass, which has a zero contact angle, clearly indicates that the surface hydrophilicity is not the major determining factor in protein adsorption as once thought. It is true, however, that the driving force for protein adsorption is decreased on the hydrophilic surface of γ-irradiated TCVS-glass. DDS-glass is much less affected

and thus does not exhibit as large a decrease in fibrinogen adsorption following γ irradiation in DDW.

TCVS-Glass Versus DDS-Glass in Surface Modification Studies

Fibrinogen adsorption to TCVS-glass treated with various Pluronic® surfactants and PEO (Fig. 2) underscores the value of the vinyl-derivatized surface in polymer grafting. All polymers were used as received, without chemical derivatization or purification. Yet, these polymers without active chemical functional groups *appear to be* covalently grafted to the TCVS-glass surface with γ radiation in a facile two step process. Other approaches to surface modification, as previously mentioned, are generally labor intensive and require several steps. Previous work in our laboratory showed that unmodified Pluronic® polymers could be grafted to DDS-glass using γ irradiation.[45] The γ-radiation dose required, up to 2.5 Mrad, was much greater than the relatively small 0.3 Mrad used for TCVS-glass in these studies, however. This advantage alone saves significant time and laboratory resources.

It is interesting to note that PEO, which was not expected to tightly adsorb to the hydrophobic surface, was grafted in

sufficient density on TCVS-glass to significantly decrease fibrinogen adsorption (Fig. 2). Further, all Pluronic® polymers decreased fibrinogen adsorption by an order of magnitude, irrespective of their PEO block lengths. When amphiphilic block copolymers adsorb in high density to hydrophobic surfaces they form a ''brush'' surface. Under this regime, which is expected in these experimental conditions, the polypropylene oxide (PPO) segments in the Pluronic® block copolymers enjoy intimate interaction with the surface while the PEO chains extend into the bulk aqueous solvent. Rinsing away the excess polymer solution is not expected to alter the adsorbed brush, due to the strong interaction between PPO segments and the surface. Thus, γ irradiation after rinsing results in grafting of only the adsorbed brush, the density of which is determined by the adsorption isotherm. This is consistent with the fibrinogen adsorption data in Figures 4 and 5. Rinsing may, however, interrupt weaker polymer–surface interactions, such as adsorption of a PEO block. The grafting method for the data shown in Figure 2 did not include rinsing. Thus, PEO–surface interactions may have persisted during γ irradiation, resulting in an unexpectedly high polymer surface density as compared to brush grafting. Rinsing away the bulk PEO homopolymer solution prior to γ irradiation is not expected to result in significant PEO grafting. This has been indeed our experience in the laboratory. The data collectively indicate that TCVS is superior to DDS for surface treatment of inorganic materials for surface modification.

TCVS in Surface Modification of Clinically Relevant Materials

Studies of platelet adhesion to a variety of surfaces affirm the utility of PEO grafting to inorganic materials. Derivatized glass is a convenient model surface because it affords sample observation by optical microscopy. DDS-glass is an obvious standard with which to compare TCVS-glass, because its platelet adhesion and activation properties have long been studied in our laboratory.[6,23,24,45] TCVS-glass equals DDS-glass in convenience of preparation and nonspecific thrombogenicity. Analogous to protein adsorption studies, however, TCVS-glass is superior to DDS-glass in the study of surface modifications to prevent platelet adhesion.

Surface-induced thrombosis on nitinol is of clinical concern, because some intravascular stents and angioplasty guide wires are fabricated of this alloy. The nitinol used in the platelet adhesion study was in the form of intravascular stents. Platelet adhesion to the untreated nitinol was significant in our assay, while PF108 grafting nearly eliminated platelet adhesion, as seen in Figure 7. The PF108-grafted nitinol stents performed equally well in a pig *ex vivo* carotid arteriovenous shunt model. In this model, PF108 grafting reduced thrombus deposition on the nitinol stents by 93%.[46] Further, the untreated stents generally occluded within the scheduled experiment time of 40 min, while all PF108-grafted samples remained patent. The clinical significance of the combined *in vitro* and *ex vivo* data is obvious: hydrophilic polymer grafting may obviate anticoagulant pharmacotherapy in patients implanted with intravascular stents.

PC disks, which are blood-contacting components of prosthetic heart valves, also benefited from grafting with PF127. The low number of adherent platelets and lack of significant multilayer formation on the untreated PC surface indicated excellent intrinsic biocompatibility. PF127 grafting, however, nearly eliminated platelet adhesion to this already excellent biomaterial. The platelets that succeeded in adhering to the grafted surfaces did not spread as they did on the untreated PC. Similar to PF108-grafted nitinol, the potential clinical implications of surface-modified PC are clear.

Role of Grafted Polymer in Thromboresistance

The increased thromboresistance of PEO-grafted surfaces cannot be attributed to increased hydrophilicity of the treated materials. All DDS-glass surfaces, whether γ irradiated or not, showed extensive platelet adhesion and activation. Any minor increase in hydrophilicity imparted by γ-radiation induced oxidation went unnoticed in the platelet response. TCVS-glass surfaces also showed no correlation between hydrophilicity and platelet adhesion. Advancing and receding contact angles on untreated TCVS-glass became zero after γ irradiation (Table I). As expected, untreated TCVS-glass exhibited gross hydrophobicity and complete platelet activation. Although TCVS-glass γ irradiated in DDW was very hydrophilic, the platelet response was identical to the untreated surface. In stark contrast, PF127 grafting only moderately increased the hydrophilicity, yet it prevented platelet adhesion and activation.

Fibrinogen adsorption profiles paralleled the platelet adhesion data, showing no simple correlation between water contact angles and performance of the grafted surface. γ Irradiation in DDW decreased fibrinogen adsorption to TCVS-glass as previously described, but the extent of the decrease was far less than was attributable to the grafted polymers. These observations are inconsistent with the hypothesis that surface hydrophilicity is responsible for protein adsorption or platelet response to surfaces. The same effect was noted on TCVS-coated nitinol, both with fibrinogen adsorption and platelet adhesion. The extent of PEO grafting, or the surface density of the grafted PEO, appeared to be the determining factor in preventing protein adsorption and platelet adhesion in these systems. In all surface modification studies using PEO or other hydrophilic polymers, the density of the grafted polymers should be maintained high enough to ensure protein-resistant and platelet-resistant properties of the modified surfaces.

CONCLUSIONS

A new method to graft hydrophilic polymers, such as PEO, to inorganic surfaces, such as metal and glass, has been

developed. The surface is first primed with a vinyl group containing layer by reacting with TCVS. The surface is then exposed to a low dose (e.g., 0.3 Mrad) of γ irradiation in the presence of grafting polymer solution. Surfaces modified by this method show substantial reduction in fibrinogen adsorption and platelet adhesion. While we do not have direct evidence proving the covalent grafting of Pluronics® or PEO to the TCVS-modified, γ-irradiated surface, the significant reduction in both fibrinogen adsorption and platelet adhesion suggests that PEO molecules remain on the surface even after overnight washing in 1% SDS solution. Because this approach does not require modification of the grafting polymers, such as PEO, it is simple, efficient, and material independent. Addition of this technique effectively expands the range of surface modification methods to include all relevant biomaterials, both organic and inorganic.

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