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EX VIVO PLATELET DEPOSITION ON FIBRONECTIN-PREADSORBED SURFACES

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Abstract

Temporal platelet deposition profiles of canine plasma fibronectin (CPFN) adsorbed to different polymers ex vivo and the in vitro characteristics of CPFN adsorption were studied in an attempt to correlate the two. The maximum platelet deposition (Γ_{plt}^{max}) obtained at a protein preadsorption time of 30 min was greater than that obtained using an adsorption time of 120 min for all surfaces studied. At 30 min of preadsorption, Γ_{plt}^{max} was 520, 560 and 1230 platelets/1000 μm^2 on Biomer, polyethylene (PE) and oxidized PE (OXPE), respectively. In contrast, the platelet deposition at 120 min. of fibronectin preadsorption was about 60~90 platelets/1000 μm^2 on all polymers studied. The surface concentrations of adsorbed CPFN measured using ^{125}I-CPFN, were in the order PE > OXPE > Biomer. The adsorbed protein concentration increased with increasing adsorption time. The surface distribution of adsorbed CPFN was visualized with antibody-labelled colloidal gold and scanning electron microscopy. The extent of staining was lowest on PE, greater on Biomer, and highest on OXPE, roughly similar to the order of platelet deposition. Platelet deposition ex vivo appears to correlate with the immunogold-stainable-adsorbed protein rather than with the total amount of adsorbed protein.

Key Words: Fibronectin, Adsorption, Polymer, Platelet Deposition, Immunogold-Bead Labelling.

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Introduction

Considerable effort has been expended toward the synthesis of polymers for use in the fabrication of vascular prostheses, cardiac assist devices, and artificial hearts. Although presently artificial polymers are used in many blood contacting applications, unfavorable biological responses, such as thromboembolism and poor histocompatibility are often elicited by the foreign materials.

We have developed a canine ex vivo arteriovenous shunt model to follow blood platelet deposition on candidate biomaterials. The series shunt configuration in this model allows several different materials to be tested simultaneously, hence under identical conditions (12). It is also possible to investigate the role plasma proteins play in polymer hemocompatibility by preadsorbing such proteins to test materials (10, 23). This role is significant because the first event in the thromboembolic response is probably the adsorption of plasma proteins; their adsorption is shortly followed by binding and activation of platelets, fibrin formation and other related phenomena (3). In the past we have used this model to study the behavior of canine plasma fibronectin (CPFN) adsorbed to polymers because this glycoprotein promotes the adhesion and spreading of several types of cells (15, 21). Our previous studies demonstrated that adsorbed CPFN enhances the thrombotic response (10, 23).

Here we report an attempt to correlate the behavior of CPFN adsorbed to various polymers and the ex vivo thrombogenicity of the adsorbed protein. Under carefully controlled conditions, CPFN was adsorbed to a series of polymers with widely different surface characteristics, and the platelet deposition profiles evoked by the protein-coated surface ex vivo were determined. In conjunction with the ex vivo experiments, the adsorption of CPFN was characterized by different techniques in vitro. The extent of adsorption as a function of the adsorption time was quantified with ^{125}I-labelled CPFN (10). The surface distribution of the adsorbed protein was examined by analysis of immunogold-labelled adsorbed protein using scanning electron microscopy.

Materials and Methods

Protein Isolation and Characterization

Fibronectin was isolated from citrated canine plasma, using a modification of the method of Ruoslahti et. al. (19), by affinity chromatography on gelatin-agarose (BioRad). The bulk concentration of CPFN used c_b was determined using absorption of CPFN solutions at 280 nm and an extinction coefficient of 1.28 cm^2/mg. The protein was

radiolabelled with ^{125}I using the Chloramine-T method (Iodobeads, New England Nuclear). The radioiodinated protein was separated from free radioiodide on a desalting column of Bio-gel P-30 (BioRad). Radioiodination levels were less than or equal to 0.13 mole of ^{125}I per mole of labelled protein.

The proteins were restored by dialysis to the adsorption buffer, phosphate buffered saline without divalent cations and containing 0.02% NaN_3, pH 7.39 (PBS) before snap-freezing in a dry ice-ethanol bath and storage at -70°C. The purity, identity and integrity of the proteins were established by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) (10).

Polymers

The polymers used were low density polyethylene (PE) (Intramedic), chromic acid oxidized PE (OXPE) and solution grade Biomer polyetherurethaneurea (Biomer) (Ethicon). OXPE was freshly made by the chromic acid oxidation procedure of Rasmussen et. al. (18). The polymer exhibits principally carboxylic groups on surface, as well as ketone and aldehyde functionalities. Biomer was coated onto OXPE using the procedure of Lelah et. al. (11).

Protein Adsorption

The method of preparation of the tubing surfaces and of protein adsorption is described briefly here. Five cm. segments of 3.2 mm inner diameter (ID) tubings of the polymers were connected flush using 2.6 mm ID Silastic outside sleeves (Dow Corning), and three-way stopcocks were attached at both ends of the resulting composite tubing. The tubings were washed with 1/8% Ivory detergent, copiously rinsed with double-distilled deionized water, rinsed and filled with the adsorption buffer (PBS), and equilibrated overnight at room temperature, under water. For adsorption, the tubings were held vertically in plexiglass racks and mixtures of radiolabelled and unlabelled prefiltered protein solutions were introduced from below with a syringe. After predetermined adsorption times had elapsed, the bulk protein solution was flushed out slowly with copious quantities of PBS. The introduction of air bubbles was avoided at all stages. The shunts were held vertically to eliminate the effects of gravity on the surface distribution of the proteins.

Ex Vivo Platelet Deposition on the CPFN-Preadsorbed Polymers

The canine ex vivo shunt model and the procedure for the determination of platelet deposition profiles has been described previously (12, 23). Briefly, platelets obtained from adult mongrel dogs weighing 20 to 35 kg were radiolabelled with ^{51}Cr and reinjected into the test animal about 15 h before the experiment. The left femoral artery and vein were cannulated with the test shunt (filled with PBS containing no NaN_3), which consisted of an 8 cm PE entrance region, a CPFN-preadsorbed section containing several polymer segments prepared as described in the protein adsorption section above, followed by an 80 cm PE return line (Figure 1). A branch artery proximal to the shunt cannulation site was connected to a flushing system. At the end of a certain blood contact time, the femoral artery was clamped atraumatically, and the shunt was flushed with 100 ml of fresh Tyrode's solution containing no divalent cations to remove the bulk blood and leave only adsorbed blood species on the shunt. The protein-preadsorbed test section of tubing was removed and adherent blood elements fixed by displacing the Tyrode's solution with 2% glutaraldehyde for 2 h at room temperature. The tubing was then drained, and the central 3 cm pieces of each 5 cm test polymer were counted for radioactivity to determine the amount of platelet deposition. The blood contact time was varied between 1 and 60 min, using a new protein-preadsorbed test section for each time point. Results were averaged using three separate surgeries in three different animals. The blood flow rate was monitored continuously and a clamp at the proximal end of the PE tubing was used to adjust it to an average of 250 ml/min (average shear rate 1320 s^{-1}). Occasionally, due to tubing occlusion, this was not possible, and then the blood flow rate was allowed to drop naturally, to a value not less than 80 ml/min. The following hematological parameters, blood pH, prothrombin and euglobin lysis times, and platelet and fibrinogen concentrations were monitored periodically using blood samples derived from a catheter in the neck of the animal. The platelet data were used in the estimation of platelet deposition and to determine if splenic uptake and release effects occurred.

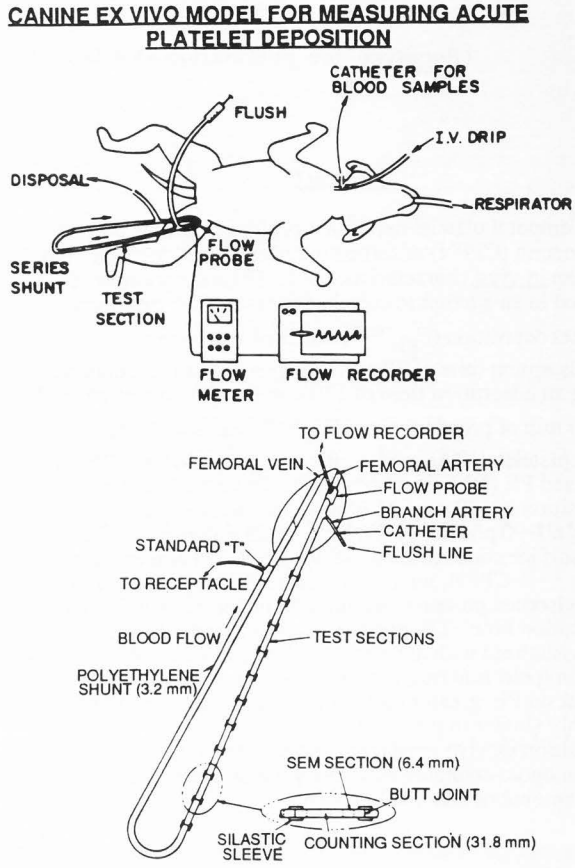


Figure 1. Schematic of animal experiment showing shunt, catheter, flow measurement and recording instrumentation. Below the schematic, displaying the joined series of fibronectin-preadsorbed tubing, is the shunt assembly.

Protein Surface Concentration and Immunogold Labeling

Each test segment was monitored for adherent radioactivity and immunogold-stained. A 2 cm piece of each segment of the prefixed protein-adsorbed tubing was cut, drained, and used for gamma counting in a Beckman 5500 gamma counter. The length in cm of this sample was measured to three significant figures, and the surface concentration of the adsorbed protein was determined. A 1 cm length from each end of each polymer segment was always cut off and discarded.

The adsorbed protein molecules were fixed by 7 min incubation with 1% glutaraldehyde in PBS, followed by rinsing with PBS. The excess glutaraldehyde was neutralized by 20 min incubation with 50 mM glycine in PBS, followed again by rinsing with PBS. Approximately 3

mm pieces of the prefixed protein-coated shunt were cut using a sharp double-edged blade, and transferred onto a ridged 96-well cover plate (Costar), without drainage. A 50 µl suspension of the immunogold particles (A_{525} equalled 6.2 such that the immunogold bead concentration was of the order 10^{12} particles/ml) was added and incubation was allowed for 45 min at room temperature (ca. 22~24°C). The sample was then rinsed carefully and slowly in PBS, and transferred into a vial containing 2% glutaraldehyde. After at least 24 h of fixation, the piece was prepared for electron microscopy. Two antibodies were bound to the colloidal gold. Antialbumin antibody was used as a control for nonspecific labeling, in addition to the antifibronectin antibody. Reactivity of both antibodies with CPFN was tested. Polymers without adsorbed protein were also tested for nonspecific immunogold labeling. Contact of protein-coated surfaces with the air-water interface was avoided to diminish artifacts in the staining results.

Immunogold Suspensions

The immunogold suspensions were prepared as previously reported (13), and were used within a week of preparation. Affinity-purified goat antihuman protein antibodies (Cooper Biomedical) were conjugated to 18 nm gold particles, as described by Park et. al. (16).

SEM Preparation

Samples were dehydrated in a graded ethanol series and were dried by the critical point method using molecular sieve-dried CO_2 as the transitional fluid. The samples were sputter-coated with 10 nm gold and examined in a JEOL JSM 35C scanning electron microscope using 10-20 kV acceleration voltage and 20,000x magnification.

Results

Platelet Deposition Ex Vivo

The platelet deposition profiles on bare polymers, obtained as a control, are displayed in Figure 2. OXPE by far was the most thrombogenic material, displaying a platelet deposition peak Γ_{plt}^{max} of 3900/1000 µm^2, at a blood contact time t_c of 20 min. PE exhibited a lower peak of 370/1000 µm^2, at 15 min, while solution grade Biomer was relatively thromboresistant, with a peak deposition of 220 platelets per 1000 µm^2, at 15 min of blood contact (9). A monolayer of rounded, close-packed platelets contains roughly 220 platelets/1000 µm^2, using a simple calculation based on a platelet diameter of 2.13 µm.

The platelet deposition profiles obtained when CPFN was adsorbed from a 0.071 mg/ml solution for an adsorption time t_i of 30 min and for 120 min onto various polymers are presented in Figure 3 and 4, respectively. At 30 min of adsorption time, the presence of CPFN enhanced the thrombogenicity of both PE and Biomer (Biomer), compared to the respective bare polymers, whereas the opposite effect was seen on OXPE. At this t_i, OXPE showed the highest deposition with Γ_{plt}^{max} being 1230 platelets/1000 µm^2 at 60 min of blood contact, followed by PE (560/1000 µm^2) and Biomer (520/1000 µm^2), with the maxima occurring on both of the latter polymers at 45 min of blood contact. CPFN preadsorbed for 30 min enhanced platelet deposition somewhat and prolonged the attainment of platelet deposition peaks. These were generally exhibited at or above t_c 45 min and were significantly greater than platelet deposition on the bare polymers at these values of t_c. These peaks substantially exceeded a monolayer of close-packed platelets and demonstrate considerable activity of the cells deposited.

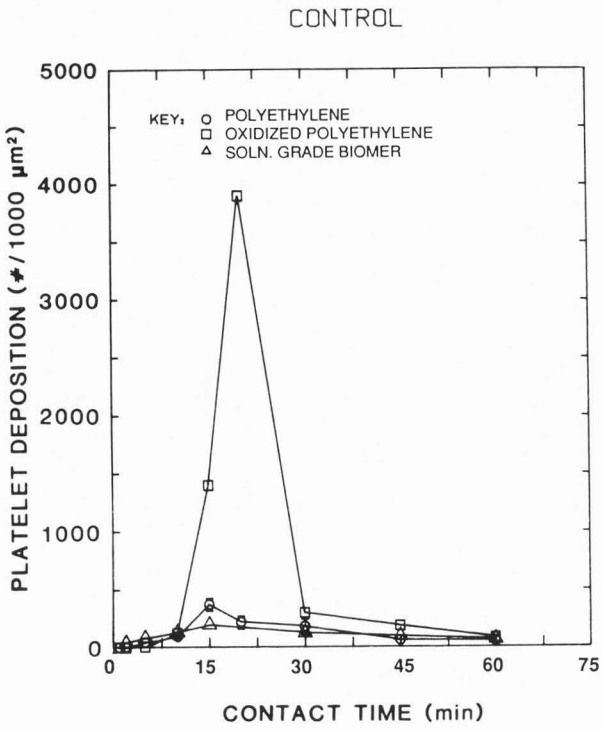


Figure 2. Platelet deposition profiles on bare OXPE, PE and Biomer.

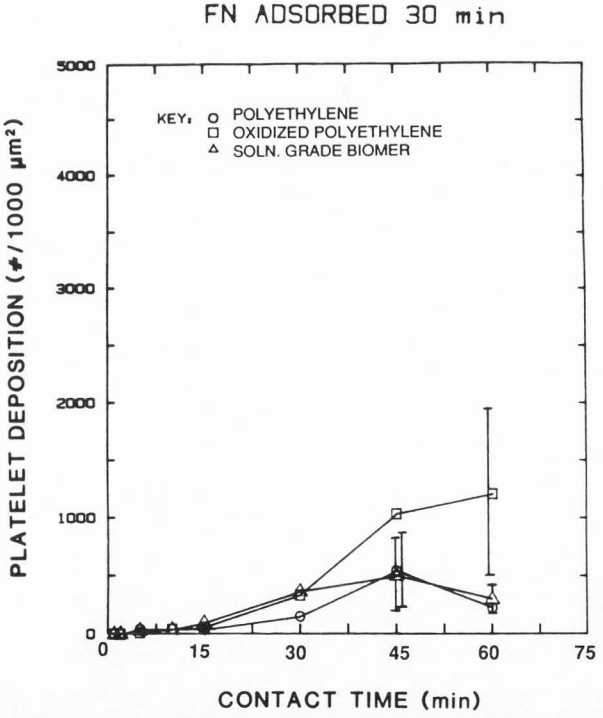


Figure 3. Platelet deposition profiles on OXPE, PE and Biomer preadsorbed with CPFN for 30 minutes.

The platelet deposition profiles for t_i 120 min differ substantially from those obtained at t_i 30 min. Platelet deposition levels for the longer t_i were greatly diminished;

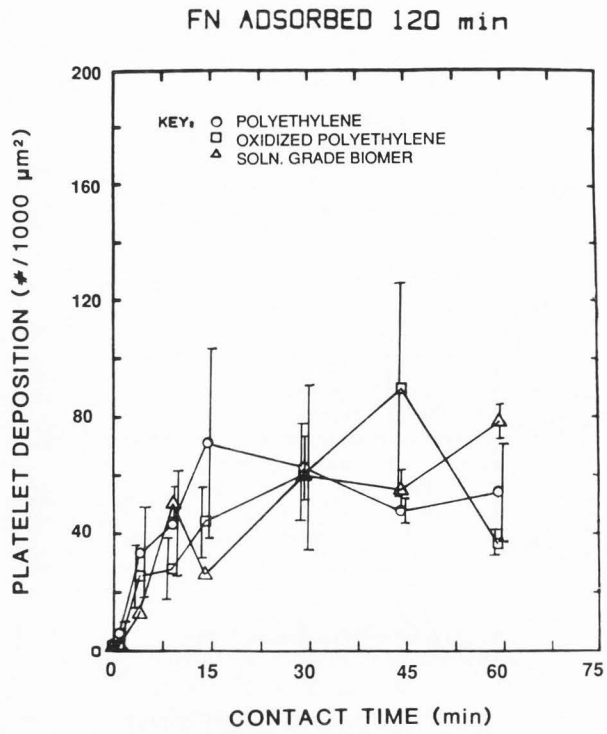


Figure 4. Platelet deposition profiles on OXPE, PE and Biomer preadsorbed with CPFN for 120 minutes.

these data are shown in Figure 4 using an expanded scale. In sharp contrast to the data at the 30 min adsorption time, the maximum platelet deposition at 120 min protein preadsorption was significantly below monolayer platelet coverage, with levels of 90, 80 and 70 platelets per 1000 μm^2 on OXPE, Biomer, and PE respectively. The statistical differences of the platelet deposition between the various polymers were very small using the student's t-test, and it appears that the effect of the polymer substrate was minimal under these conditions.

However little these distinctions were, thrombus morphology demonstrated considerable dependence on adsorption conditions. For instance, differences between materials preadsorbed for 120 min were evident in scanning electron micrographs. Though platelet deposition at t_i 120 min was well below monolayer surface coverage, CPFN-preadsorbed polymers still activated platelets. For example, at t_c 30 min dendritic platelets spread on a lawn of fully spread platelets are shown in Figure 5 in which PE is the test polymer. Figure 6 shows OXPE preadsorbed under the same testing conditions. More thrombosis was exhibited in response to fibronectin adsorbed to OXPE. Small thrombi formed in several instances on a layer of fully spread thrombocytes. In contrast to these figures, thrombus formation had proceeded from incipient stages to maturity at t_i 30 minutes. This shift is illustrated when the other figures are compared to Figure 7, showing PE preadsorbed 30 min. and contacted with blood 30 min. A huge thrombus was present in which there were many fibrin strands and entrapped crenated erythrocytes. Deposited platelets were present in various stages of activation. The thrombus had adhered to confluent spread platelets beneath it, and white blood cells had attached to these platelets and the thrombus. On even more active materials such as OXPE, still larger thrombi had formed.

Table 1

Immunogold Staining of Fibronectin Adsorbed to Polymers

Polymer	Adsorption Time [a] (min)	Adprotein Concentration ($\mu g/cm^2$)	Immunogold Concentration (#/4 μm^2)	Connected Particles (#/4 μm^2)
PE	30	0.206 ± 0.028	23 ± 8	16 ± 4
	120	0.288 ± 0.018	nd.[b]	nd.
OXPE	30	0.193 ± 0.004	160 ± 10	153 ± 8
	120	0.234 ± 0.010	128 ± 5	121 ± 7
Biomer	30	0.164 ± 0.007	107 ± 6	98 ± 4
	120	0.185 ± 0.016	137 ± 5	128 ± 4

[a]The bulk concentration of fibronectin used was 0.071 mg/ml.
[b]Not determined.

Table 2

Monolayers of Fibronectin Adsorbed in Different Orientations

Orientation	Type of Packing	Packing Coefficient	Monolayer Surface Concentration $\Gamma^{*,c}$ ($\mu g/cm^2$)
Side-on	Closed	0.78	0.11
End-on	Closed	0.78	0.70
Side-on	Random	0.56	0.08
End-on	Random	0.56	0.50

[c]The fibronectin molecule was assumed to be a prolate spheroid with minor axes of 10.3 nm. and a major axis of 66.0 nm.

Figure 5. Scanning electron micrograph of thrombosis on polyethylene preadsorbed with canine fibronectin for 120 min and contacted with blood for 30 min. The bar shown is 10 micrometers in length.

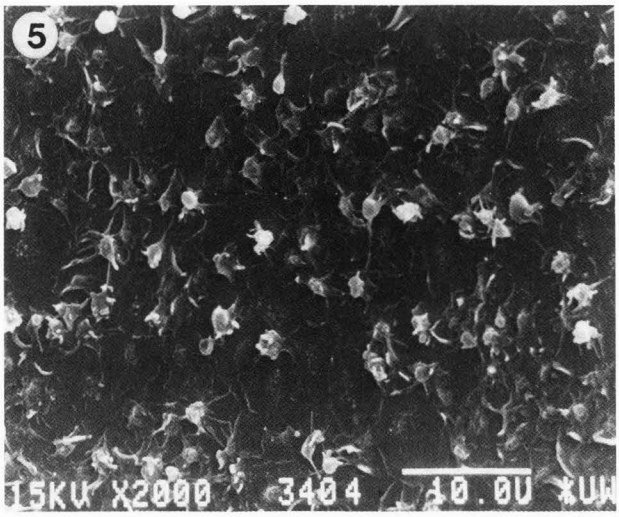
Figure 6. Thrombosis on oxidized polyethylene preadsorbed and tested like the polyethylene shown in Fig. 5. Scale bar is 10 micrometers.

Figure 7. Scanning electron micrograph of the morphology of the thrombus that formed on polyethylene preadsorbed with CPFN for 30 min at t_c 30 min. Bar is 10 micrometers long.

Figure 8. Scanning electron micrograph of canine plasma fibronectin adsorbed to oxidized polyethylene for 30 minutes and stained with antihuman plasma fibronectin immunogold. The scale bar is 1 micrometer long.

Figure 9. Immunogold-bead-labelled CPFN adsorbed to OXPE for 120 min. Bar is 1 micrometer.

Figure 10. Electron micrograph of antifibronectin immunogold-stained CPFN adsorbed to polyethylene at t_i 120 min. Scale bar is 1 micrometer.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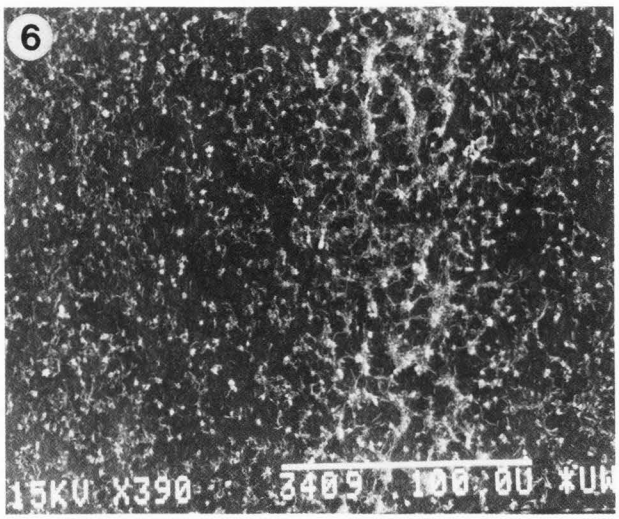
15KV X2000 3404 10.0U *UW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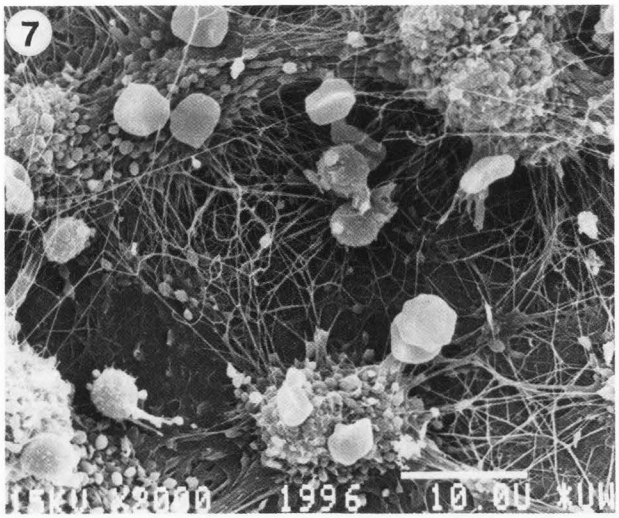
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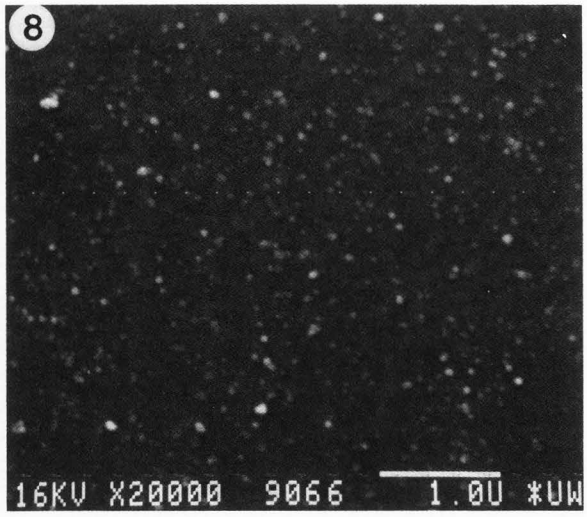
1996 10.0U *UW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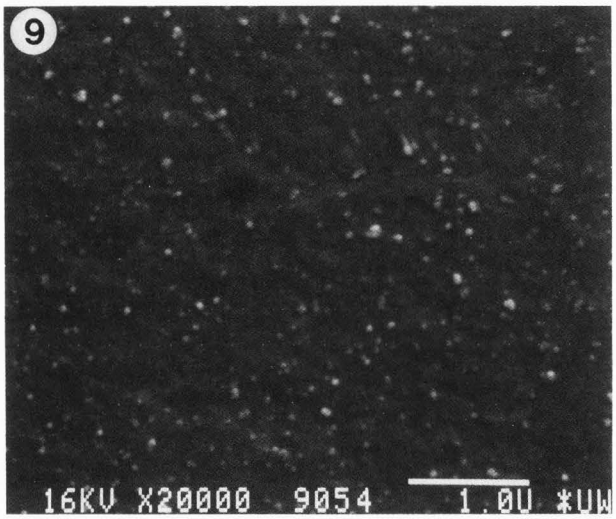
16KV X20000 9066 1.0U *UW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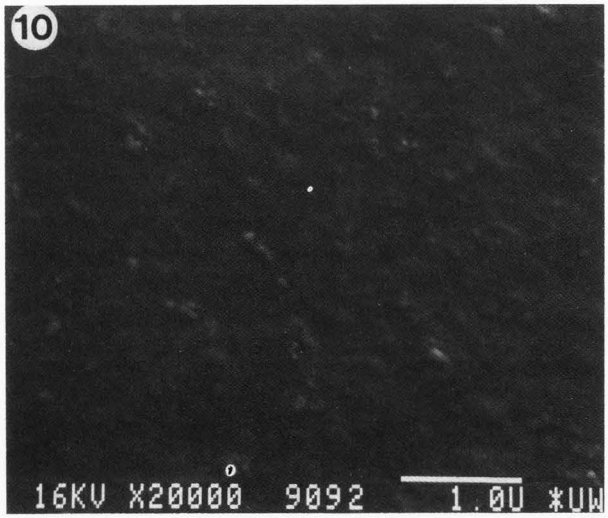
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16KV X20000 9092 1.0U *UW

Fibronectin Adsorption Kinetics

The surface concentration Γ of adsorbed CPFN on the different polymers at adsorption times of 30 and 120 min are given in Table 1 (bulk protein concentration was 0.071 mg/ml), along with the immunogold distribution data. Surface concentrations necessary for a monolayer coverage were estimated for different orientations of the adsorbing protein molecules, and for different packing densities, using the dimensions of a human fibronectin molecule in solution at the physiological conditions of ionic strength of 0.15 and pH 7.4. These dimensions were calculated for an equivalent prolate spheroid, from diffusivity and intrinsic viscosity data (20, 22). Each resulting monolayer surface concentration Γ* is presented in Table 2. The average packing coefficient of 0.56 for random packing of prolate spheroids in a plane was obtained from literature computer simulations of packing in two dimensions (5). There is evidence that protein molecules adsorb in an open conformation, more like side-on rather than end-on, to maximize interface contact at air-water interfaces (2, 14), and perhaps at solid-water interfaces also. Comparing the calculated coverages for a random or close-packed side-on oriented monolayer (Table 2) with the observed surface concentrations under experimental conditions (Table 1) shows that the protein adsorption was generally over twice monolayer surface coverage if the side-on orientation indeed prevailed, with higher values at the longer adsorption time. But Γ* is orientation-dependent: for instance, CPFN adsorption was a fraction of monolayer coverage if protein adsorbed predominantly end-on. Since no direct information was available regarding the exact orientation in coverage, based on the different values in Table 2 for calculated monolayer coverage, one can only simply infer that the observed adsorption was in the monolayer regime. Adsorption was highest on PE, less on OXPE, and least on Biomer. The adprotein concentration increased with the protein adsorption time. It increased much more on PE than on the other two polymers.

Immunogold Visualization of the Adprotein Layer

The distribution of immunogold particles on OXPE at the adsorption time of 30 min is shown in Figure 8. The immunogold label was distributed in a nonuniform fashion, forming strings and exhibiting large unlabeled patches on the surface. Patterns were also seen on Biomer to a lesser extent. This pattern formation appears typical of fibronectin adsorbed to the polymers employed. Further, we did not observe such extensive pattern formation with fibrinogen. On OXPE similar patterns were exhibited for t_i 120 min (Figure 9), but on PE very low staining densities were evident at either t_i (Figure 10). The patterns may indicate intermolecular association of adsorbed fibronectin molecules on the surfaces. We are currently studying this phenomenon in more detail to insure that it is not an artifact caused by the procedures involved in sample preparation.

An attempt was made to quantitate the immunogold distribution (Table 1). The number of gold beads per unit area was counted, as well as the number of particles that appeared to be associated in some kind of a pattern with adjacent particles ("connected" particles). Although the latter parameter is highly qualitative in nature, it serves as a basis for comparison. Gold beads coated with antialbumin antibodies did not stain the protein-adsorbed surfaces, indicating the absence of nonspecific labeling. The general tendency for the gold bead concentration to increase with an increase in protein surface concentration on most surfaces (16) was not exhibited. At 30 min adsorption time, the number of gold beads (as well as the connected beads) was highest on OXPE, less on Biomer, and far less on PE (Table 1). The same order prevailed at 120 min adsorption time also. This trend is in contrast with that for the surface protein concentration on the different polymers. In particular, despite the very high surface adprotein concentration on PE, the labelling density was the lowest. Although crossreactivity between CPFN and antihuman plasma fibronectin was displayed, we believe that the difference between species might, in part, be responsible for this trend. In the future, we intend to examine and compare adhuman plasma fibronectin labelled with antiHPFN immunogold and adsorbed canine plasma fibronectin visualized under antiCPFN immunogold in order to test our hypothesis.

Discussion

Plasma fibronectin (PFN) is a large dimeric molecule with a wide range of biochemical activities (15, 21). PFN binds many species involved in blood coagulation. These include structural molecules like heparin, an anticoagulant, and collagen, a procoagulant; fibrin, as well as other blood proteins such as factor XIIIa, and formed elements such as platelets (15, 21). These binding properties have prompted the study of the role of PFN in the hemocompatibility of polymers. The conformation of PFN is appreciably mutable, and is substantially affected by the chemical environment of the molecule. For instance, solution pH, ionic strength, buffer composition, water-soluble homopolymers and denaturants strongly influence the size and macroscopic shape of the fibronectin molecule (1, 4 , 9, 22). The conformation and state of fibronectins are similarly affected by adsorption to air-water interfaces (8, 24).

Grinnell and Feld have demonstrated that solid surfaces affect the activity of human PFN (6, 7). In particular, they have shown that the level of activity of adsorbed PFN (adPFN) depended more strongly on the type of polymer used rather than on the adPFN surface concentration. For instance, though more PFN adsorbed to untreated hydrophobic polystyrene, kidney cell adhesion was supported more strongly by the protein adsorbed to hydrophilic polystyrene. Moreover, antibody binding to adPFN was correlated roughly to the activity of the adsorbed human PFN according to their studies (6, 7).

In this report, the effects of adsorbing substrate as well as of adsorption time on the biological activity of PFN were studied in terms of both the ex vivo thrombogenic activity and in vitro antibody binding ability of the adsorbed canine PFN (CPFN). The effect of polymer surface properties on the phenomenological activity of adsorbed CPFN ex vivo is unclear presently although it is known that material characteristics substantially influence the activity.

At the lower adsorption time of 30 min, platelet deposition on CPFN-coated PE was significantly less than that on protein preadsorbed OXPE or Biomer, while more CPFN adsorbed to PE than to OXPE or Biomer. The results with respect to platelet deposition with CPFN adsorbed polymers are very different from those on the bare polymers, both qualitatively and quantitatively. Thus, we may say that our ex vivo results were not the effects of bare (not covered by the preadsorbed protein) polymer area being exposed to blood contact.

The extent of immunogold staining of the adsorbed protein (surface concentration of immunogold particles in # per 4 μm^2) is related to the total immunochemically active protein on the surface. It may thus be an indicator of the platelet attachment activity of adCPFN, particularly if some antibodies in the polyclonal preparation used reacted with determinants in the CPFN platelet binding site. This interpretation is borne out by our results at 30 min adsorption

of CPFN. Thus the immunogold-bead labelling of the fibronectin adsorbed to PE was least dense; the labelling on Biomer was greater and on OXPE most dense. The ex vivo platelet deposition was in the same order, and thus appears to correlate well with the antibody-binding activity of the adsorbed CPFN, but not with the adCPFN surface concentration. Further, both the ex vivo platelet deposition levels and the in vitro immunochemical activity of adsorbed CPFN appear to increase in the order of increasing hydrophilicity of the materials, as seen from the air-water contact angles on the polymers (Table 3). The effects of CPFN adsorption to polymers presented here therefore roughly correspond to the results of Grinnell and Feld (6, 7). These authors used a short adsorption time of ten minutes and obtained very active adsorbed protein.

Increasing the adsorption time to 120 min yielded a different picture. While the surface concentration of adCPFN increased with increased adsorption time, the extent of immunogold staining remained nearly the same as that at 30 min and the platelet deposition dropped markedly to 60~90 platelets/1000 μm^2 on all surfaces. Neither the ex vivo nor the in vitro biological activity of the adCPFN corresponded to the protein surface concentration on the different materials. The relatively unaffected immunogold concentration implies that the adprotein at the solution-adprotein interface exhibited similar immunochemical activities at both adsorption conditions. In the ex vivo response, not only the adprotein at the solution-surface interface but also that in the buried layers may play a role, due to flow-induced desorption of adprotein into the complex mixture of blood (17). Thus adsorption conditions can produce considerably more than a monolayer adsorbed only to see predominantly admolecules in the monolayer remain adsorbed by the time cellular elements arrive in large numbers from blood.The activity of the protein directly in contact with the substrate may decrease with increasing adsorption time due to the effect of the surface on protein conformation. Such slow conformational changes have been found to occur as shown by several studies of adsorbed proteins (2). If the buried protein is less active, a decrease in biological activity ex vivo may be expected with increased adsorption time, when the protein coverage is well above monolayer. Further experiments are under way to test this hypothesis. First will be an attempt to correlate the extent of adprotein desorption and of immunogold staining. Second is a comparison of the degree of immunogold labelling experienced by fibronectin adsorbed for 120 min and by fibronectin adsorbed for as short a t_i as feasible (<<120 min) and let incubate with the polymer adsorbent for 120 min.

Overall, these results suggest that adsorption time, as well as the adsorption substrate, substantially influences the behavior and biological activity of the adsorbed protein.

Table 3

Air-Water Contact Angles on Polymer Surfaces

Polymer	Air-Water Contact Angle
PE	90°
OXPE	55°
Biomer	34°

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Discussion with Reviewers

D. A. Handley: As one extends adsorption time, CPFN increases and platelet deposition decreases. Is it possible that other reactions between the polymer and CPFN are taking place to account for decreased platelet binding?
Authors: Yes, these reactions were addressed in our discussion. Slow adsorption-induced conformational changes of adsorbed CPFN and flow-induced desorption of adfibronectin are both able to attenuate platelet binding.

It is important to differentiate between the two time parameters used in our study. The first is t_i, the time that solutions of radioiodinated fibronectin in PBS were incubated with the test polymers, or simply the adsorption time. The dependence of Γ, the amount of CPFN-adsorbed per unit area of given test polymer, on t_i characterizes the kinetics of CPFN adsorption to the polymers. Typically in protein adsorption to polymers conducted with bulk concentrations comparable to those employed in our experiments, increases in adsorption time increase Γ when t_i is short. Γ displays a plateau with respect to t_i at values in excess of about 120 min.

The second time parameter used is the blood contact time t_c, the time that a polymer preadsorbed with fibronectin under predetermined adsorption conditions was contacted with flowing blood ex vivo. $\Gamma_{plt}(t_c)$, the temporal platelet deposition profile, is a measure of thromboembolism on the CPFN-preadsorbed polymers tested. Several animal experimental models assessing the hemocompatibility of synthetic materials such as those used by Horbett, Callow, Greer and ourselves employ this measure or a similar methodology. These profiles apparently contain substantial information, but are supplemented with flow data and electron micrographs of samples at pertinent data points to facilitate interpretation. Presently platelet deposition profiles indicate that bare or protein-preadsorbed polymers are highly thrombogenic when the number of adherent platelets in deposition peaks is substantially greater than 220 platelets/1000 μm^2, that in a single layer of close-packed, unactivated platelets. Nonactive polymers or adproteins evoke deposition peaks far less than this single layer value. For instance, the highest number of platelets deposited on preadsorbed albumin, a blood protein that empirically appears to passivate polymers, are significantly below monolayer values.

Accordingly we have estimated the effect of t_i on the thrombotic activity of CPFN adsorbed to each polymer selected by comparing Γ_{plt} at certain t_c's and furthermore Γ_{plt}^{max}, which is independent of t_c. We had inferred, from a large set of studies conducted well before the study analyzed and discussed here, that Γ_{plt}^{max} depended more on polymer and preadsorbed-protein types than on the amount of protein adsorbed. Thus this study examined factors affecting the phenomenological platelet-binding activity of adCPFN. The possibility that other factors and reactions saliently affect this activity exists, but these reactions do not appear to play large roles according to results obtained in the present study.

D. A. Handley: Are the polymers examined in this study commonly used in vascular prostheses or artificial hearts?
Authors: Since polymer surface properties influence protein adsorption and presumably the biological activity of proteins adsorbed, we selected polymers among which surface properties--surface charge, surface free energy, crystal structure, microdomain size and distribution--varied widely. We also desired to study a material with good prospects for artificial heart fabrication and therefore in our study included Biomer which has been often used in blood-contacting prostheses.