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AJNR Am J Neuroradiol 1996, 17 (5) 859-863
http://www.ajnr.org/content/17/5/859

Platelet Deposition and Fibrinogen Binding on Surfaces Coated with Heparin or Friction-Reducing Polymers

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Summary: The blood-contacting properties of polyethylene coated with a lubricious, hydrophilic coating; an uncoated polyethylene; or a photoheparin-treated polyethylene-negative control sample were compared by measuring fibrinogen adsorption, antifibrinogen binding, and platelet attachment from human plasma. The polyethylene surfaces coated with a hydrophilic polymer were found to be similar to surfaces coated with heparin. Fibrinogen adsorption on the hydrophilic coating was 60% lower than adsorption on either the uncoated or heparin-coated polyethylene samples. Antifibrinogen binding from buffer to the hydrophilic coating was also reduced more than 85% from binding to uncoated polyethylene samples. Both the hydrophilic coating and heparin coating showed a reduction in platelet attachment by a factor of 100 over the uncoated sample as well as significantly reduced platelet activation

Index terms: Blood, coagulation; Heparin; Interventional materials; Platelets

Hydrophilic, lubricious polymer coatings have been applied to guidewires and, more recently, to microcatheters for use in interventional neuroradiology to minimize friction along the access pathway (1). Hydrophilically modified polymers have been shown to decrease protein adsorption and platelet adhesion onto their surfaces (2, 3). Pinto et al (4) showed that minimal thrombus formed on a guidewire with a hydrophilic copolymer as compared with a polytef (Teflon)-coated wire. Similarly, Leach and coworkers (5) showed that guidewires coated with a hydrophilic polymer had reduced clot formation compared with uncoated stainless steel guidewires. Interestingly, they found no reduction in clot formation on catheters unless the animal had been treated with heparin.

The Fastracker infusion catheter and FasGuide guiding catheter (Target Therapeutics, Inc, Fremont, Calif) are coated with Hydrolene, a hydrophilic polymer formulation, to reduce friction. We compared the platelet adhesion and fibrinogen deposition on polyethylene covalently bonded with heparin, Hydrolene-coated polyethylene, or uncoated polyethylene.

Materials and Methods

Sample Preparation

The National Institutes of Health primary reference polyethylene in 1-cm-diameter disks was used as the substrate for all experiments. Control samples of polyethylene were used as received. Test samples were surface-modified with Hydrolene, a proprietary formula of covalently bonded polyvinylpyrrolidone and polyacrylamide (Guire PE, Dunkirk SG, Josephson MW, Swanson MJ, "Preparation of Polymeric Surfaces via Covalently Attaching Polymer," US Patent 5 002 582, 1991). Negative control samples having a covalently bound heparin coating were prepared using a photoheparin reagent (HP-01, BSI Corp, Eden Prairie, Minn) (Guire PE, "Method for Improving the Biocompatibility of Solid Surfaces," US Patent 4 973 493, 1990; and Guire PE, "Biocompatible Coating for Solid Surfaces," US Patent 4 979 959, 1990) (6).

Fibrinogen Adsorption from Buffer and Platelet-Poor Plasma

Adsorption of ^{125}I-fibrinogen.—Adsorption of fibrinogen radiolabeled with iodine-125 onto the surface-modified materials was studied from two solutions: tris-buffered saline solution and platelet-poor plasma. The procedure was based on the method of Brash and coworkers (7).

Fibrinogen (Sigma, St Louis, Mo) solution (500 μg/mL) was prepared with tris-saline (10 mM tris, 150 mM NaCl, pH 7.5) and the solution was spiked with the radiolabeled fibrinogen (Amersham, Arlington Heights, Ill) (specific activity 5.8×10^5 cpm/μg) such that the specific activity was 92 or 703 cpm/μg for the entire solution.

Received June 1, 1995; accepted after revision September 22.

Supported by Target Therapeutics, Inc.

From the BSI Corporation, Eden Prairie, Minn (A.B.A., T.H.T., M.J.H., S.J.C., B.P.H., M.J.M.) and Target Therapeutics, Fremont, Calif (R.W.H.).

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AJNR 17:859–863, May 1996 0195-6108/96/1705–0859

The radioactivity of each sample was quantified using a scintillation counter (Packard 1900CA) and the surface concentration of fibrinogen was calculated using the known specific activity of the fibrinogen and the surface area of the sample pieces.

Binding of antifibrinogen antibodies to adsorbed fibrinogen.—The binding of monoclonal, anti-fibrinogen antibodies to previously adsorbed fibrinogen was quantified using an enzyme-linked immunosorbent assay technique. The experiments were carried out in two steps. First, fibrinogen was adsorbed to the polymer samples (uncoated and surface-modified) using a procedure analogous to that described above for the ^{125}I-fibrinogen sample. Second, the adsorbed fibrinogen was challenged with a horseradish peroxidase (HRP)–conjugated, monoclonal antibody that is specific for a region on fibrinogen responsible for platelet aggregation. The surface concentration of antibody was determined by quantifying the HRP activity with a chromogenic enzyme substrate (tetramethylbenzidine), and measuring absorbance at 655 nm.

Platelet Attachment and Activation

Platelet attachment and activation experiments were conducted using human platelet-rich plasma based on the method of Ebert and coworkers (8). The test samples were placed into six-well plates, one sample per well. The platelet-rich plasma solution was added onto the top of the samples until the entire surface was covered. The samples were fixed, dehydrated, and mounted for observation with a JEOL 840 (Tokyo, Japan) scanning electron microscope. The quantity of platelets was assessed visually. Three substrate samples were analyzed for a total of 60 measurements. The degree of activation was judged qualitatively on the basis of morphology by using definitions described previously (9).

Statistical Analyses

For each set of data, analysis of variance methods (the least significant difference method or Dunnet's method for comparing treatments with a control) were used to determine statistical significance.

Results and Discussion

Fibrinogen Adsorption

In the lower activity experiments (92 cpm/μg), the radioactive counts for the samples that were incubated in platelet-poor plasma were barely above the background signal and thus were not included in the analysis. The radioactivity counts for the samples incubated with fibrinogen in tris-saline solution, however, were at least two orders of magnitude above background. The values for the two experiments in buffer were averaged. The results are shown in Table 1.

TABLE 1: Adsorption of ^{125}I-fibrinogen (μg/cm^2)

Surface	Conditions	
	Tris-Saline Buffer (average of 92 and 703 cpm/μg experiments)	Platelet-Poor Plasma (703 cpm/μg)
Uncoated	23.4	0.68
Hydrolene	9.5	0.38
Heparin	26.0	0.51

TABLE 2: Antifibrinogen antibody binding (A_{655})

Surface	Conditions	
	Tris-Saline Buffer	Platelet-Poor Plasma
Uncoated	2.13 ± 0.16	0.055 ± 0.002
Hydrolene	0.30 ± 0.07	0.045 ± 0.015
Heparin	0.45 ± 0.25	0.152 ± 0.067

The surface concentration of fibrinogen adsorbed from tris-saline on the Hydrolene-modified polyethylene was substantially lower than the surface concentration on either the heparin-modified (63% lower) or unmodified (60% lower) polyethylene surfaces. These results indicated that the Hydrolene coating reduced the binding of fibrinogen from tris-saline solution. In contrast, the heparin coating showed no significant effect compared with the uncoated polyethylene.

The fibrinogen surface concentrations attained during adsorption from platelet-poor plasma were two orders of magnitude lower than during adsorption from tris-saline. This was expected, in that the competition from other proteins in the platelet-poor plasma for surface sites should reduce the binding of fibrinogen (7, 10). The surface concentrations attained during adsorption from platelet-poor plasma were comparable for all surfaces, with no significant differences ($P > 0.05$) among them. Again, however, the Hydrolene had at least a 25% lower surface concentration than either the heparin or uncoated surface.

Antifibrinogen Antibody Binding

The antifibrinogen binding results are shown in Table 2. Antibody binding to previously adsorbed fibrinogen was dependent on whether the fibrinogen was bound from tris-buffer solu-

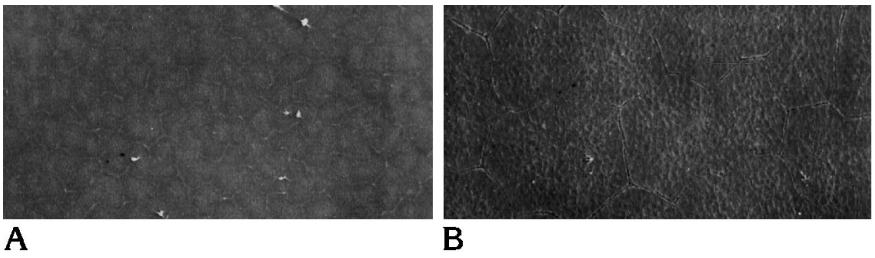

Fig 1. Scanning electron micrographs of Hydrolene-coated polyethylene exposed to platelet-rich plasma for 1 hour at magnification ×500 (*A*) and at ×2000 (*B*).

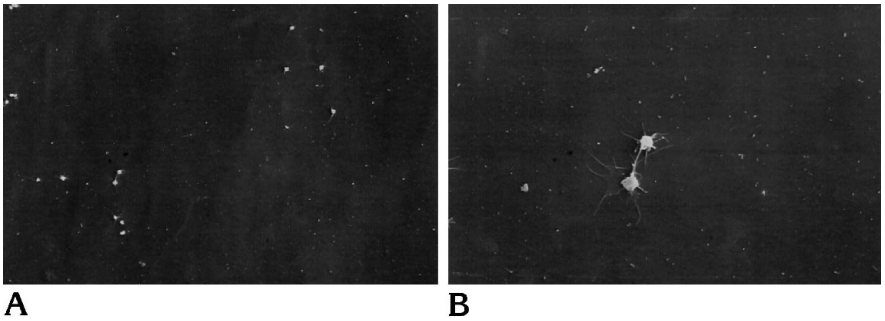

Fig 2. Scanning electron micrographs of heparin-coated polyethylene exposed to platelet-rich plasma for 1 hour at magnification ×500 (*A*) and at ×2000 (*B*).

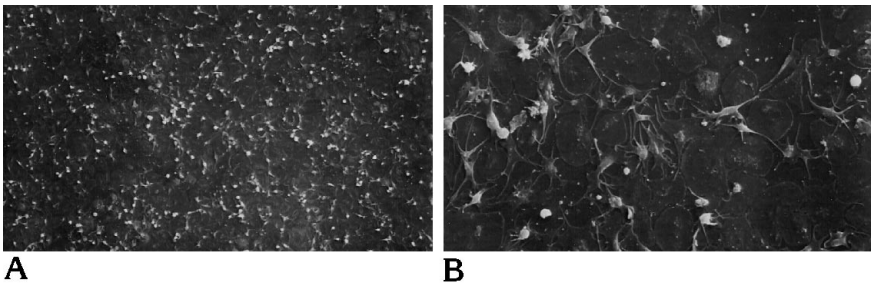

Fig 3. Scanning electron micrographs of uncoated polyethylene exposed to platelet-rich plasma for 1 hour at magnification ×500 (*A*) and at ×2000 (*B*).

tion or platelet-poor plasma. Upon adsorption from buffer, uncoated polyethylene showed at least 4.5 times greater binding than the heparin and the Hydrolene surfaces. These results were significant for both the heparin and Hydrolene surfaces ($P < 0.02$ and $P < 0.01$, respectively). The difference between heparin and Hydrolene was not significant ($P > 0.05$).

These antibody binding results indicated that the fibrinogen adsorbed from tris-saline on the uncoated polyethylene surface retained greater recognizable antigenic structure compared with the fibrinogen adsorbed on the Hydrolene and heparin surfaces. This result was not due merely to higher fibrinogen surface concentrations on the uncoated polyethylene. For example, the fibrinogen surface concentration on uncoated polyethylene was 2.5 times higher than on the Hydrolene surface, but the antibody binding to the fibrinogen on the polyethylene surface was 7.1 times higher than antibody binding to the fibrinogen on the Hydrolene surface. Consequently, it can be inferred that the fibrinogen on the uncoated polyethylene surface had a preferential orientation or conformation that allowed substantially greater antibody recognition.

The fibrinogen adsorbed to the heparin surface showed greatly reduced antibody binding relative to the amount of fibrinogen present. The fibrinogen surface concentration on the heparin-modified polyethylene was comparable to the uncoated polyethylene (Table 1), but the antibody binding to fibrinogen adsorbed on the heparin-modified polyethylene was nearly five times less than the antibody binding to fibrinogen adsorbed on the unmodified polyethylene. The reduced antibody recognition of fibrinogen on the Hydrolene and heparin-modified polyethylene suggested that both these surfaces would bind fewer platelets than unmodified polyethylene. This hypothesis was confirmed

TABLE 3: Platelet deposition

Surface	Average No. of Platelets per Square Centimeter
Uncoated	$2.50 \pm 0.79 \times 10^6$
Hydrolene	$1.17 \pm 1.02 \times 10^4$
Heparin	$3.17 \pm 1.40 \times 10^4$

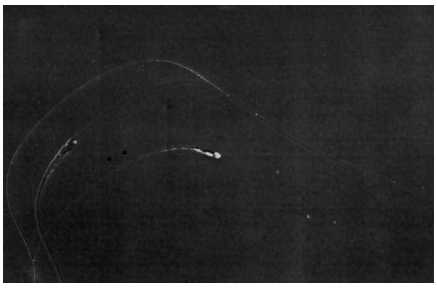

Fig 4. Scanning electron micrograph of Hydrolene-coated polyethylene not exposed to platelet-rich plasma dried at ambient temperature (magnification ×2000).

by the platelet attachment results described below.

For fibrinogen preadsorbed from platelet-poor plasma, the heparin surface showed the greatest binding of antibody, approximately three-fold higher than both the uncoated and Hydrolene surfaces. These differences, however, had tenuous significance ($.05 < P < .10$).

For fibrinogen adsorption from tris-saline or platelet-poor plasma, the Hydrolene-modified polyethylene showed the lowest average levels of antibody binding.

Platelet Attachment and Activation

The Hydrolene and heparin coatings had reduced platelet attachment and activation compared with the uncoated polyethylene control. Figures 1 through 3 show comparable magnifications of the three surfaces that were exposed to the platelet-rich plasma. It was clear that there were both qualitative and quantitative differences between the uncoated surface and each of the coated surfaces. The platelets on the Hydrolene (Fig 1) and heparin (Fig 2) surfaces were primarily in the discoid or dendritic state, with a few in the spread dendritic state, whereas the platelets on the uncoated polyethylene surface (Fig 3) were almost universally in the fully spread state. The quantities of platelets on each of the surfaces were estimated and the results are shown in Table 3. On both the Hydrolene and heparin surfaces, platelet attachment was reduced by approximately a factor of 100.

(Notes about scanning electron micrographs: the cracks in the Hydrolene coating [Fig 1] were artifacts of the critical point-drying step; Hydrolene-coated pieces that were dried at ambient conditions and examined with scanning electron microscopy had smooth surfaces [Fig 4]).

Conclusions

The fibrinogen adsorption and platelet adhesion to Hydrolene-coated, heparin-coated, and uncoated polyethylene were studied. Hydrolene was as effective as covalently bound heparin in the in vitro blood compatibility experiments. The Hydrolene-modified polyethylene showed reduced fibrinogen adsorption compared with uncoated polyethylene and heparin-modified polyethylene. This effect was more pronounced upon adsorption from buffer than from platelet-poor plasma. The Hydrolene surface bound less antifibrinogen antibody than uncoated and heparin-modified surfaces after adsorption of fibrinogen from buffer solution or platelet-poor plasma. The Hydrolene surface and the heparin surface reduced platelet attachment by 100-fold compared with uncoated polyethylene, and platelet activation was also considerably reduced.

By coating a surface, such as a microcatheter, with Hydrolene, the blood-contacting properties are dramatically improved over uncoated surfaces. In fact, these studies suggest that Hydrolene-coated surfaces are as effective as heparin-coated surfaces in reducing fibrinogen adsorption and platelet adhesion.

A follow-up study has been planned in which the in vivo thrombogenicity of Hydrolene-coated catheters and appropriate controls will be measured.

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