Delivery of Passivating Proteins to Sutures During Passage Through the Vessel Wall Reduces Subsequent Platelet Deposition by Blocking Fibrinogen Adsorption


Intraluminal vascular suture material, which attracts fewer than the expected number of platelets compared with the same biomaterial exposed to blood in vitro, differs from the untreated biomaterial in that it has been passed once through the vessel wall. The mechanism by which this apparently trivial maneuver reduces platelet deposition was investigated. Polypropylene suture (7-0 Prolene) was passed through human arteries (fetal and adult), and platelet deposition to the suture was measured in a standardized perfusion chamber. Single vessel passage of the sutures reduced platelet deposition by 68 ± 23%, which contrasts sharply with the power of prostaglandin E (1 μM PGE, is sufficient to abolish platelet shape change and aggregation), which inhibited only 11% of platelet deposition to the sutures. Aspirin treatment of the vessel (to prevent PG, formation) or endothelial stripping (to remove the ability to produce nitric oxide) had no effect on the degree of inhibition. Passage of the suture through a vessel analogue (expanded polytetrafluoroethylene) did not inhibit platelet deposition. 125I-fibrinogen adsorption to the suture after vessel passage was reduced to a degree similar to that of platelet deposition. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of proteins eluted from vessel-passed sutures revealed bands at 66, 47, and 16 kd. Western blotting indicated the presence of large amounts of albumin and hemoglobin, a moderate amount of haptoglobin, and only trace amounts of fibrinogen. When sutures were exposed to each of these proteins in vitro before perfusion, albumin and hemoglobin were found to reproduce the effect of vessel passage alone on platelet deposition. We conclude that albumin and hemoglobin adsorb to sutures during their passage through the vessel subendothelium. This reduces the subsequent adsorption of fibrinogen from blood, thereby reducing the ability of platelets to attach to the suture.

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KEYWORDS • biomaterials • platelets • microsurgery • vascular anastomosis • subendothelium

The mechanism of anastomotic thrombosis after small-vessel (<2-mm i.d.) repair remains unclear despite its importance to surgeons in several disciplines.1-4 Up to 10% of such anastomoses become occluded by thrombus despite good technique and various antithrombotic therapies.5,6 The sutured vessel anastomosis is peculiar among thrombosis models in that it exposes several potentially thrombogenic surfaces to flowing blood, one of which is the suture material itself.2,7-11 The present study focuses on the role of intraluminal suture material in anastomotic thrombosis.

Biomaterials, when exposed to flowing blood either in vivo or in vitro, are rapidly covered with a dense coat of platelets.12-14 It was, therefore, surprising to note in a review of published scanning electron photomicrographs of small-vessel anastomoses that with few exceptions,15,16 suture material was minimally covered with platelets at several relevant time points after exposure to blood.17-23

Standard suture placement technique requires that the suture pass once through a vessel wall from outside to inside, followed by the reverse maneuver on the opposite vessel end (Figure 1). Unlike the case with other blood-contacting biomaterials, therefore, the portion of the suture that remains intraluminal has actually been treated by a single passage through the vessel wall.20 The cellular and proteinaceous components of the vessel wall to which the suture has been exposed may include tissue factor, ADP, von Willebrand factor, or collagen, all of which should make the suture more thrombogenic.24-26 Conversely, albumin pretreatment is known to reduce platelet deposition to biomaterials. Because albumin is present in the vessel wall, it is also possible that adsorption of albumin to the suture during

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LUMEN

FIGURE 1. Diagram showing technique of vascular suture placement. Panel A: Vascular suture material used to anastomose vessels is placed through one vessel (right) from the adventitia to the lumen and then through the opposite vessel (left) from the lumen to the adventitia and is finally externally tied. Panel B: The portion of the suture that remains in the vascular lumen is, therefore, no longer an untreated biomaterial surface but has been exposed to a single outside-to-inside passage through the vessel wall.

passage may reduce subsequent platelet deposition.27-30 The scanning electron microscopic (SEM) evidence favors the latter hypothesis, as do the present experiments.17-23

Methods

Materials

7-0 Prolene suture was purchased from Ethicon, Inc., Somerville, N.J. One-millimeter-i.d. expanded polytetrafluoroethylene (ePTFE) grafts having a 30-μm pore size were donated by W.L. Gore & Associates, Inc., Flagstaff, Ariz. Luciferin-luciferase, ristocetin, ADP, human hemoglobin, human haptoglobin, SEM reagents, and buffer reagents were purchased from Sigma Chemical Co., St. Louis, Mo. Human fibrinogen (Kabi) was purchased from Pharmacia Fine Chemicals, Piscataway, N.J. "In-oxine and 125I-fibrinogen were obtained from Amersham, Inc., Arlington Heights, 111. Glass capillary tubes (0.07-cm i.d., normally used for quantitative platelet counting) were purchased from Drummond Scientific, Broomall, Pa. All electrophoresis reagents were purchased from Bio-Rad, Richmond, Calif. Polaroid Type 55 black-and-white film was used to record electron microscope-generated images. Human albumin (Albuminair-5; 5% [wt/vol] solution) was purchased from Armour Pharmaceutical Co., Kankakee, 111., and as fat-free human albumin from Sigma. Tween-80 was a kind gift from Rosemary Hoffman.

Antibodies used for immunoblotting were 1) goat antiserum to albumin and high-molecular-weight kininogen (Nordic Immunology, Tilberg, The Netherlands), fibronectin and transferrin (Cappel Laboratories, Cochranville, Pa.), immunoglobulin G (IgG) and β-lipoprotein (Miles Scientific, Rexdale, Canada), and haptoglobin (Sigma) and 2) rabbit antiserum to fibrinogen (Cappel Laboratories), β-2-microglobulin (Miles Scientific), and hemoglobin (Sigma).

Second antibodies for colorimetric assay were affinity-purified rabbit anti-goat IgG–alkaline phosphatase conjugate and affinity-purified goat anti-rabbit IgG–alkaline phosphatase conjugate (both from Sigma).

Constituents of N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)–Tyrode’s (HT) buffer were NaHCO 3 8.9 mM, KCl 2.8 mM, KH 2PO 4 0.8 mM, HEPES 10 mM, dextrose 5.6 mM, NaCl 129 mM, and MgCl 2 0.8 mM, with pH adjusted to 7.4 unless stated otherwise.

Constituents of the SEM fixative were 0.1 mM sodium cacodylate, 2.0% paraformaldehyde, 1.5% glutaraldehyde, and 2.5 mM CaCl 2 at pH 7.2.

Methods

Institutional review board approval was obtained for venepuncture and placental studies from the University of Pittsburgh Biomedical Institutional Review Board and the Research Review and Human Experimentation Committee of Magee-Womens Hospital.

Acquisition and Preparation of Human Placental and Systemic Vessels: Placental Vessels

As previously described, human placental artery segments (1 cm long) were dissected from the chorionic plate under loupe (x2-3) or microscopic (x40) magnification and flushed with HT buffer, pH 7.4, to remove retained blood.23-31 Suture material was then passed through the edge of the cut vessel from the adventitia to the endothelium by needle puncture, and its entire length was drawn through (Figure 1A). This maneuver mimics the first step in the construction of a vascular anastomosis.1-20 The suture was then studied as described below.

In certain experiments, human placental arteries were treated with 5 mM aspirin in buffer for 30 minutes at 37°C to block the endothelial production of prostacyclin (PGI2).32 In additional experiments in which the effect of endothelial removal was studied, a cotton-tipped swab (three wipes) was used to strip the endothelium from the vessel surface. SEM revealed that the endothelium was completely removed after this maneuver (M. Zente and P.C. Johnson, unpublished data). A 15-minute incubation in HT buffer followed the removal of the endothelium to allow the short-lived nitric oxide to be completely metabolized.33

Systemic Vessels

Segments of the anterior tibial artery and vein were harvested from a fresh below-knee traumatic amputation specimen. The vessels were harvested > 10 cm from the region of gross trauma to minimize the possibility of a zone-of-injury effect.34 These vessels were also washed free of blood using HT buffer, and suture material was passed through the individual vessel walls as described above.
**Artificial Microvascular Grafts**

One-millimeter-diameter ePTFE grafts were moistened with saline, and 7-0 Prolene suture was passed through the wall from the outside to the inside. The platelet deposition assay was then performed to determine if the physical forces of vessel passage per se were responsible for the observed effects after passage of the suture through a human vessel.

**Suture Choice and Preparation**

Ethicon 7-0 Prolene double-armed suture was divided at its midpoint, washed in isopropyl alcohol, and allowed to dry. This suture will be considered “untreated” in the subsequent analysis. In pilot studies, the isopropyl alcohol wash had no effect on the relative amounts of platelet deposition in the presence or absence of vessel passage. This gauge of suture, which lies midway between those suture sizes used for macrovascular and microvascular surgery, provided sufficient surface area to obtain a measurable radioisotope signal in the platelet deposition studies.

Nineteen-centimeter lengths of untreated suture or suture that had been passed through the vessel wall were individually placed inside 18-cm-long glass tubes of 0.7 mm i.d. The needle and excess suture were passed through a polypropylene tubing collar (Figure 2A) to protect them from exposure to blood flow. The collar was also used to attach the glass tubing to a blunt needle on the perfusion syringe (see below).

**Collection of Blood, Platelet Labeling, and Functional Assays**

Human venous blood was collected into sodium citrate anticoagulant, platelets were harvested and labeled with $^{111}$In, and whole blood was reconstituted (hematocrit, 40±5; platelet count, 180,000±50,000/µl) as previously described. Plasma lactate dehydrogenase activity after reconstitution of whole blood according to this method is not elevated above that of normal freshly drawn blood, thus indicating that red blood cell lysis is minimal (Technicon RA 500, Technicon Instruments Corp., Tarrytown, N.Y.).

Before whole-blood reconstitution, platelets in platelet-rich plasma were checked for their ability to aggregate and to secrete ATP from dense granules (luciferin-luciferase assay, Chronolog Platelet Ionized Calcium Aggregometer) in response to ADP (5–20 µM) and ristocetin (1.8 mg/ml final concentration).

**Perfusion Apparatus**

A neonatal incubator (Isolette, model C-86, Narco Medical, Warminster, Pa.) was custom modified to house two constant-perfusion syringe pumps (models 907 and 975, Harvard Apparatus, South Natick, Mass.) to enable control of blood flow and, therefore, shear rate (Figure 2B). The incubator maintained a 37°C environment during the perfusion. An adjustable-length rack, which holds up to four syringes, allowed simultaneous perfusion of multiple treated and untreated sutures. The glass tubes containing sutures were mounted between

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**Figure 2. Diagram showing suture perfusion device.** Panel A: Suture is placed within an 18-cm-long glass tube (0.07 cm i.d.), and its needle is passed out of the lumen through a polypropylene tubing collar. Panel B: This assembly is then attached to a syringe containing fresh whole human blood whose platelets are radiolabeled with $^{111}$In, placed on a perfusion pump (pump B) in a neonatal incubator (37°C), and perfused (1,060 sec⁻¹ wall shear rate) against a continuously evacuating column to maintain a constant 80 cm H₂O transmural pressure. At the completion of the blood perfusion, a separate pump (pump A) delivers a 5-ml buffer flush at the same temperature and shear rate.
three-way stopcocks in line with the blood-containing syringe. A syringe containing HT buffer for a postperfusion flush was mounted on the second Harvard pump and was attached via warmed tubing to the proximal stopcock. The distal stopcock was attached to tubing that was filled with HT buffer and suspended at a height of 80 cm. During perfusion, blood and then buffer were discharged through the tubing into a collection receptacle at the top of the column, thereby maintaining a constant 80 cm H₂O transmural pressure at the level of the suture. Untreated sutures were run in parallel with treated sutures using blood from the same donor.

**Perfusion Technique**

In each case, 5 ml reconstituted human whole blood containing ¹¹¹In-labeled platelets was perfused at a flow rate of 0.6 ml/min for a contact time of 500 seconds. On the assumption that flow in an annulus having inner and outer diameters of 0.007 cm and 0.07 cm, respectively, has a Reynolds number of 4.1, the flow may be assumed to be laminar. The shear rate at the suture surface (the inner wall of the annulus) for laminar flow is estimated to be 1,060 sec⁻¹.37 This shear rate was chosen to mimic that which develops in a small-artery anastomosis after complete restoration of flow to ischemic tissue in microsurgery during the interval that is also the highest risk period for anastomotic thrombosis (i.e., ≤30 minutes after flow restoration).36-40

In some experiments, untreated sutures were perfused with whole blood containing prostaglandin E₁ (PGE₁, 1 μM), a concentration that nearly abolishes platelet shape change and completely abolishes aggregation in response to 20 μM ADP (determined by optical aggregometry using platelet-rich plasma).

At the completion of the blood perfusion, 5 ml HT buffer was perfused at the same flow and shear rates to clear the tube of nonadherent formed elements. SEM of sutures thereafter revealed a minimal number of contaminating red blood cells.

When the perfusion was completed, the glass tubes were drained, then sutures were removed and placed in SEM fixative, and gamma counts were obtained. Platelet deposition (platelets per square centimeter) was then calculated based on the known surface area of the suture, the gamma count, and the platelet specific radioactivity. Representative sutures were prepared for SEM as previously described.16,17

**Fibrinogen Adsorption to Sutures: Effect of Previous Single Vessel Passage Through Human Placental Artery**

Human fibrinogen adsorption from static buffer. A human fibrinogen solution (1.5 mg/ml in HT buffer, pH 7.4) containing 5 μCi ¹²⁵I-fibrinogen was added to glass capillary tubes containing control or vessel-passed sutures. After a 10-minute static incubation at 37°C, the glass tube was perfused with 5 ml HT buffer at a wall shear rate of 1,060 sec⁻¹. The sutures were then removed, and suture-associated gamma counts were obtained.

Human fibrinogen adsorption from flowing blood. Five microcuries of ¹²⁵I-fibrinogen were added to 5 ml fresh human whole blood (which was otherwise unlabeled) and perfused (at 1,060 sec⁻¹) through glass capillary tubes containing control or vessel-passed sutures, as detailed above. After a 5-ml HT buffer flush, the sutures were removed and gamma counts were obtained.

**Elution and Western Blot of Proteins Adsorbed to Sutures: Elution and Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis**

Unreated and vessel-passed sutures (19 cm long) were placed in glass tubes (0.7 mm i.d.) containing physiological saline to which 2% sodium dodecyl sulfate (SDS) and 20 mM EDTA were added.41 After overnight incubation at 4°C to elute the adsorbed proteins, the eluates of untreated and vessel-passed sutures (n=10 and n=20 on two separate occasions) were pooled separately and concentrated to approximately 500 μl in an Amicon cell using a membrane with a cutoff of 5 kd. An aliquot of each was then subjected to SDS-polyacrylamide gel electrophoresis (PAGE) on a 7.5-15% gradient gel with a 5% stacking gel in a Mini-Protean II electrophoresis chamber (Bio-Rad). Thirty-five microliters of the concentrated protein samples were first reduced by heating at 95°C for 5 minutes in the presence of β-mercaptoethanol, applied to the gels, and run for approximately 1 hour. The gels were then fixed and stained for protein with Coomassie blue.41 The untreated sutures revealed no evidence of protein, and subsequent discussion will refer only to vessel-passed sutures.

**Western Blot Preparation**

Two hundred microliters of concentrated eluate from the vessel-passed sutures was reduced by heating at 95°C for 5 minutes in the presence of β-mercaptoethanol and loaded onto a polyacrylamide gel (5% stacking, 7.5-15% gradient). Electrophoresis was performed for 1 hour at 200 V. Electrophoretic transfer using Immobilon PVDF transfer membranes cut to size was carried out for 1 hour at 100 V. For molecular weight determination, the marker lane and a small portion of the sample lane were removed and stained with amido black. They were subsequently de-stained and air dried. The rest of the blot was cut into 2.5-mm-wide strips and immunostained as previously described.41

Three separate immunoblots were performed on two concentrated protein samples. Based on the presence of a strong band at 66 kd and faint bands at 47 and 16 kd, antibodies were directed against possible plasma proteins whose molecular weights might fall in these areas.41 In two blots, antisera to eight proteins were used (fibrinogen, albumin, fibronectin, high-molecular-weight kininogen, IgG, β-2-microglobulin, haptoglobin, and hemoglobin), and in the third blot, antisera to 10 proteins were used (the eight above plus transferrin and β-lipoprotein). Two second-antibody-alkaline phosphatase conjugate systems were used for color generation, depending on the animal source of the specific antisera (i.e., goat or rabbit). These were affinity-purified rabbit anti-goat IgG-alkaline phosphatase and affinity-purified goat anti-rabbit IgG-alkaline phosphatase.

The substrates used for color generation were 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Bio-Rad) prepared according to the supplier's instructions. A rinse in distilled water was used to stop the reaction.
Demonstration of Protein(s) Responsible for Suture Passivation

To determine which of the proteins detected on the Western blot were responsible for passivation, 7-0 Prolene sutures were incubated (37°C, 15 minutes) in solutions containing no protein (control), bovine serum albumin (30 mg/ml), human fat-free serum albumin (30 mg/ml, which gave results similar to bovine albumin), human hemoglobin (1.5 mg/ml), human haptoglobin (1.5 mg/ml), or human fibrinogen (1.5 mg/ml). The sutures were washed (HT buffer) and then perfused with 5 ml human whole blood containing 111In-labeled platelets (see “Methods”). The sutures were washed again (HT buffer), and gamma counts of the treated sutures were compared as ratios to those of the control sutures.

Data Management and Statistical Interpretation

Suture gamma counts were analyzed using BMDP statistical software. Single comparisons (but not multiple comparisons) were performed using the paired t test when Levene’s test indicated equal variances. For multiple comparisons, an analysis of variance was run in parallel with Levene’s test for the equality of variances. If the latter indicated equal variances at α=0.05, then defined contrasts were performed using Tukey’s Studentized Range Test. When variances were unequal, the Welch or Brown-Forsythe equality of means tests were run, and if a significant difference was present, then defined contrasts were tested as described above.

Results

Effect of Single Passage of Sutures Through Human Vessels on Subsequent Platelet Deposition

Platelet deposition to 7-0 Prolene suture after whole-blood perfusion was reduced 68±23% by a previous single passage through a human placental artery (Figure 3). In contrast, a dose of PGE1 (1 μM), which fully inhibited platelet aggregation, did not provide significant protection against platelet deposition to suture material. The inhibitory effect observed with placental vessels also occurred after suture passage through human anterior tibial artery and vein (Table 1), indicating that the effect is not restricted to the fetal (i.e., placental artery) circulation. Conversely, passage of sutures through saline-moistened ePTFE did not reduce subsequent platelet deposition (Table 1). SEM images of control and vessel-passed sutures subsequently exposed to a blood perfusion corroborate the platelet deposition data measured using 111In (Figure 4).

Effect of Aspirin Treatment of the Vessel and Removal of the Endothelium

The inhibitory effect of vessel passage on platelet deposition to suture was not reduced by aspirin treatment of the vessel or removal of its endothelium (Table 1).

Effect of Suture Passage on Subsequent Fibrinogen Adsorption

Prior passage of sutures through the vessel wall reduced fibrinogen adsorption from the static buffer relative to untreated control sutures (Table 2). The extent of inhibition of fibrinogen adsorption was found to be similar to the reduction in platelet deposition (68%) measured after vessel passage.

A similar reduction was observed in fibrinogen adsorption from flowing blood (Table 2). Also, the effect of vessel passage on the reduction of fibrinogen adsorption after a single passage or multiple (five times) passage (Table 2) was similar to that observed for platelet deposition to sutures (68%).

Determination of Identity of Adsorbed Proteins: Molecular Weight Spectrum

SDS-PAGE analysis of suture eluates after vessel passage revealed protein bands at 66, 47, and 16 kd. Using a database of plasma protein molecular weights seen after desorption from an artificial surface,41 candidate proteins were listed and evidence of their actual presence was sought using the Western blot technique.

Determination of Identity of Adsorbed Proteins: Western Blot

As described in “Methods,” specific antisera were used to stain electrophoretic gels for multiple candidate
proteins. Albumin and hemoglobin were shown to be present in large amounts while haptoglobin was less evident, and only traces of fibrinogen, transferrin, and \( \beta \)-lipoprotein were detected (Figure 5). Proteins that were absent from among those for which specific antisera were used were high-molecular-weight kininogen, IgG, fibronectin, and \( \beta \)-2-microglobulin. It is unclear from the present data if the haptoglobin that was adsorbed to the suture from the vessel wall was present in a 1:1 complex with hemoglobin, as is known to occur in the vascular space.

**Determination of Proteins Responsible for Passivation**

Pretreatment of sutures with either albumin or hemoglobin was able to reproduce the inhibition of platelet deposition measured after vessel passage alone (Figure 6). Haptoglobin caused a small but insignificant depression of platelet deposition, while fibrinogen augmented (but not significantly so) platelet deposition to sutures. These data indicate that either albumin or hemoglobin (or both) is able to mediate the observed inhibitory effect on platelet deposition to sutures after vessel passage.

**Discussion**

The thrombogenicity of a microvascular anastomosis has been related to the presence of both intraluminal suture material and local vessel injury.\(^1\) Because SEM images indicate that suture material is the last portion of the anastomosis to be covered by the endothelium during healing, it is theoretically well situated to remain the focus of thrombogenicity at a vascular anastomosis. However, relative to platelet deposition on other intraluminal biomaterials, the coverage of anastomotic sutures with platelets in published electron photomicrographs was found to be unexpectedly light.\(^2\) We hypothesized that the simple and clinically relevant passage of suture material through the vessel wall in some way reduced its ability to support platelet deposition. The foregoing experiments indicate that suture passage through the vessel wall passivates the suture toward platelets by precoating the suture with albumin and/or hemoglobin, which appears to be selectively available to suture as it passes through the vessel wall. This inhibitory effect was shown not to require the presence of the endothelium at the time of passage.

The ability of an artificial surface such as suture material to support platelet adhesion is known to depend on the quantity, orientation, and conformation of surface-bound fibrinogen, which populates the surface within seconds of plasma exposure.\(^4\) The present data indicate indirectly that, as on other biomaterials, sur-

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**TABLE 2. Relative Fibrinogen Adsorption to Sutures After Vessel Passage**

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>( % ) of control</th>
<th>( P ) (vs. control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no passage)</td>
<td>5</td>
<td>1.00±0.19</td>
<td></td>
</tr>
<tr>
<td>Buffer (static)</td>
<td>3</td>
<td>0.24±0.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood (flow)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single passage</td>
<td>6</td>
<td>0.38±0.25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fivefold passage</td>
<td>3</td>
<td>0.30±0.12</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

AdSORPTION of \(^{125}\)I-fibrinogen to 7-0 Prolene suture before and after placental artery passage. Adsorption under static conditions carried out in HEPES-Tyrode's buffer, pH 7.4. Adsorption under flow conditions carried out using human whole blood. See “Methods” for details of experiments and statistical evaluation.

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**FIGURE 4.** Electron micrographic appearance of sutures after perfusion. Scanning electron photomicrographs were obtained of 7-0 Prolene sutures (Ethicon, Inc., Somerville, N.J.) under the following conditions: Panel A: Untreated suture; panel B: Untreated suture perfused with whole blood as described in “Methods”; panel C: Suture passed once through a placental artery; and panel D: Suture passed once through a placental artery and then perfused with whole blood. Note the large number of platelets that have attached to a perfused but otherwise untreated suture, that simple passage through the vessel wall does not coat the suture with obstructive debris, and that platelet deposition after vessel passage is significantly reduced relative to the untreated perfused suture (10 kV, ×1,650–4,124 magnification).

**FIGURE 5.** Western blot of suture-adsorbed vessel wall proteins. As described in “Methods” Western blots were made of suture eluates and stained for the presence of suspected proteins based on the molecular weight distribution seen on sodium dodecyl sulfate-polyacrylamide gel electrophoresis preliminary gels. Blots indicate large amounts of albumin and hemoglobin, small amounts of haptoglobin, and trace amounts of fibrinogen. Much smaller amounts of transferrin and \( \beta \)-lipoprotein, detected on a separate blot, are not shown. Molecular weight markers are shown at left. HMWK, high-molecular-weight kininogen; IgG, immunoglobulin G.
Figure 6. Bar graph showing ability of the proteins detected on Western blot to inhibit platelet deposition to sutures. Sutures were incubated with albumin (n=8), hemoglobin (n=8), haptoglobin (n=4), or fibrinogen (n=6) as described in “Methods.” After whole-blood perfusion, platelet deposition relative to untreated sutures (n=6) was measured. These results indicate that albumin and hemoglobin can independently reproduce the extent of the effect on platelet deposition measured after vessel passage. While haptoglobin appears to inhibit platelet deposition to some degree, this is not statistically significant in this small group. Fibrinogen may augment platelet deposition, but its value is also not significantly different from controls.

The suture material used in this study was chosen because of its common application in vascular and microvascular surgery. The gauge was chosen in part to serve as a bridge between the sizes used in both disciplines. Platelet deposition to polypropylene is known to be moderate with respect to other biomaterials; therefore, it is not a “worst-case” material. The effect has also been observed to a smaller degree when silk sutures are studied (P.C. Johnson et al, preliminary observations).

These studies indicate that lower-molecular-weight proteins such as albumin and hemoglobin, when administered to the suture during vessel passage, play a role in the prevention of fibrinogen adsorption to sutures. Once blood contact occurs, however, the passivated suture becomes exposed to a high fibrinogen concentration. Fibrinogen is generally believed to have greater surface activity than albumin and thus could be expected to displace adsorbed albumin. Hemoglobin, on the other hand, has been shown to be strongly adsorbed from plasma and to have substantially greater binding affinity for polyethylene than does fibrinogen. Thus, hemoglobin may be the key element in the layer transferred to the suture that prevents subsequent fibrinogen adsorption.

The life span of proteins preadsorbed on biomaterials and then exposed to blood has been shown to be short. For example, about 80% of preadsorbed fibrinogen was shown to be lost from a glass surface within 5 minutes of plasma contact. Such preadsorbed proteins are almost certainly replaced by other blood constituents having higher binding affinity. Similar exchange phenomena occur when bare surfaces are contacted with blood. Abundant proteins like albumin and fibrinogen are rapidly adsorbed initially but are soon replaced by more slowly arriving proteins having greater adsorption affinity.

In adsorption from solution, fibrinogen can compete favorably for surface sites even in the presence of a significantly higher albumin concentration. The absence of a significant amount of fibrinogen on vessel-passed sutures, therefore, suggests that the vessel layers beneath the endothelium serve as a reservoir of plasma proteins other than fibrinogen. This concept is supported by endothelial permeability studies, which indicate that the intact endothelium is selectively capable of restricting the egress of plasma proteins on the basis of molecular weight and charge. On the basis of these studies, albumin and hemoglobin (65–68 kd) would, therefore, be expected to be present in the subendothelium, while larger proteins such as fibrinogen (340 kd) would be expected to be present in only a small concentration except under extreme circumstances. Furthermore, the fibrinogen that does escape has been shown to undergo rapid conversion to fibrin with subsequent fibrinolysis. This has the effect of further reducing potential surface-adsorbable platelet ligands (fibrinogen or fibrin) in the vessel wall.

The present findings, therefore, suggest that vessel-passed sutures are relatively protected from platelet deposition, at least in the short term, and that this effect is more powerful than that of one of the most potent soluble platelet antagonists (PGE). While not all platelet deposition is prevented, these data and the published electron photomicrographs of sutures suggest that vascular suture material is unlikely to be the nidus for platelet-mediated occlusion of small-arterial anastomoses. It may prove possible now to turn our attention toward those components of the vessel anastomosis that are more capable of promoting the development of a local, occlusive thrombus: exposed subendothelium (which bears multiple binding sites for platelets, thrombin, and tissue factor) and dysfunctional endothelium (which may have lost its antithrombotic properties as a consequence of ischemia and/or trauma).

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References


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