

# BASIC RESEARCH STUDIES

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From the Midwestern Vascular Surgical Society

## Silyl-heparin bonding improves the patency and in vivo thromboresistance of carbon-coated polytetrafluoroethylene vascular grafts

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**Objectives:** Our purpose was to improve the performance of carbon-coated expanded polytetrafluoroethylene vascular grafts by bonding the grafts with silyl-heparin, a biologically active heparin analog, using polyethylene glycol as a cross-linking agent.

**Material and method:** Silyl-heparin-bonded carbon-coated expanded polytetrafluoroethylene vascular grafts (Bard Peripheral Vascular, Tempe, Ariz), were evaluated for patency and platelet deposition 2 hours, 7 days, and 30 days after graft implantation in a canine bilateral aortoiliac artery model. Platelet deposition was determined by injection of autologous, <sup>111</sup>Indium-radiolabeled platelets, followed by a 2-hour circulation period prior to graft explantation. Histologic studies were performed on a 2-mm longitudinal strip of each graft (7-day and 30-day groups). Heparin activity of the explanted silyl-heparin grafts was determined by using an antithrombin-III based thrombin binding assay. **Results:** Overall chronic graft patency (7-day and 30-day groups) was 100% for the silyl-heparin bonded (16/16) grafts versus 68.75% for control (11/16) grafts ( $P = .043$ ). Acute 2-hour graft patency was 100% for the silyl-heparin bonded (6/6) grafts versus 83.3% for control (5/6) grafts. Radiolabeled platelet deposition studies revealed a significantly lower amount of platelets deposited on the silyl-heparin grafts as compared with control grafts in the 30-day group ( $13.8 \pm 7.18$  vs  $28.4 \pm 9.73$ , CPM per cm<sup>2</sup> per million platelets, mean  $\pm$  SD,  $P = .0451$ , Wilcoxon rank sum test). In the 2-hour group of dogs, a trend towards a lower deposition of platelets on the silyl-heparin grafts was observed. There was no significant difference in platelet deposition between the two grafts in the 7-day group. Histologic studies revealed a significant reduction in intraluminal graft thrombus present on the silyl-heparin grafts as compared with control grafts in the 30-day group of animals. In contrast, there was no difference in amount of graft thrombus present on both graft types in the 7-day group of dogs. Pre-implant heparin activity on the silyl-heparin bonded grafts was 2.0 IU/cm<sup>2</sup> (international units [IU]/cm<sup>2</sup>). Heparin activity remained present on the silyl-heparin grafts after explantation at all 3 time points (2 hours: above upper limit of assay, upper limit = 0.57,  $n = 6$ ; 7 days:  $0.106 \pm 0.015$ ,  $n = 5$ ; 30 days:  $0.007 \pm 0.001$ ,  $n = 5$ ; mean  $\pm$  SD, IU/cm<sup>2</sup>).

**Conclusion:** Silyl-heparin bonding onto carbon-coated expanded polytetrafluoroethylene vascular grafts resulted in (1) improved graft patency, (2) increased in vivo graft thromboresistance, and (3) a significant reduction in intraluminal graft thrombus. This graft may prove to be useful in the clinical setting. (*J Vasc Surg* 2004;39:1059-65.)

**Clinical Relevance:** Expanded polytetrafluoroethylene (ePTFE) remains the most commonly used prosthetic graft material in infrainguinal arterial reconstructions. Reported long-term patency rates of ePTFE bypass grafts are inferior to those observed with autogenous vein. Modification of the luminal surface of ePTFE bypass grafts may prevent early graft failure and ultimately improve long-term graft performance. Silyl-heparin is a biologically active heparin analog that is readily adsorbed onto hydrophobic surfaces while retaining its anticoagulant properties. Silyl-heparin bonding onto carbon-coated ePTFE grafts improves the patency and in vivo thromboresistance and results in a decrease in intraluminal graft thrombus. This graft may be useful in the clinical setting.

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Competition of interest: Dr Greisler has been a consultant to Bard Peripheral Vascular.

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Approximately 219,000 peripheral arterial bypass procedures are performed in the United States each year.<sup>1</sup> The majority of these operations are infrainguinal reconstructions done for critical limb ischemia and limb salvage. Autogenous vein remains the conduit of choice. However, in situations where vein is unsuitable or unavailable, prosthetic graft material is utilized. Expanded polytetrafluoroethylene (ePTFE) remains the most commonly used prosthetic graft material in infrainguinal arterial reconstructions. Although reported long-term patency rates of ePTFE bypass grafts are inferior to those observed with autogenous vein,<sup>2</sup> modification of the luminal surface of ePTFE bypass grafts may prevent early graft failure and ultimately improve long-term graft performance.

Silyl-heparin is a biologically active heparin analog that is readily adsorbed onto hydrophobic surfaces while retaining its anticoagulant properties. We recently reported our experience with a silyl-heparin adsorbed, carbon-coated ePTFE graft, where an improvement in graft thromboresistance was observed.<sup>3</sup> The advantage in thromboresistance, however, was short-lived due to poor retention of the heparin analog on the grafts. We have successfully bonded silyl-heparin onto carbon-coated ePTFE grafts, significantly improving its retention. We now report our experience with a silyl-heparin bonded, carbon-coated ePTFE graft.

## MATERIAL AND METHODS

**Grafts.** Control grafts were carbon-coated, 4-mm internal diameter, 5-cm-long ePTFE, 30- $\mu$ m internodal distance, without outer support. Experimental grafts were control grafts that underwent silyl-heparin bonding. All grafts were supplied by Bard Peripheral Vascular, Tempe, Ariz. Briefly, grafts were wetted by immersion in acetone and transferred to an acetonitrile solution containing 0.5 mg/mL of bis (benzotriazole carbonate)-polyethylene glycol (BTC-PEG) (Nektar Therapeutics, San Carlos, Calif) for 30 minutes. The grafts were then immersed in 60% acetonitrile solution containing 1% silyl-heparin (benzyltetra[dimethylsilylmethyl]oxycarbonyl-heparin) for 1 hour and reimmersed a BTC-PEG solution for 30 minutes. The grafts were rinsed in several changes of acetonitrile and air-dried at 56°C. All grafts were ethylene-oxide sterilized. Grafts were evaluated for patency and platelet deposition 2 hours, 7 days, and 30 days after graft implantation in a canine bilateral aortoiliac artery model.

**Animal model.** Adult mongrel dogs (19-26 kg) were obtained from a local supplier and underwent standard preoperative evaluation, which included platelet aggregometry as previously described,<sup>4</sup> and laboratory measurement of prothrombin time, activated partial thromboplastin, and complete blood count. All dogs displayed nonaggregator profiles, and all laboratory measurements were consistent with normal controls. All dogs were medicated with 81-mg acetylsalicylic acid daily and 25-mg dipyridamole three times a day for 3 days before operation.

Both medications were continued postoperatively in keeping with our standard protocols for long-term canine implants.<sup>4</sup> Platelet aggregometry was repeated on the day of surgery, and all dogs remained nonaggregators.

After an overnight fast, the dogs were anesthetized with IV thiopental sodium (30 mg/kg), intubated, and ventilated. Perioperative cefazolin (500 mg IV) was given after induction and 4 hours later in both the 7-day and 30-day groups of dogs. Anesthesia was maintained with nitrous oxide and isoflurane. Exposure of the infrarenal aorta and iliac arteries was obtained through a midline incision. The infrarenal aorta, its branches, and iliac arteries were dissected, and the median sacral artery was ligated and divided. Heparin (100 IU/kg) was given 5 minutes before clamping of the aorta and iliac vessels. Bilateral aortoiliac grafting was then performed, with one experimental and one control graft placed on either (alternating) side of the dog. Graft anastomoses were performed with a continuous 6-0 polypropylene suture (on a BV-1 needle) proximally in an end-to-side fashion on the aorta and distally in an end-to-end fashion with the common iliac arteries. The transected proximal common iliac arteries were ligated. Unclamping and exposure to blood was identical for both experimental and control grafts.

Blood flows through the iliac arteries were quantitated immediately before aortic and iliac artery clamping and 5 minutes after unclamping with an electromagnetic flowmeter (model SP2204, Gould Inc, Medical Products Division, Oxnard, Calif) to document the absence of technical or hemodynamic differences affecting results. Pre-implantation and post-implantation iliac flows were symmetrical in all animals.

In the acute 2-hour group of animals, <sup>111</sup>Indium-labeled autologous platelets were injected intravenously 10 minutes before aortic and iliac artery unclamping. Circulation was re-established through both grafts simultaneously and maintained for 120 minutes prior to explantation.

Six dogs were used in the acute studies (2-hour group of animals) and 16 dogs in the chronic studies (both the 7- and 30-day groups). Animal care complied with the "Principles of Laboratory Animal Care" and "The Guide for the Care and Use of Laboratory Animals."<sup>5</sup>

**Graft explantation.** In the acute 2-hour group of animals, another dose of heparin (100 IU/kg) was given 5 minutes before re-clamping of the aorta and iliac arteries after the 120-minute circulation period. In both the 7-day and 30-day groups, where both control and experimental grafts were patent, dogs were anesthetized as described. Radiolabeled platelets were injected intravenously followed by a 120-minute circulation period. At 90 minutes, the infrarenal aorta and iliac arteries were dissected. Heparin (100 IU/kg) was given 5 minutes before clamping of the aorta and iliac arteries. Pre-explantation iliac artery flows were quantitated as described, and flows were found to be symmetrical in all animals.

Grafts were explanted in continuity, with 2- to 3-cm segments of aorta and iliac arteries that had not been clamped. Dogs were euthanized with a lethal dose of IV

potassium chloride. Explanted specimens were clamped at the aorta and iliac arteries and immersed in normal saline to remove any external blood. The specimens were flushed of luminal blood with normal saline through the aortic opening (20 mL) and through both iliac artery openings (10 mL each). Both proximal and distal anastomoses were taken down, and each explanted graft was opened longitudinally along the inferior aspect. Explanted grafts were then cut into five 1-cm-long segments, which were numbered 1 to 5 starting at the distal end and were measured for radioactivity by a gamma radiation counter (Packard Auto Gamma 5650, Packard Instrument Co, Downers Grove, Ill). Segments were then photographed with a digital camera (Nikon Coolpix 5700, Nikon Corp, Tokyo, Japan), to allow for surface area determination by computerized planimetry (Scion Image 4.0, Scion Corp, Frederick, Md). Graft segment radioactivity was expressed as count per minute (CPM). Total graft radioactivity was normalized to graft surface area and number of platelets injected and was expressed as CPM per cm<sup>2</sup> per million platelets injected.

**Radiolabeling of platelets.** Canine autologous platelets were labeled with radioactive <sup>111</sup>Indium as previously described.<sup>4</sup> Briefly, on the day of explantation 86 mL of blood was collected by jugular puncture and mixed with 14 mL of acid-citrate-dextrose anticoagulant solution, followed by isolation of platelet-rich plasma by centrifugation (200 × g, 30 minutes). Platelets were pelleted by centrifugation (650 × g, 10 minutes) and then resuspended in normal saline and incubated at 37° C for 10 minutes with 800 μCi of radioactive <sup>111</sup>Indium oxyquinoline solution (Amersham Healthcare, Arlington Heights, Ill). After recentrifugation, the pellet was washed with 2 mL of normal saline and then suspended in 7 mL of normal saline/acid-citrate-dextrose solution. <sup>111</sup>Indium radiolabeling efficiency was on the order of 88% to 97%.

**Histologic analysis of grafts.** Histologic analysis was performed on explanted grafts from the 7-day and 30-day groups. The acute group of explanted grafts did not undergo histologic evaluation because microscopic differences observed within a 2-hour implantation are negligible. The carbon coating on the ePTFE grafts made thrombus-free surface area determinations problematic; therefore, histologic analysis was performed on a 2-mm-wide longitudinal strip of graft taken at the time of explantation. The strip of graft was excised from the inferior edge of the graft starting at the heel of the proximal anastomosis and extending distally. The strip of graft was then fixed in 4% paraformaldehyde and stained with hematoxylin and eosin, after subsequent decay of radioactivity. Photomicrographs were taken of stained graft segments, and histologic parameters were determined by computerized planimetry (Scion Image 4.0, Scion Corp, Frederick, Md). To avoid sample bias, the histologist was blinded to both group of animals and graft type. Histologic parameters measured were graft length, thrombus length, thrombus area, and maximal thrombus height. From these measurements, the percentage of graft length free of thrombus could be calculated

(graft length–thrombus length/graft length × 100) as well as average height of thrombus (area of thrombus/thrombus length).

**Heparin activity of grafts.** The heparin activity of the silyl-heparin-coated grafts was determined by using an anti-thrombin III-based thrombin binding assay that was modified from one previously described.<sup>6</sup> The assay utilizes a defined concentration of antithrombin III and thrombin in excess. In the assay, antithrombin III binds with heparin. The resulting heparin-antithrombin III complex then binds and inactivates thrombin. Residual thrombin is free to react with the chromogenic substrate S2238 (DiaPharma, West Chester, Ohio), liberating a chromophore that absorbs at 405 nanometers.

A 1-cm segment of silyl-heparin graft was placed in an Eppendorf tube containing 700 μL of HEPES buffer (25mmol/L HEPES, 190 mmol/L NaCl, 0.5 mg/mL bovine serum albumin, pH 7.5). Antithrombin III (DiaPharma), 100 μL, (2 international units [IU]/mL in distilled water) was then added followed by thrombin (American Red Cross, Washington, DC), 200 μL, (10 IU/mL in 0.9% NaCl). The reaction tube was agitated at room temperature for 3 minutes. An aliquot (20 μL) of the graft-antithrombin III-thrombin solution was then taken and added to 380 μL of Tris-EDTA buffer (50-mmol/L Tris Base, 175-mmol/L NaCl, 7.5-mmol/L Na<sup>2</sup>EDTA, pH 8.4). To this solution, 100 μL of S2238 (2 nmol/L in sterile water) was then added. After 3 minutes at 37°C, 300 μL of 20% acetic acid was added to stop the chromogenic reaction. An aliquot (200 μL) of the final solution was placed in a 96-well microtiter plate, and the absorption of the solution at 405 nanometers was read on a Bio-Tek EL800 (Bio-Tek Instruments Inc, Winooski, Vt) microtiter plate reader. A heparin standard curve was generated by incubating heparin (Upjohn, Kalamazoo, Mich) at various concentrations (range, 0.005-0.02 IU/mL) with antithrombin III and thrombin. An aliquot (20 μL) of each solution was then assayed as previously described. The upper limit of the assay was 0.57 IU per cm<sup>2</sup> of graft. Intra-assay and interassay coefficients of variation were less than 10%.

Heparin activity of silyl-heparin grafts was measured before and after explant. Heparin activity was expressed in IU per cm<sup>2</sup> of graft.

**Statistical analysis.** Statistical comparisons of platelet deposition within each group (acute 2-hour, 7-day, and 30-day), were performed between silyl-heparin adsorbed grafts and control grafts by using the nonparametric Wilcoxon rank sum test. The Fisher exact test was used for statistical comparisons of patency within each group (acute 2-hour, 7-day, and 30-day) between both graft types. All statistical tests were performed on a computer using the Statview statistical software program for the Wilcoxon rank sum test (SAS Institute, Cary, NC) and the SISA Fisher exact test Internet program.<sup>7</sup> Data are presented as mean ± standard deviation.

**Table I.** <sup>111</sup>Indium radiolabeled platelet deposition

Group	Control grafts	Silyl-heparin grafts
2-Hour	40.1 ± 58.1	3.32 ± 2.42
7-Day	13.9 ± 22.2	21.8 ± 12.1
30-Day*	28.4 ± 9.73*	13.8 ± 7.18*

Counts per minute per cm<sup>2</sup> per million platelets injected, mean ± SD, n = 5.

\**P* = .0451, Wilcoxon rank sum test.

## RESULTS

The silyl-heparin bonded grafts were indistinguishable from the control carbon-coated vascular grafts in appearance during implantation. Surgeons were blinded at the time of operation as to the side to which either graft was assigned. Graft handling characteristics—namely stiffness, ability to hold sutures, and pliability—were similar between the silyl-heparin bonded and control carbon-coated grafts. Suture hole bleeding was problematic in the silyl-heparin bonded grafts, which required multiple applications of oxidized regenerated cellulose (Surgicel, Johnson and Johnson, New Brunswick, NJ) to the proximal suture line for up to 90 minutes and to the distal suture line for up to 60 minutes before cessation of bleeding. Oxidized regenerated cellulose was removed after hemostasis was achieved. In contrast, suture hole bleeding was minimal in the control grafts.

In the acute 2-hour group of dogs, patency was 100% (6/6) for silyl-heparin and 83.3% (5/6) for control grafts. This difference was not significant as measured by the Fisher exact test (*P* = .50). Overall chronic 7-day and 30-day graft patency was 100% for the silyl-heparin bonded (16/16) grafts versus 68.75% for control (11/16) grafts. This difference was statistically significant (*P* = .043). All control graft occlusions occurred within the first postoperative day, as determined by loss of palpable femoral pulses on physical examination of the animals. All grafts (both patent and occluded) were explanted, and both proximal and distal anastomoses were examined. No technical errors were observed in any of the explanted grafts. In addition, no hematomas or seromas were noted postoperatively.

<sup>111</sup>Indium-radiolabeled platelet studies were performed only in dogs in which both experimental and control grafts were patent. A linear relationship between total number of platelets injected and total graft radioactivity was observed (data not shown). Therefore, the total radioactivity of each graft was normalized to both graft surface area and number of platelets injected (Table I). In the acute 2-hour group of animals, a lower amount of radiolabeled platelets was deposited on the silyl-heparin bonded grafts than on the control grafts (3.32 ± 2.42 vs 40.1 ± 58.1 CPM per cm<sup>2</sup> per million platelets injected, mean ± SD). This difference did not reach statistical significance. In the 7-day group of dogs, there was a trend towards a higher amount of radiolabeled platelets deposited on the silyl-heparin bonded grafts (although not statistically significant) compared with control grafts. In the 30-day group of

**Table II.** Heparin activity of silyl-heparin bonded grafts

Silyl-heparin grafts	IU/cm <sup>2</sup> ± SD
Pre-implant	2.0
Acute 2 hour (n = 6)	0.57*
7-Day (n = 5)	0.106 ± 0.015
30-Day (n = 5)	0.007 ± 0.001

IU, International units.

\*Upper limit of assay.

animals, a significantly lower amount of platelets was deposited onto the silyl-heparin bonded grafts compared with control grafts (*P* = .0451, Wilcoxon rank sum test).

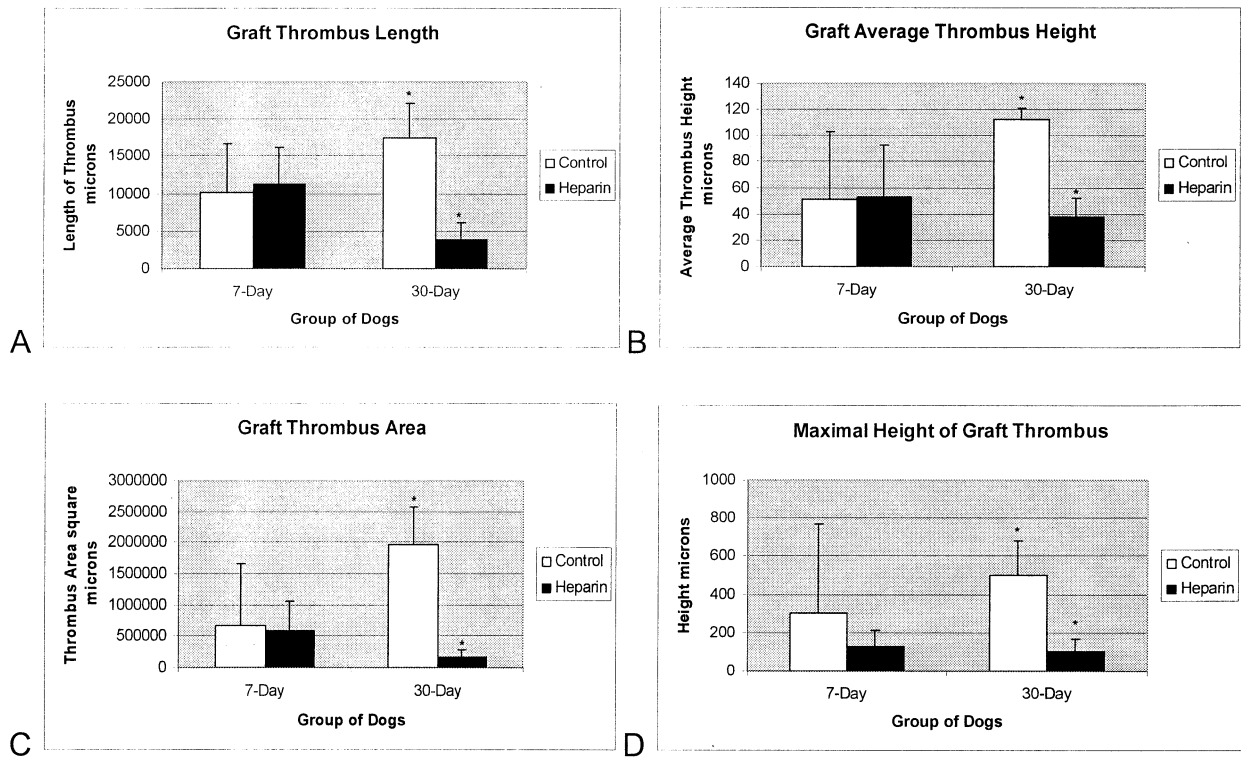
Histologic analysis of grafts revealed a significantly lower amount of intraluminal thrombus on the silyl-heparin bonded grafts than on control grafts in the 30-day group of animals. This was consistent in all histologic parameters measured, including graft percentage free of thrombus (Figs 1 and 2). In contrast, there was no difference in the amount of intraluminal thrombus observed on both types of grafts in the 7-day group of dogs (Figs 1 and 2).

Based on calculations obtained during the bonding process, the pre-implant activity of the silyl-heparin bonded grafts was estimated to be 2.0 IU per cm<sup>2</sup> of graft (Table II). After a 2-hour implantation, the activity of the silyl-heparin bonded grafts remained above the upper limit of the heparin assay (0.57 IU per cm<sup>2</sup> of graft). After 7 days implanted, the activity decreased to 0.106 ± 0.015 IU per cm<sup>2</sup> of graft (5.3% of pre-implant activity). The activity of the silyl-heparin bonded grafts further decreased, after 30 days implanted, to 0.007 ± 0.001 IU per cm<sup>2</sup> of graft (0.35% of pre-implant activity).

## DISCUSSION

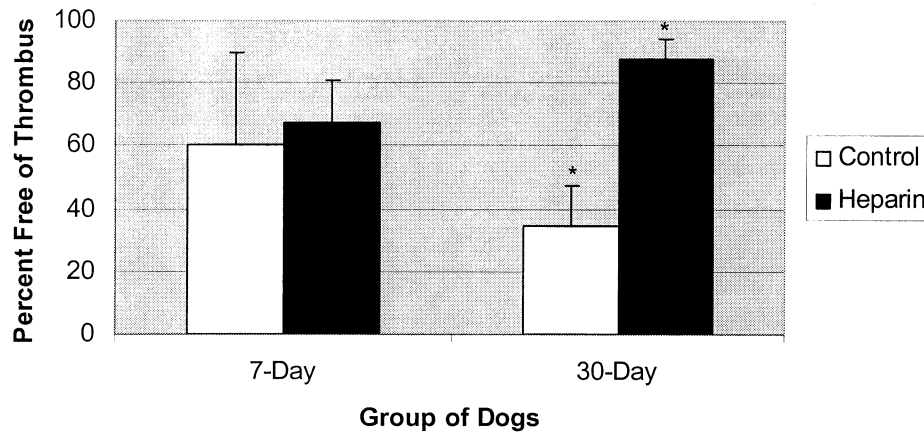
In order to improve surface hemocompatibility, heparin has been immobilized onto the blood-contact surface of numerous medical devices such as coronary stents, cardiopulmonary bypass circuits, ventricular assist devices, and central venous catheters.<sup>8-11</sup> Biologically active heparin has been immobilized onto Dacron and ePTFE vascular grafts.<sup>12-14</sup> The heparin-bonded Dacron graft, intended primarily for infra-inguinal arterial reconstruction, has been available clinically for the past several years. The results of a randomized clinical trial comparing the heparin-bonded Dacron graft with ePTFE grafts in femoropopliteal artery bypass grafting have recently been reported, in which an advantage in 3-year graft patency and 3-year amputation rate was observed with the heparin-bonded Dacron graft over the ePTFE grafts.<sup>11</sup> The heparin ePTFE graft was recently evaluated in a canine carotid artery interposition grafting model where improved patency and thromboresistance were observed.<sup>14</sup>

Carbon coating of ePTFE vascular grafts has been shown to decrease surface thrombogenicity due to its negative charge and hydrophobic nature. Carbon-coated prosthetic grafts were shown to reduce platelet deposition.<sup>15</sup> In



**Fig 1.** Histologic parameters measured in silyl-heparin and control grafts for both chronic 7-day and 30-day groups are summarized. **A,** Graft thrombus length. **B,** Graft average thrombus height. **C,** Graft thrombus area. **D,** Maximal height of graft thrombus (n = 5, mean ± SD, P = .0451 by Wilcoxon rank sum test).

### Graft % Free of Thrombus



**Fig 2.** The percentage of silyl-heparin and control graft length free of thrombus is shown for both 7-day and 30-day groups of dogs (n = 5, mean ± SD, P = .0451 by Wilcoxon rank sum test).

addition, improved 12-month primary and secondary graft patency and higher 12-month limb salvage rates have been reported in a multicenter clinical trial.<sup>16</sup>

Surface modification using hydrophilic polymers effectively prevents protein adsorption and cell adhesion.<sup>17-19</sup>

Polyethylene glycol (PEG) has been extensively used for surface modification because of its unique properties, such as hydrophilicity, flexibility, nontoxicity, and non-immunogenicity.<sup>20</sup> When coated on hydrophobic surfaces, PEG has been shown to decrease protein adsorption and platelet

adhesion.<sup>21,22</sup> In addition, leukocyte-induced procoagulant activities, such as tissue factor expression and Factor Xa generation by monocytes, have been shown to be reduced on PEG-coated polystyrene versus uncoated polystyrene.<sup>22</sup> In contrast, PEG immobilization onto ePTFE resulted, according to one report, in an increase in surface platelet adhesion.<sup>20</sup> Despite the increase in platelet adhesion, the PEG-coated ePTFE surface demonstrated improved blood compatibility (increased albumin adsorption and decreased fibrinogen adsorption).<sup>20</sup>

Silyl-heparin, benzyl-tetra(dimethylsilylmethyl)oxycarbonyl-heparin, is a biologically active heparin analogue synthesized by conjugating silyl-prosthetic units to the heparin molecule. The resultant molecule is amphipathic and readily adsorbed onto hydrophobic surfaces and biodegradable polymers, while retaining its anticoagulant properties. We have recently evaluated a silyl-heparin adsorbed carbon-coated ePTFE graft in a canine aortoiliac model where a significant improvement in thromboresistance was observed.<sup>3</sup> The advantage in thromboresistance, however, was short-lived due to poor retention of the silyl-heparin on the carbon-coated ePTFE grafts. In the present study, we have improved the retention of the silyl-heparin by using polyethylene glycol to bond the molecule onto the grafts and by increasing the total amount of active silyl-heparin present on the grafts by tenfold. Heparin assay of the explanted silyl-heparin grafts confirmed the presence of active silyl-heparin up to 30 days post implantation (Table II).

Silyl-heparin bonding onto carbon-coated ePTFE grafts resulted in a significant improvement in graft patency and thromboresistance (Table I). The advantage in silyl-heparin graft thromboresistance, as measured by reduced platelet deposition, reached statistical significance at 30 days. A significant reduction in intraluminal graft thrombus in the silyl-heparin bonded grafts was also observed in the 30-day group of animals (Fig 1 and 2). In the 7-day group of dogs, there was no difference in platelet deposition or the amount of intraluminal graft thrombus present on the silyl-heparin bonded grafts compared with control grafts. In the acute 2-hour group of animals, a reduction in platelet deposition was observed in the silyl-heparin bonded grafts compared with control grafts, although this difference was not statistically significant. These findings support a time-dependent mechanism for the development of a thromboresistant surface on the silyl-heparin bonded grafts.

The lack of advantage in thromboresistance observed in the 7-day group of animals is possibly due to a combination of time-dependent PEG and silyl-heparin effects that result in a delay in initial surface platelet deposition measured at the 7-day time point. The silyl-heparin bonding process is such that the heparin analog is sandwiched between two separate applications of PEG to the carbon-coated ePTFE grafts (see Material and Methods).

From a theoretical standpoint, the initial silyl-heparin-mediated advantage in thromboresistance observed in the acute 2-hour group would lead to a delay in the accumula-

tion of coagulant proteins such as thrombin and Factor Xa on the luminal surface after graft implantation. Thrombin and Factor Xa have been shown to contribute to graft-associated procoagulant activity.<sup>23</sup> The delay in the accumulation of these coagulant proteins would allow deposition of less thrombogenic proteins, such as albumin and immunoglobulin G, and formation of a hemocompatible surface.<sup>24</sup> In addition, the PEG-mediated attenuation of leukocyte-induced procoagulant effects would further contribute to surface thromboresistance.<sup>22</sup> By 7 days, when only 5% of the initial silyl-heparin remains, the PEG and silyl-heparin effects result in an increase in measured platelet deposition (Table II). Although an increase in platelet deposition is observed, improved blood compatibility remains. After this time, a passivated surface would be present, resulting in lower intraluminal thrombus accumulation and lower platelet deposition. At 30 days, only 0.35% of the silyl-heparin remains on the experimental graft surface. At this time, the silyl-heparin effect is less important, as evidenced by the small amount remaining compared with the additive or synergistic effect of both PEG and silyl-heparin. This process may prevent early graft failure leading ultimately to improved long-term graft patency rates.

In conclusion, silyl-heparin bonding onto carbon-coated ePTFE grafts resulted in an increase in graft patency, improved thromboresistance, and a significant reduction in intraluminal graft thrombus. This graft may prove to be useful in the clinical setting.

## REFERENCES

1. National Center for Health Statistics. Series 13, No. 139;1996:25-8.
2. Whittemore AD, Belkin M. Infrainguinal bypass. In: Cronenwett JL, Gloviczki P, Johnson KW, Kempczinski RF, Krupski WC, editors. Rutherford Vascular Surgery, 5th ed. Philadelphia: W.B. Saunders Company; 2000. 998-1018.
3. Laredo J, Xue L, Husak VA, Ellinger J, Greisler HP. Silyl-heparin adsorption improves the in vivo thromboresistance of carbon-coated polytetrafluoroethylene vascular grafts. *Am J Surg* 2003;186:556-60.
4. Gosselin C, Ren D, Ellinger J, Greisler HP. In vivo platelet deposition on polytetrafluoroethylene coated with fibrin glue containing fibroblast growth factor 1 and heparin in a canine model. *Am J Surg* 1995;170:126-30.
5. The guide for the care and use of laboratory animals. Washington (DC): National Institutes of Health; 1985. Publication no. 85-23.
6. Lindhout T, Blezer R, Schoen P, Willems GM, Fouache B, Verhoeven M, et al. Antithrombin activity of surface-bound heparin studied under flow conditions. *J Biomed Mater Res* 1995;29:1255-66.
7. Uitenbroek DG. SISA-Binomial. 1997 [cited 2002 Jan 1]. Available from: URL:<http://home.clara.net/sisa/binomial.htm>.
8. Serruys P, Emanuelsson H, van der Giessen W, Lunn AC, Kiemeneij F, Macaya C, et al. Heparin coated Palmaz-Schatz stents in human coronary arteries. *Circulation* 1996;93:412-22.
9. Videm V, Svennevig JL, Fosse E, Semb G, Osterud A, Mollnes TE, et al. Reduced complement activation with heparin-coated oxygenator and tubings in coronary bypass operations. *J Thorac Cardiovasc Surg* 1992;103:806-13.
10. Hetzer R, Loebe M, Potapov EV, Weng Y, Stiller B, Hennig E, et al. Circulatory support with pneumatic paracorporeal ventricular assist device in infants and children. *Ann Thorac Surg* 1998;66:1498-1506.
11. Appelgren P, Ransjo U, Bindslev L, Espersen F, Larm O. Surface heparinization of central venous catheters reduces microbial colonization in vitro and in vivo: results from a prospective, randomized trial. *Crit Care Med* 1996;24:1482-9.

12. Lambert AW, Fox AD, Williams DJ, Horrocks M, Budd JS. Experience with heparin-bonded collagen-coated grafts for infrainguinal bypass. *Cardiovasc Surg* 1999;7:491-4.
13. Devine C, Hons BA, McCollum C. Heparin-bonded Dacron or polytetrafluoroethylene for femoropopliteal bypass grafting: a multicenter trial. *J Vasc Surg* 2001;33:533-9.
14. Begovac PC, Thomson RC, Fisher JL, Hughson A, Gallhagen A. Improvements in Gore-tex vascular graft performance by carmeda bioactive surface heparin immobilization. *Eur J Vasc Endovasc Surg* 2003;25:432-7.
15. Tsuchida H, Cameron BL, Marcus CS, Wilson SE. Modified polytetrafluoroethylene: Indium 111-labeled platelet deposition on carbon-lined and high porosity polytetrafluoroethylene grafts. *J Vasc Surg* 1992;16:643-9.
16. Groegler FM, Kapfer X, Meichelböck W. Crural prosthetic revascularization: randomized, prospective, multicentric comparison of standard and carbon impregnated ePTFE grafts. In: Montefiore Annual Symposium on Current Critical Problems, New Horizons and Techniques in Vascular and Endovascular Surgery ('01). New York, NY, Nov 15-18, 2001; Abstract 01-2, I2.1-I2.3.
17. Jacobs H, Grainger D, Okano T, Kim SW. Surface modification for improved blood compatibility. *Artif Org* 1988;12:506-7.
18. Holmberg K, Bergstrom K, Brink C, Osterberg E, Tiberg F, Harris JM. Effects on protein adsorption, bacterial adhesion and contact angle of grafting PEG chain to polystyrene. *J Adhes Sci Technol* 1993;7:503-17.
19. Lee JH, Lee HB, Andrade JD. Blood compatibility of polyethylene oxide surfaces. *Prog Polym Sci* 1995;20:1043-79.
20. Zhang Q, Wang C, Babukutty Y, Ohyama T, Kogoma M, Kodama M. Biocompatibility evaluation of ePTFE modified with PEG in atmospheric pressure glow discharge. *J Biomed Mater Res* 2002;60:502-9.
21. Llanos GR, Sefton MV. Immobilization of polyethylene glycol onto a polyvinyl alcohol hydrogel: 2. evaluation of thrombogenicity. *J Biomed Mater Res* 1993;27:1383-91.
22. Gorbet MB, Sefton MV. Leukocyte activation and leukocyte procoagulant activities after blood contact with polystyrene and polyethylene glycol-immobilized polystyrene beads. *J Lab Clin Med* 2001;137:345-55.
23. Toursarkissian B, Eisenberg PR, Abendschein DR, Rubin BG. Thrombogenicity of small-diameter prosthetic grafts, relative contributions of graft-associated thrombin and factor Xa. *J Vasc Surg* 1997;25:730-5.
24. Xue L, Greisler HP. Blood Vessels. In: Lanza R, editor. Principles of tissue engineering. 2nd ed. San Diego: Academic Press; 2000. p. 427-46.

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