Nitric oxide–releasing biopolymers inhibit thrombus formation in a sheep model of arteriovenous bridge grafts

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Objectives: **Nitric oxide (NO), produced by normal vascular endothelial cells, reduces platelet aggregation and thrombus formation. NO-releasing biopolymers have the potential to prolong vascular graft and stent patency without adverse systemic vasodilation.**

Methods: **5-mm polyurethane vascular grafts coated with a polymer containing the NO-donor dialkylhexanediamine diazeniumdiolate were implanted for 21 days in a sheep arteriovenous bridge-graft model.**

Results: **Eighty percent (4/5) of grafts coated with the NO-releasing polymer remained patent through the 21 day implantation period, compared to fifty percent (2/4) of sham-coated grafts and no (0/3) uncoated grafts. Thrombus-free surface area (±SEM) of explanted grafts was significantly increased in NO-donor coated grafts (98.2% ± 0.9%) compared with sham-coated (79.2% ± 8.6%) and uncoated (47.2% ± 5.4%) grafts ($P = .00046$). Examination of the graft surface showed no adherent thrombus or platelets and no inflammatory cell infiltration in NO-donor coated grafts, while control grafts showed adherent complex surface thrombus consisting of red blood cells in an amorphous fibrin matrix, as well as significant red blood cell and inflammatory cell infiltration into the graft wall.**

Conclusion: **In this study we determined that local NO release from the luminal surface of prosthetic vascular grafts can reduce thrombus formation and prolong patency in a model of prosthetic arteriovenous bridge grafts in adult sheep. These findings may translate into improved function and improved primary patency rates in small-diameter prosthetic vascular grafts. (J Vasc Surg 2004;40:803-11.)**

Clinical Relevance: **Early (within 30 days) small-diameter prosthetic graft occlusion is common; low conduit flow volume and inherent thrombogenicity are believed to be the major factors in the cause of occlusion. In this study small-diameter arteriovenous bridge grafts were coated with a new nitric oxide–releasing biopolymer, resulting in an increased thrombus-free graft surface area. With the results of this study it is reasonable to hypothesize that prevention of early thrombus formation may translate into improved primary patency rates in small-diameter prosthetic vascular grafts.**

Over the last decade the numbers of surgical revascularization procedures, including coronary and lower extremity bypass, have increased dramatically, owing in part to the aging population.[1] Furthermore, the number of secondary vascular procedures is estimated at 15% after initial coronary revascularization and approximately 50% after lower extremity bypass.[2,3] Similarly, the number of patients requiring vascular access for hemodialysis has increased significantly, and is currently estimated at 250,000 persons in the United States, of whom 70% receive prosthetic vascular grafts as the primary vascular access.[4] Together, these data suggest an increasing clinical need for small-diameter prosthetic vascular grafts in the future.

Because of superior patency, autogenous bypass conduits are preferred for primary revascularization procedures. The increasing incidence of secondary procedures in the aging patient population suggests a growing need for alternatives when autogenous conduits are unavailable or are inadequate.[5] Despite this accelerating need for vascular conduits in small-diameter bypass and hemodialysis grafts, early thrombosis (<30 days) remains a formidable obstacle to their widespread applicability. Early thrombosis is estimated to occur in as many as 25% of prosthetic infrapopliteal bypass grafts and 18% of prosthetic hemodialysis access grafts.[3,6-8] Early thrombosis in small-diameter (<6 mm) conduits is primarily a result of graft thrombogenicity and reduced flow volume.[3,7,9] Recent research to prolong patency of small-diameter prosthetic grafts has focused primarily on the activity of platelets and the nature of the graft material to prevent thrombus formation.[10-14] On the other hand, subacute thrombosis (1-18 months) is thought to result from altered flow dynamics produced by development of intimal hyperplasia, primarily at the distal anastomosis.[6]

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Nitric oxide (NO), produced by normal vascular endothelial cells, limits platelet activation, adhesion, and aggregation by activating guanylyl cyclase, inhibiting phosphoinositide 3-kinase, impairing capacitative calcium influx, and inhibiting cyclooxygenase-1,[15,16] preventing the adhesive receptor-ligand interactions that promote thrombus formation and growth.[17] However, profound vasodilatory effects limit the use of NO in pharmacologic doses in vivo.[18] Nevertheless, because NO is rapidly scavenged by oxygen and hemoglobin in plasma, local release of NO by NO-releasing biopolymers has the potential to prolong vascular graft and stent patency without adverse systemic vasodilation.[13] For example, in a previous study in baboons, NO-releasing arteriovenous grafts reduced indium 111–labeled platelet aggregation, compared with control grafts.[16] In a similar study that used extracorporeal circulation circuits, NO-releasing polymer significantly reduced platelet adhesion without adverse physiologic effects in a rabbit model.[10]

In the present study we explored the hypothesis that local NO release from the luminal surface of prosthetic vascular grafts reduces thrombus formation and prolongs graft patency in vivo. Small-diameter vascular grafts were coated with a new NO-releasing biopolymer. When exposed to physiologic conditions the polymer releases NO gas without releasing appreciable amounts of the dialkyldiamine-based diazeniumdiolate NO donor compound or other by-products of the NO release reaction. The grafts were placed as arteriovenous bridge grafts in adult sheep, and were explanted at 21 days for evaluation. Patency was determined at clinical and ultrasound examinations, and thrombus formation was measured as gross thrombus-free surface area (TFSA) and graft histologic findings. NO-releasing biopolymers significantly reduced thrombus formation and increased overall patency in these grafts. These results suggest that NO-releasing biopolymers may improve patency of small-diameter prosthetic vascular grafts.

MATERIAL AND METHODS

Graft preparation. Small-diameter polyurethane vascular grafts (5-mm internal diameter, 25 cm long; Vectra vascular access graft, Bard-Impra) were coated with a previously characterized NO-releasing biopolymer (MC3 Corp).[14] Vascular grafts were dip-coated with multiple layers of plasticized polyvinyl chloride containing the NO donor dialkylhexanediamine diazeniumdiolate. Control grafts were either uncoated or sham-coated polyurethane grafts of equivalent diameter and length. Sham-coated control grafts were prepared with the same polymer layer used in the NO-releasing grafts; however, the NO-releasing compound present within the polymer was absent. The NO-releasing compound, or NO donor, is a previously described and well-characterized dialkylhexanediamine diazeniumdiolate compound.[13] The principles of the activity of this new NO-releasing biopolymer are outlined in Fig 1. After sterilization, a 1-cm piece of coated graft material was removed and retained for in vitro assay of NO release by on-line chemiluminescence. The 1-cm long sections of the vascular graft were soaked in phosphate-buffered saline solution (PBS; pH 7.4, 37°C) for selected time intervals. NO surface flux was calculated on the basis of surface area of the graft and the NO release profile.[13] The grafts were blinded after coating, and were implanted, harvested, and processed in blinded fashion. The grafts were not unblinded until data collection was complete.

Animal model. All animal experiments were approved by the William Beaumont Hospital Animal Care Committee and complied with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*[19]

Six adult male sheep (35-45 kg) underwent an initial procedure for blinded placement of bilateral arteriovenous bridge grafts from the distal common carotid arteries to the ipsilateral external jugular veins. Aspirin (325 mg) per rectum was given daily to each animal, starting on the day of the operation. All sheep were anesthetized with intravenously administered ketamine hydrochloride (22 mg/kg), and all received orotracheal tubes. Mechanical ventilation with inhaled isoflurane was used for maintenance anesthesia. An intravenous infusion was maintained via a forefoot vein throughout the procedure.

Sheep were initially positioned in the right lateral decubitus position, to expose the left side of the neck, with the neck slightly extended. The proximal left external jugular vein and distal left common carotid artery were exposed through individual incisions, and were connected with a subcutaneous tunnel for the graft. Once the vessels were exposed and the tunnel created, intravenous heparin sodium at 200 U/kg was given, and supplemented every 90 minutes with an additional 100 U/kg. Standard end-to-side vascular anastomoses were created with continuous 6-0 polypropylene suture. After completion of the anastomosis flow was restored, and a palpable thrill was appreciated within the graft. Local hemostasis was obtained, and the left-side incision was closed in layers. The sheep were then turned to the left lateral decubitus position for exposure of the right side of the neck. The graft on the right side was placed in an identical manner. Feeding was reinstituted on the first postoperative day. With the same anesthesia and positioning as described for the initial procedure, all grafts were explanted at 21 days. Complete in situ exposure of each side enabled visualization to assess for any mechanical problems.

Patency. Palpation and auscultation were performed daily to assess for graft patency. Patency was defined as the presence of a palpable thrill, audible bruit, or both. In any sheep for which examination findings were equivocal, duplex ultrasound scanning was performed with the animal under ketamine sedation, to further assess graft status. Pre-arterial and post-arterial anastomotic flow velocity and venous outflow velocity were measured. Because these polyurethane grafts are highly echogenic, direct ultrasound measurements cannot be made of flow velocity within the graft. Failure to demonstrate a decrease in flow velocity across the arterial anastomosis or lack of arterial flow velocity at the venous anastomosis was considered consistent

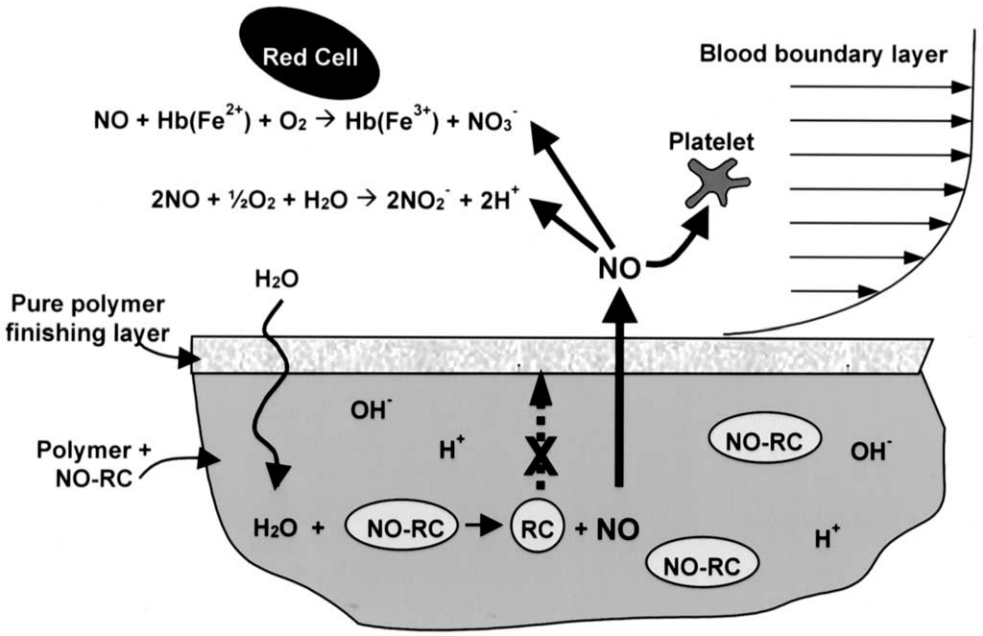

Fig 1. Schematic representation of nitric oxide (NO) release from polymer coating. At luminal surface of the NO-releasing graft is a thin layer of pure polymer outer coating consisting entirely of polyvinyl chloride (PVC) polymer. Beneath the outer coating is a PVC polymer suspension containing the NO-releasing compound (*NO-RC*). As water diffuses from the blood, it contacts the NO-releasing compound within the suspension. This enables release of free NO into the lumen of the vessel, where local activity on platelets is established. Systemic NO effects are averted, because NO is quickly scavenged by oxygen and hemoglobin of local red blood cells.

with graft thrombosis. Final graft patency was confirmed at explantation by the presence of an intraoperative palpable thrill, in addition to aspiration of arterial blood at mid-graft before ligation. Coagulation status was not monitored during the period of implantation.

TFSA. At explantation all grafts were immediately flushed with 10 mL of normal saline solution to remove fresh thrombus and blood. To preserve the grafts for gross and histologic sectioning, perfusion fixation with 2.5% glutaraldehyde solution was performed at physiologic pressure for 5 minutes. To determine the effect of NO-releasing biopolymer on surface thrombus formation, percent TFSA for each graft was measured with Optimas image processing software (Media Cybernetics). Grafts were opened longitudinally, and a high-resolution digital image was captured. Image characteristics identified as indicative of surface thrombus were assigned color levels in the programming, and percent of graft surface area corresponding to TFSA was computed. On average 5 repeat, nonsequential analyses of each graft segment were performed. Validation studies of this technique have shown that repeat measures performed in blinded fashion on the same sample typically show a variance of less than 5%.[20]

Histologic analysis. Graft specimens were taken for histologic analysis through the graft at the venous anastomosis. This is a common site of flow disturbances, and is thought to represent an area of increased thrombogenic potential. Specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Five-micrometer sections were cut perpendicular to the axis of the vessel through the spatulated toe of the graft. Sections were stained with hematoxylin-eosin and examined for location, thickness, and structure of any thrombus. Cellular organization and matrix formation were examined by staining with Movat pentachrome stain, which differentiates collagen, elastin, mucin, and ground substance, fibrin, muscle, and cell nuclei.

Scanning electron microscopy. Surface characteristics such as fibrin layering and platelet adherence were evaluated with scanning electron microscopy. After gross photography, scanning electron microscopy specimens (8 mm × 6 mm) were taken from the arterial and venous anastomoses and from the central graft area. Specimens were processed with standard methods of osmium tetroxide after fixation, dehydration, and critical point drying. Dried specimens were affixed to specimen mounts with double-sided tape. Specimens were coated with 15 nm of gold-palladium, and were examined with a JEOL 6400 scanning electron microscope at 8 kV. Images were recorded on Polaroid P/N 55 film.

Statistical analysis. Differences in percentage of TFSA between NO-releasing grafts and control grafts were analyzed with analysis of variance followed with the Student unpaired *t* test (two-tailed), and the Fisher exact test was used to determine the significance of patency differ-

Table I. Distribution of grafts in experimental animals

Identifier	*Graft type*	*Implantation order*	*Patency status*
Animal 1	Uncoated control graft	Simultaneous	Occluded day 3
	Uncoated control graft		Occluded day 12
Animal 2	Uncoated control graft	Sequential	Occluded day 9
	Sham-coated graft		Occluded day 12
Animal 3	NO-releasing graft	Sequential	Occluded day 18
	NO-releasing graft		Open at explantation
Animal 4	Sham-coated graft	Simultaneous	Open at explantation
	NO-releasing graft		Open at explantation
Animal 5	Sham-coated graft	Simultaneous	Open at explantation
	NO-releasing graft		Open at explantation
Animal 6	Sham-coated graft	Simultaneous	Occluded day 12
	NO-releasing graft		Open at explantation

NO, Nitric oxide.

Table II. Mean percent thrombus-free surface area for all grafts with statistical comparison between groups

Graft type	*% TFSA ± SEM*
NO-releasing graft	98.2 ± 0.9
Sham-coated control graft	79.2 ± 8.6
Uncoated graft	47.2 ± 5.4

TFSA, Thrombus-free surface area; *NO*, nitric oxide.
$P = .000446$ at 1-way variable analysis of variance.

Table III. Graft patency at 21 days with statistical comparison between groups

Graft type	*Patency (21 days)*
NO-releasing graft	4 of 5
Sham-coated control graft	2 of 4
Uncoated graft	0 of 3

$P = .071$ (NO-releasing graft vs uncoated graft; Fisher exact test).
$P = .405$ (NO-releasing graft vs sham-coated graft; Fisher exact test).

ences between groups. Statistical significance was defined as $P < .05$.

RESULTS

Twelve grafts (5 NO-releasing grafts, 4 sham-coated control grafts, 3 uncoated control grafts) were placed in 6 sheep in a blinded, randomized fashion. In 4 animals both grafts were implanted simultaneously, and in 2 sheep the grafts were implanted sequentially (Table I). Because the graft distribution was blinded, randomization of the grafts resulted in some sheep receiving 2 identical grafts and other sheep receiving different grafts.

There were no obvious mechanical problems (eg, twisting, kinking) in any graft at removal. Patency was confirmed by the presence of audible bruit, palpable thrill, and needle aspiration of arterial blood at explantation. All grafts determined to be thrombosed before 21 days were confirmed as occluded at explantation by lack of audible bruit and palpable thrill, and failure to produce arterial blood at needle aspiration. Duplex ultrasound scanning was used on 4 separate occasions to confirm graft status. On all 4 occasions, lack of arterial flow velocity change across the arterial anastomosis and failure to demonstrate arterial flow velocity at the venous anastomosis confirmed the clinical suspicion of thrombosis of the graft.

Patency. Patency of each graft was determined at clinical and ultrasound examination, and confirmed intraoperatively at explantation. At explantation, both grafts were occluded in 2 sheep, both grafts were patent in 2 sheep, and 1 graft was occluded and 1 graft was patent in 2 sheep. All 3 uncoated control grafts were determined to be thrombosed at clinical and duplex ultrasound scanning before 21 days (3, 7, and 12 days; mean, 7 days). Of the 4 sham-coated control grafts, 2 were thrombosed, both at 12 days, and 2 remained patent at 21 days. Of the 5 NO-releasing grafts, 1 was thrombosed at 18 days and 4 remained patent at 21 days. Overall, improved patency between NO-releasing grafts and control grafts did not reach statistical significance (Table II).

TFSA. With the Optimas system TFSA was determined for the entire luminal surface of each graft. A statistically significant increase in TFSA was seen with the NO-releasing grafts compared with both uncoated and sham-coated control grafts. In 5 NO-releasing grafts, TFSA averaged 98.2% (range, 95.1%-99.6%). In 4 sham-coated control grafts TFSA averaged 79.2% (range, 56.6%-97.9%). In 3 uncoated control grafts TFSA averaged 47.2% (range, 37.6%-56.3%; (Fig 2). Statistical comparison of mean percent TFSA is shown in Table III.

Histologic analysis. Examination of hematoxylin-eosin–stained light microscopy sections at 40× magnification showed significant thrombus adherent to the luminal surface of the uncoated control graft. The complex thrombus had a maximum thickness of 200 μm, and consisted of red blood cells in a heavy fibrin matrix, with a few inflammatory cells. Within the graft wall a significant increase was noted in the number of inflammatory cells and red blood cell infiltration. Similar histologic sections of the NO-releasing graft showed the luminal surface to be free of thrombus, with occasional minor deposits of thin matrix

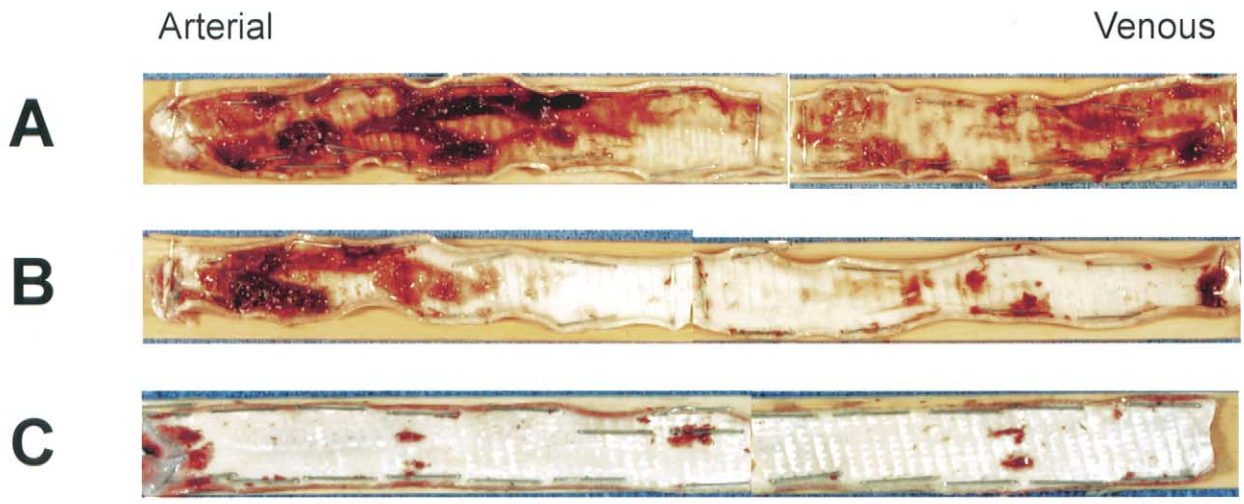

Fig 2. Representative gross photographs of explanted polyurethane vascular grafts. Grafts were opened longitudinally to display luminal thrombus. **A,** Uncoated control graft. **B,** Sham-coated graft without nitric oxide–releasing biopolymer. **C,** Graft incorporating nitric oxide–releasing polymer coating on luminal surface.

fragments and the graft containing only a few scattered red blood cells and inflammatory cells (Fig 3). Specimens from sham-coated grafts showed variable amounts of thrombus, which did not appear to be strongly adherent to the graft surface at the venous anastomosis. Staining of NO-releasing grafts with Movat pentachrome showed occasional areas of the polymer coating covered with a noncontinuous, approximately 2-μm layer of fibrin matrix.

Scanning electron microscopy. Surface characteristics such as fibrin layering and platelet adherence were evaluated with scanning electron microscopy; Fig 4 shows representative images. Although there was variability between specimens, the arterial anastomosis of sham-coated grafts generally showed a fibrin coating and dense scattering or clusters of activated platelets, and 20% to 50% coverage with red blood cells, and some specimens showed complete coverage with complex thrombus consisting of red blood cells and occasional inflammatory cells in a heavy fibrin matrix. The arterial anastomosis of NO-releasing grafts also showed some variability, but had less than 30% coverage with red blood cells, and scattered or few small clusters of mostly inactivated platelets, with small thrombi around the sutures. Venous anastomoses of sham-coated grafts also showed a fibrin coating and 20% to 30% coverage with red blood cells or thrombus; platelets were generally scattered. Venous anastomoses of NO-releasing grafts were similar. Specimens from the central position of sham-coated grafts showed 10% to 50% red blood cell coverage, with generally scattered, inactivated platelets and some platelet clusters in occasional specimens. Specimens from the central portion of NO-releasing grafts were highly variable, and ranged from mostly clear with a few scattered platelets to 1 specimen with 85% to 90% coverage with red blood cells and platelet clusters.

NO release. NO release rates measured in vitro from samples of grafts reserved at implantation demonstrated persistent NO production after the first week at 29.2×10^{-12} mol cm−2 sec−1, and this decreased slightly to 5.5×10^{-12} mol cm−2 sec−1 by 3 weeks. Kinetics of release was linear for approximately the first week (Fig 5).

DISCUSSION

Autogenous conduits are the ideal replacements for diseased small-diameter arteries, because of the relatively low inherent thrombogenicity of native endothelium.[5] However, when previous procedures or underlying conditions have depleted autogenous options, there is need for alternative conduits. Prosthetic grafts are routinely used for replacement and bypass of large-diameter arteries, with a fairly limited incidence of early thrombosis.[21] In contrast, as graft diameter decreases thrombosis is more common, because shear stress increases under higher flow velocity and lower flow volume.[9] Moreover, the addition of a relatively thrombogenic synthetic luminal surface makes prosthetic conduits even less desirable for bypass of small-diameter arteries.

Early (within 30 days) prosthetic graft occlusion is not uncommon. As many as 18% of synthetic vascular access conduits for dialysis become thrombosed within the first 3 weeks and can never be used.[7] Moreover, as many as 25% of infrapopliteal synthetic grafts become thrombosed within the first month.[3,6,8] Once believed to be due entirely to technical errors, only 20% of early graft thromboses are now thought to be the direct result of poor technique. Conduit flow volume and inherent thrombogenicity are believed to be the major factors, especially since smaller conduits are being used to bypass smaller vessels.[6]

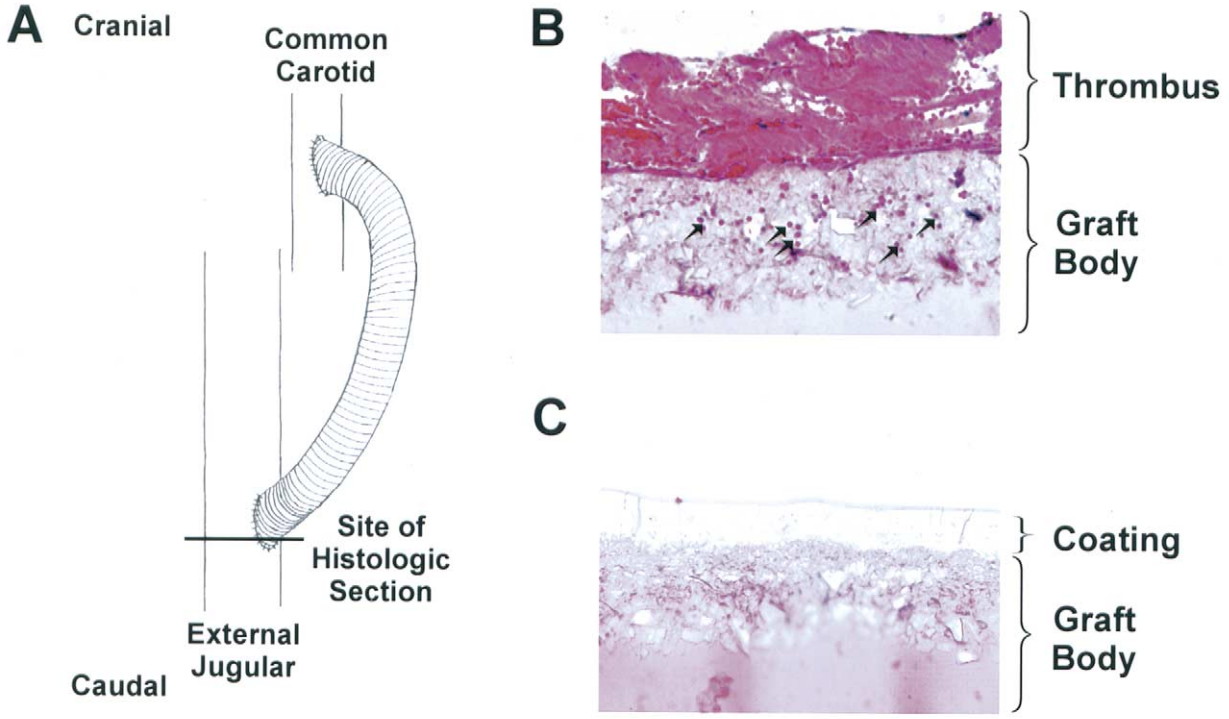

Fig 3. A, Schematic diagram demonstrating site of histologic sections of arteriovenous graft specimens. **B,** Hematoxylin-eosin–stained histologic sections from uncoated graft. Note abundance of thrombus at luminal surface of uncoated graft; *arrowheads,* red blood cell infiltration. Magnification ×40. **C,** Hematoxylin-eosin–stained histologic sections from nitric oxide–releasing graft. Luminal surface has thin fibrin coating in some specimens, but is free of attached thrombus. Cellular infiltration is not seen within the graft wall. Magnification ×40. Luminal surface toward top of photograph.

In this study, small-diameter arteriovenous bridge grafts were coated with a new NO-releasing biopolymer in an effort to reduce thrombus formation and improve overall patency. Inasmuch as platelets are an important contributor to early thrombus formation, we introduced a dialkylhexanediamine diazeniumdiolate NO-releasing biopolymer that reduces platelet adhesion.[13,22] Previous studies have demonstrated that the kinetics of NO release are linear for approximately the first week; however, the levels of NO generated after 25 days are still greater than the estimated rate of NO production for endothelial cells in vivo of 5.3 to 6.8 $\times$ 10^{-12} mol cm$-$2 sec$-$1.[23,24] In the present study, surface thrombus accumulation was significantly less in the NO-releasing grafts compared with both uncoated and sham-coated control grafts over a 3-week follow-up. This interval was chosen because it represents not only the period of maximal platelet adhesion and early thrombus formation but also the period of maximal NO-releasing activity from the biopolymer (Fig 5). Increased TFSA in the early postoperative period may lead to improved primary patency rates in these grafts. Moreover, reduction in thrombus formation and early platelet adhesion may improve flow volume during the period of maximal intimal hyperplastic response that follows. Although we did not observe a statistically significant improvement in patency in this study, the results for TFSA certainly suggest a beneficial effect of local NO release on thrombus accumulation on the surface of these grafts. Thus we believe that this preliminary study in a limited cohort of animals provides compelling evidence to suggest the merit of additional investigation into the effects of NO-releasing grafts on long-term patency.

A hypercoagulable state can be induced in sheep experimentally with infusion of endotoxin[25] after severe trauma,[26] in fetal lambs with infusion of insulin,[27] and naturally after viral infection.[28-30] Thus a potential weakness of this study is that an unrecognized hypercoagulable state, if present in some of the animals, could markedly bias the TFSA results, depending on the randomization schedule. However, the pattern of graft occlusion observed in this study suggests that a hypercoagulable state did not exist in these animals. In 4 of the animals 2 grafts were implanted simultaneously, and in the remaining 2 animals grafts were implanted sequentially. In 1 of the animals with sequentially implanted grafts (Table I, Animal 3), the NO-releasing graft became occluded on day 18 post-implantation, whereas the subsequently implanted NO-releasing graft remained open until explantation, with TFSA of 97.4%.

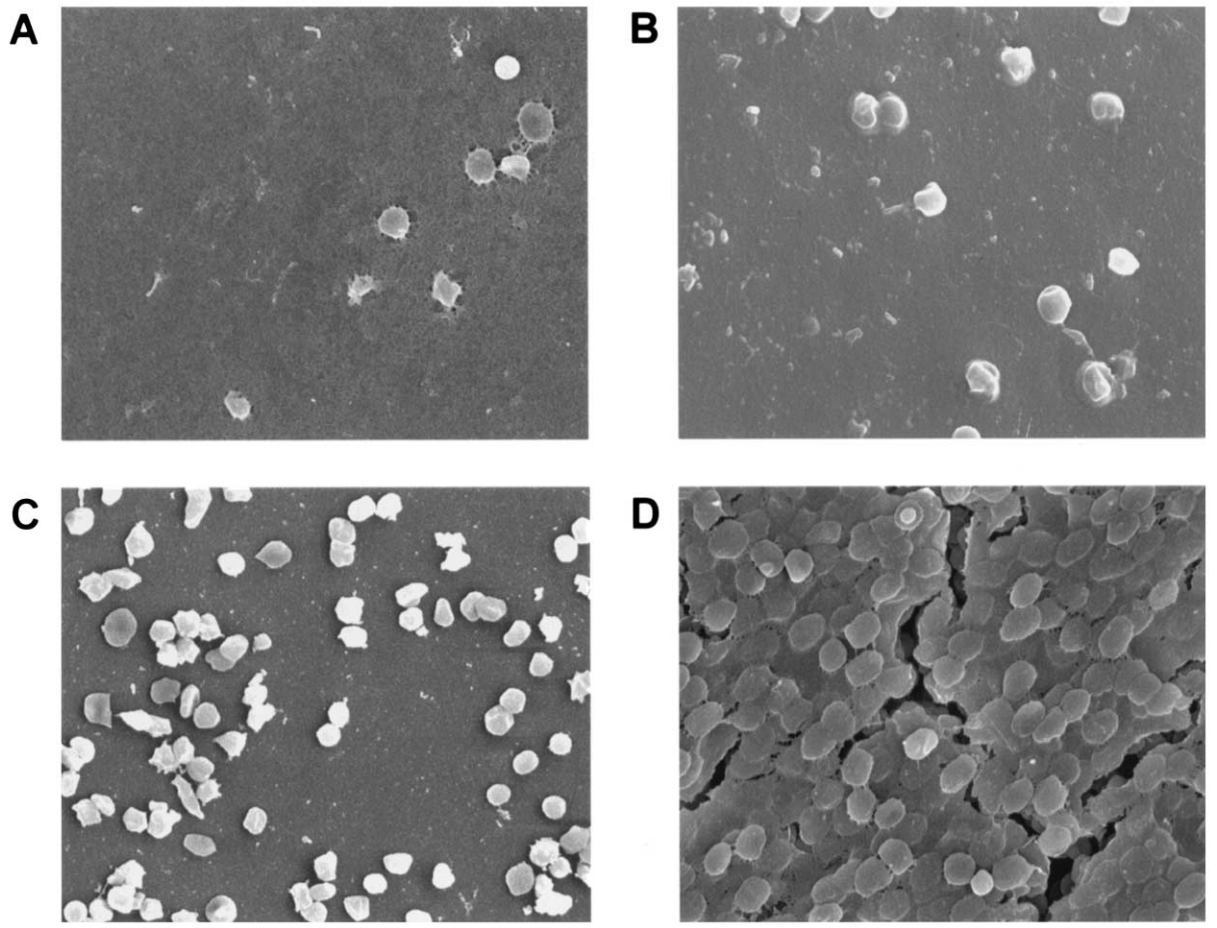

Fig 4. Explanted grafts were examined with scanning electron microscopy to show platelet adhesion and thrombus accumulation on graft surfaces. **A-D,** Magnification ×1000. **A and B,** Representative specimens from arterial anastomoses of 2 different nitric oxide–releasing grafts. Thin coating of fibrin is visible in specimen *A* as a mottled background of thin fibrils. Isolated platelets and 5% to 20% coverage of red and white blood cells are noted in both specimens. Most platelets appear inactivated when viewed at higher magnification (not shown). **C and D,** Representative specimens from arterial anastomoses of 2 sham-coated grafts. Specimen *C* shows approximately 50% coverage of red and white blood cells, isolated platelets, and platelet clumps. Some platelets appear activated when viewed at higher magnification (not shown). Specimen *D* shows complete coverage with complex thrombus consisting of red blood cells and occasional inflammatory cells in a heavy fibrin matrix.

Moreover, a second animal (Table I, Animal 6), in which only the sham-coated graft occluded at day 12, had a second, NO-releasing graft, which remained patent throughout the study, with TFSA of 99.3%. It is reasonable to assume that any significant hypercoagulable state should affect both grafts. Thus, although we did not specifically test for the presence of a hypercoagulable state, we believe that the randomization patterns and patency results argue against the presence of a significant hypercoagulable state in these animals.

The present study has a number of methodologic limitations. First, the sheep model may not be representative of the human condition. Sheep were ultimately chosen because of their uniformity, suitable vessel sizes, similarity of coagulation system to that in human beings, ease of neck dissection, and the possibility of producing chronic renal failure to study the effects of uremia on graft function in the future. Second, the arteriovenous bridge graft model, while relevant to the flow hemodynamics of hemodialysis access grafts, may not be representative of the hemodynamic conditions in small-diameter prosthetic vascular grafts used to bypass coronary arteries or tibial arteries in the lower extremity. Moreover, there is now increasing evidence that factors that influence graft patency may be specific to the site and the vascular bed.[31-34] While the present model has the obvious advantage of being analogous to the standard arteriovenous loop grafts used for vascular access for hemodialysis in human beings, these observations may not be generalizable to prosthetic arterio-arterial bypass grafts used in conventional coronary or peripheral revascularize-

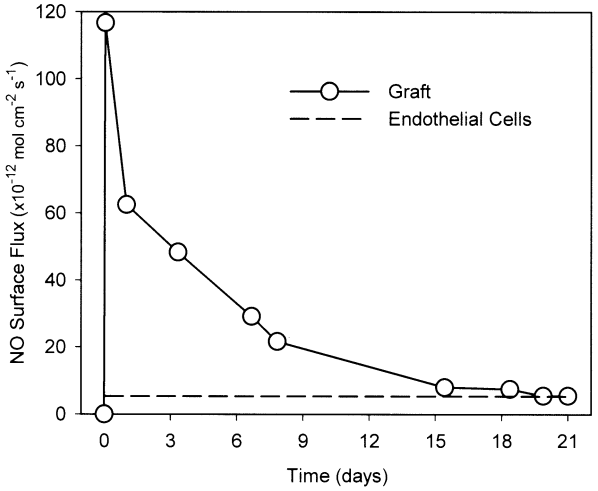

Fig 5. In vitro nitric oxide (NO) release over 25 days from surface-coated polyurethane graft. Sample of the polyurethane graft coated with the NO-releasing compound was removed from the graft before implantation, and tested for NO release in vitro over 25 days. NO release is linear for approximately 1 week. Although kinetics of release appear to level off after approximately 12 days, NO surface flux level is still at or above that generated by endothelial cells in vivo.

tion procedures. These limitations notwithstanding, early thrombus accumulation is a necessary precursor to complete graft thrombosis. Thus we believe the results of the present study provide supportive preliminary data for future studies in an arterio-arterial bypass model.

Finally, NO also reduces smooth muscle cell proliferation and migration, which are important factors in the intimal hyperplastic response.[18] NO also inhibits leukocyte adhesion, which has a major role in the intimal hyperplastic response.[18] Of interest, no study to date has shown a significant reduction in intimal hyperplasia from NO-releasing surfaces.[11,18] An important question as to whether NO-releasing biopolymers can reduce intimal hyperplasia in the subacute period was not addressed in this study, but is currently being evaluated in additional experiments. In studies by Cruz et al,[35] inhibiting platelet adhesion with Saratin reduced intimal hyperplasia in a rat carotid endarterectomy model. With the results of the present study, it is reasonable to hypothesize that prevention of early thrombus formation may have a role in abrogating the intimal hyperplastic response. Together, the results of these studies suggest that the effects of NO-releasing biopolymers on intimal hyperplasia in this model may provide a fruitful area for future investigation.

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