Alterations in wall tension and shear stress modulate tyrosine kinase signaling and wall remodeling in experimental vein grafts

Tam T. T. Huynh, MD, Mark G. Davies, MD, PhD, Matthew J. Trovato, Einar Svendsen, MD, PhD, and **Per-Otto Hagen, PhD,** *Durham, NC, and Bergen, Norway*

Purpose: **Hemodynamic alterations have been implicated as major stimuli for the development of intimal hyperplasia in vein grafts that are implanted in the arterial circulation. Tyrosine kinase is known to mediate cell signaling. However, its role with in vivo mechanotransduction is not yet well defined. We used a novel bioprosthetic collagen tube to provide an external support to vein grafts and examined the subsequent changes in hemodynamics, tyrosine kinase signaling, wall remodeling, and vasomotor function.**
Methods: **Carotid interposition bypass grafting was performed with the reversed jugular vein in New Zealand white rabbits. In the experimental group (n = 15), after the completion of the proximal anastomosis, the vein was passed through a 4-mm collagen tube and the distal anastomosis was performed. The tube support was fashioned to completely cover the vein grafts. The control animals (n = 14) had no tube support. After surgery, the blood pressure and flow rate were measured and the wall tension and shear stress were calculated in the vein grafts on day 3 or day 28 (n = 5 per group). Tyrosine phosphorylation was assessed with the Western blot test in vein grafts at day 3 (n = 4 per group). The intimal and medial dimensions of the vein grafts were assessed with videomorphometry on day 28 (n = 5 per group). The cumulative dose response curves of the vein grafts to contractile and relaxant agonists were determined in isometric tension studies on day 28 (n = 5 per group).**
Results: **The use of tube support reduced wall tension 1.7-fold ($P < .01$) and increased shear stress 4.8-fold ($P < .001$) without altering the flow rate or blood pressure. The tyrosine kinase activity was reduced 15-fold ($P < .001$) in the tube-supported vein grafts. The intimal thickness was reduced by 45% in the tube-supported vein grafts as compared with the control grafts (46 ± 2 μm vs 84 ± 5 μm, respectively; $P < .0001$), and the media thickness was reduced by 20% (63 ± 8 μm vs 79 ± 4 μm, respectively; $P < .05$). Isometric tension studies showed preservation of contractile function and modulation of endothelial-dependent dysfunctional relaxation in tube-supported vein grafts.**
Conclusion: **These results show that reduced wall tension and increased shear stress with an external tube support can effectively modulate the signaling, functional, and hyperplastic responses in vein grafts. We conclude that this simple strategy deserves further study and clinical consideration. (J Vasc Surg 1999;29:334-44.)**

From the Vascular Biology and Atherosclerosis Research Laboratory, Departments of Surgery and Biochemistry (Dr Hagen), Duke University Medical Center, and the Department of Pathology (Dr Svendsen), The Gade Institute, University of Bergen, Bergen, Norway.
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Reprint requests: Per-Otto Hagen, PhD, Duke University Medical Center, PO Box 3473, Durham, NC 27710.

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Autologous veins remain the most commonly used conduits for revascularization of occlusive arterial lesions. Vein grafts universally develop wall thickening in response to the new arterial hemodynamic milieu. This process is at first adaptive. However, it can also lead to graft failure, which occurs in up to 50% of the implanted vein grafts at 10 years.[1,2] Wall thickening is a result of the migration and proliferation of smooth muscle cells with deposition of extracellular matrix in the intima and media of vein grafts. Although the hemodynamic elements involved in lesion formation are complex, studies have shown that wall tension and shear stress are the two principal factors that modulate wall remodeling in vein grafts.[3-5]

In vitro models have provided invaluable insights into the molecular mechanisms that link hemodynamic forces to cellular responses.[6-8] In particular, tyrosine kinase mediation in endothelial cells has been shown in the activation of mitogen-activated protein kinases with shear stress[9] and differential endothelial responses to alterations in circumferential deformation and shear stress have been shown.[10] Although much knowledge also has been gained from in vivo models, the link between hemodynamic elements and the induced events remain incompletely defined as a result of the complexities of the in vivo milieu. Schwartz et al[5] demonstrated that increased wall tension is the major stimulus for vein graft wall thickening in the rabbit. Recently, Mehta et al[11] reported an increase in platelet-derived growth factor–BB (PDGF-BB) expression in vein grafts that was reduced with external stenting. Various devices that circumferentially support vein grafts have been used and shown to decrease wall thickening in experimental models.[4,11-14] The hemodynamic, signaling, and functional alterations that occur in vein grafts supported by these external devices are, however, not yet well defined.

In this study, we used a novel, nonexpanding, bioprosthetic collagen tube to provide an external mechanical support to vein grafts. We then examined the changes in hemodynamics, tyrosine kinase signaling, wall remodeling, and vasomotor function.

METHODS

Experimental design. Twenty-nine New Zealand white male rabbits underwent interposition bypass grafting of the right common carotid artery with the reversed ipsilateral jugular vein as previously described.[15] In the experimental group (n = 15), once the proximal anastomosis was performed, the vein was passed through a collagen tube (diameter, 4 mm; length, 35 to 40 mm; Organogenesis, Canton, Mass). The distal anastomosis then was completed. The leaks were repaired, and the collagen tube was fashioned to completely cover the vein graft, including both anastomoses. The control animals (n = 14) were treated identically but without tube support. After surgery, the flow rate and the intraluminal blood pressure were measured on either day 3 or day 28 (n = 5 per group). The vein grafts were harvested on day 3 for an assessment of tyrosine phosphorylation with Western blot test (n = 4 per group) and on day 28 for scanning and transmission electron microscopy, morphometric measurement (n = 5 per group), and isometric tension studies (n = 5 per group).

On the day of harvest, the animals were administered anesthesia and subsequently were killed with an intravenous overdose of barbiturates as previously described.[15] All the procedures performed on the animals in this study were approved by the Duke University Institutional Animal Care and Use Committee. The animal care and handling complied with the "Guide for the Care and Use of Laboratory Animals."[16]

Hemodynamic assessment. The rate of blood flow was measured by applying flow probes (diameter, 3 or 4 mm), which are connected to a flowmeter (Transonic Systems, Inc, Ithaca, NY), to the external surface of the vessels. Flow was measured with the collagen tube in situ in tube-supported vein grafts. The intraluminal blood pressure was measured with a 27-gauge needle, which was connected to a pressure transducer and monitor (Propaq 106, Protocol Systems, Inc, Beaverton, Ore). Flow rates and mean blood pressures were determined in carotid arteries (proximal and distal to the vein graft) and in vein grafts immediately after implantation and at harvest in a pilot study. There were no significant differences in the flow rates or pressure levels in vein grafts immediately after implantation, at harvest, or compared with the proximal or distal segments of carotid arteries. Thus, the measurements at harvest on day 3 or day 28 were used (n = 5 per group). Flow rates (in mL/min) were measured from the mid segments of vein grafts, and the mean arterial blood pressures (in mm Hg) were measured from the proximal segments of the carotid arteries.

Shear stress was calculated as $\tau = 4\eta Q \div \pi r_i^3$ in dyne/cm^2 (τ, shear stress; η, blood viscosity; Q, flow rate; r_i, internal radius). Wall tension was calculated as $T = P \cdot r_i$ in 10^3 dyne/cm[1] (T, wall tension; P, mean arterial blood pressure; r_i, internal radius). The blood viscosity (0.03 in poise) was assumed to be constant. The internal radius was determined with morphometry; we previously have demonstrated that the histologic diameter underestimated the in situ diameter by 10%.[5] For analytical purposes, the internal radius and wall tension were recognized as approximations and the flow of blood was assumed to be laminar. To normalize the wall tension by wall thickness, the wall tensile stress also was calculated (wall tensile stress = pressure × internal radius ÷ wall thickness). *Wall thickness* was defined as the sum of the thickness of the intima, the media, and the collagen tube, respectively.

Protein extraction and Western blot test. On the third day after surgery, the vein grafts (n = 4 per group) were excised, cleared of adventitial tissues, washed in ice-cold phosphate-buffered saline solu-

tion, cut into 1-cm rings, snap-frozen in liquid nitrogen, and stored at –80°C. Proteins were extracted from the frozen samples by grinding the tissues to a fine powder in a mortar and pestle in liquid nitrogen followed by sonication in ice-cold RIPA (Santa Cruz Biotechnology, Santa Cruz, Calif) lysis buffer (1:4 weight:volume; 50 mmol/L Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, 1 mmol/L ethyleneglycol-tetraacetic acid, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, 1 mmol/L sodium fluoride, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 μg/mL pepstatin). Insoluble debris were pelleted in a microcentrifuge at 14,000*g* at 4°C for 10 minutes. The supernatants were collected as cell lysates and stored at –80°C until used. Protein concentration was determined with the Bradford assay (Biorad Laboratories, Richmond, Calif) with 0.2, 0.5, and 1.0 mg/mL of bovine serum albumin (BSA) as the standard.

Equal amounts of protein extracts (15 μg) were mixed in a gel loading buffer (1:4 volume:volume; 20% glycerol, 100 mmol/L Tris-HCl, pH 7.4, 100 mmol/L NaCl, 100 mmol/L dithiothreitol) and boiled for 9 minutes. The samples then were loaded onto an 8% sodiumolodecyl sulfate-polyacrylamide minigel, separated by means of electrophoresis, and transferred onto a nitrocellulose membrane. Nonspecific binding was blocked by incubating the membrane in Tris buffered saline with Tween (TBST) (10 mmol/L Tris-HCl, pH 8.0, 0.05% TWEEN-20 and 150 mmol/L NaCl), which contained 1% BSA, overnight at 4°C. A monoclonal mouse antiphosphotyrosine antibody (PY20; 1 μg/mL; Chemicon International Inc, Temecula, Calif) then was applied to the blot for 1 hour at room temperature. Antibody binding was detected by incubating the blot with a horseradish peroxidase conjugated goat antimouse immunoglobin G (1:5000 dilution; Santa Cruz Biotechnology, Santa Cruz, Calif). The blot was washed several times with TBST between the blocking steps. Finally, the blot was visualized with an enhanced chemiluminescence kit (Amersham, Arlington Heights, Ill) and autoradiographed. The autoradiographs were scanned and analyzed (Adobe Photoshop 3.0, Adobe Systems, Inc, Mountain View, Calif), and the integrated density of the visualized bands was measured (NIH Image 1.61). The chemicals were obtained from Sigma Chemical Company (St Louis, Mo) unless otherwise stated.

Morphologic assessment. On the 28th day after surgery, the vein grafts (n = 5 per group) were cleared of blood with an initial infusion of Hanks Balanced Salt Solution (Gibco Laboratories, Life Technologies, Inc, Grand Island, NY) and then perfusion-fixed in situ with 2% glutaraldehyde in a 0.1 mol/L cacodylate buffer (pH 7.2), which was supplemented with 0.1 mol/L sucrose to give an osmolality of approximately 300 mOsm at a pressure of 80 mm Hg. After immersion in the fixative for 48 hours, the cross-sections (3 per graft) from the middle segments of the vein grafts were further processed either for scanning and transmission electron microscopy or for morphometric assessment as previously described.[15] Briefly, morphometric assessment was performed on the sections that were stained with a modified Masson stain and Verhoeff's stain. The intima and the media were delineated by identification of the demarcation between the criss-cross orientation of the hyperplastic smooth muscle cells in the intima and the circular smooth muscle cells of the media. The outer limit of the media was defined by the interface between the circular smooth muscle cells of the media and the connective tissue of the adventitia. The luminal, intimal, and medial areas of the entire cross-section of each vein graft (3 sections per graft) were measured by means of videomorphometry (Innovision 150, American Innovision Inc, San Diego, Calif). The internal radius and the thickness of the intima and media of vein grafts were derived from each of the measured luminal, intimal, and medial areas as previously described.[17] The intimal ratio (intimal ratio = intimal area ÷ [intimal + medial areas]) also was calculated for each section. The value presented for each parameter was the mean of all the cross-sections ± the standard of the mean for the group (n = 5 grafts per group).

After further specimen processing, a scanning electron microscopic examination (Philips 500 scanning electron microscope, N. V. Philips, Eindhoven, The Netherlands) and a transmission electron microscopic examination (Philips 300 transmission electron microscope, N.V. Philips) were performed on the representative mid sections as previously described.[15]

Isometric tension studies. The vein grafts were sectioned into four 5-mm rings for in vitro isometric tension studies on the 28th day after surgery. The vasomotor function of the tube-supported vein grafts is unknown at present. In the tube-supported group, the collagen tubes were carefully dissected and removed to allow unimpeded vessel contraction and relaxation. Each ring was immediately mounted between two stainless steel hooks in 5-mL organ baths that contained oxygenated Krebs solution (122 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 2.5 mmol/L $CaCl_2$, 15.4 mmol/L $NaHCO_3$,

1.2 mmol/L KH_2PO_4, and 5.5 mmol/L glucose, maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2) as previously described with some modifications.[17] In brief, after equilibration, the resting tension was adjusted in increments from 0.5 to 1.25 g and the maximal response to a modified oxygenated Krebs solution (60 mmol/L KCl, 66.7 mmol/L NaCl, 1.2 mmol/L $MgCl_2$, 2.5 mmol/L $CaCl_2$, 15.4 mmol/L $NaHCO_3$, 1.2 mmol/L KH_2PO_4, and 5.5 mmol/L glucose) was measured to establish a length-tension relationship. Cumulative dose response curves to the contractile agonists norepinephrine (10^{-9} to 10^{-4} mol/L), serotonin (10^{-9} to 10^{-4} mol/L), and bradykinin (10^{-9} to 10^{-5} mol/L) were constructed. Relaxation responses to acetylcholine (10^{-8} to 10^{-4} mol/L), an endothelium-dependent agonist, and nitroprusside (10^{-8} to 10^{-4} mol/L), an endothelium-independent agonist, were assessed on rings precontracted with norepinephrine, at the concentration that produced 80% to 100% of maximal contraction. All the rings were allowed to re-equilibrate for a minimum of 60 minutes between each experimental run, and the same sequence of agonist testing was maintained for all the experiments. All the chemicals were obtained from Sigma Chemical Company.

The *median effective concentration value* for an agonist, defined as the concentration that produces the half maximal response for that agonist, was calculated with logistic analysis for each ring. The *sensitivity* of a vessel ring to an agonist was defined as $-\log_{10}$ (median effective concentration value). The *standardized contractile ratio* of each ring to an agonist was defined as the maximal actual contractile response to the agonist ÷ the contractile response to 60 mmol/L KCl.

Statistical analysis. The values presented were the mean ± the standard error of the mean for the group. The unpaired Student *t* test was applied to compare the differences in the hemodynamic assessment, the Western blot test, and the functional data between the two groups. For the morphometric analysis, the differences were compared with the Mann-Whitney test. A *P* value of less than .05 was regarded as significant.

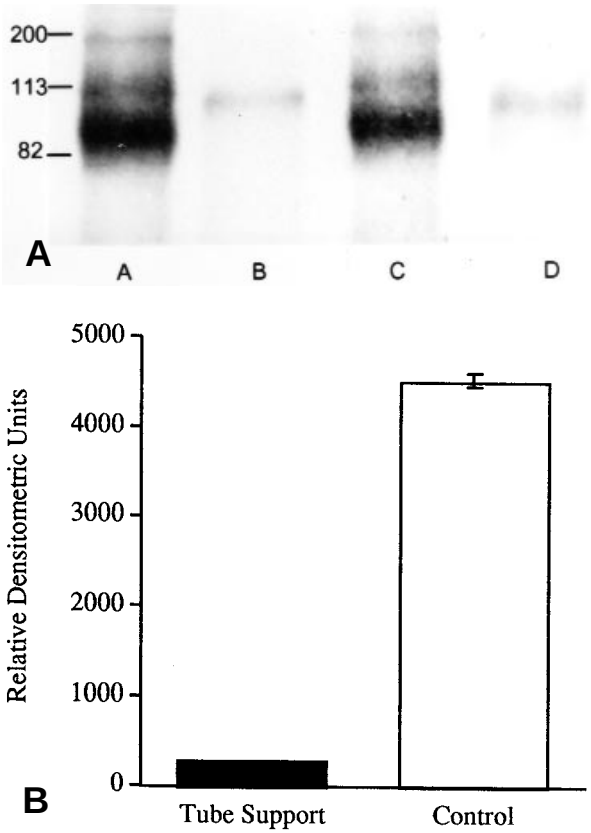

Fig 1. A, Representative Western blot test of phosphorylated tyrosine residues in control vein grafts *(A and C)* and in tube-supported vein grafts *(B and D)*. Molecular weight markers (in kDa) are shown at *left*. Three days after bypass graft surgery, phosphorylated tyrosine residues were detected with PY-20 antibody (1 μg/mL; Chemicon International, Inc, Temecula, Calif) in control vein grafts in proteins at just above 82, 113, and 200 markers. Only proteins with approximately 113 kDa molecular weight were tyrosine phosphorylated in tube-supported vein grafts. Samples were run in duplicate, and similar results were found in other Western blot tests. **B,** Densitometric analysis of protein bands (integrated density in relative units) showed 15-fold reduction in tyrosine kinase activity with tube support as compared with grafts without tube support (control) by means of unpaired Student *t* test ($P < .001$). Values are mean ± standard error of mean (n = 4 per group).

RESULTS

General results. One intraoperative death resulted from an unrecognized leak in the mid segment of a tube-supported vein graft. This occurred in the early stage of the project. All the subsequent leaks were recognized and repaired successfully. Otherwise, there were no other significant complications, including infection and bleeding, in either group. All the animals survived until the end-points, and all the vein grafts were patent at harvest.

Hemodynamics. The flow rates and pressures were not significantly altered in the vein grafts with tube support as compared with the control grafts (Table I). With the application of the equations formulated in the Methods section, the calculated wall tension was decreased by 1.7-fold, and the shear

Table I. Hemodynamic parameters

Parameter	*Tube-supported grafts*	*Control grafts*	*P value*
Flow ($mL \cdot min^{-1}$)	12.8 ± 1.1	11.5 ± 1.0	.41
Pressure (mm Hg)	53.2 ± 3.8	57.8 ± 1.4	.29
Wall tension ($\times 10^3$ $dyne \cdot cm^{-1}$)	11.7 ± 0.9	19.8 ± 0.5	<.01
Shear stress ($dyne \cdot cm^{-2}$)	1.9 ± 0.25	0.4 ± 0.04	<.001

Wall tension was calculated as $T = P \cdot r_i$. Shear stress was calculated as $\tau = 4\eta Q \div \pi r_i^3$. T, Wall tension; *P,* blood pressure; r_i, internal radius; τ, shear stress; η, blood viscosity (0.03 in poise); Q, blood flow.
Values are mean ± standard error of the mean (n = 5 per group).
Statistical differences between tube-supported vein grafts and control vein grafts were compared with unpaired Student *t* test.

Table II. Dimensional analysis of vein grafts at day 28

Parameter	*Tube-supported grafts*	*Control grafts*	*P value*
Luminal area (mm^2)	8.6 ± 0.6	23.2 ± 1.6	<.001
Intimal area (mm^2)	0.48 ± 0.02	1.42 ± 0.08	<.001
Medial area (mm^2)	0.70 ± 0.11	1.36 ± 0.07	<.001
Intimal ratio	0.46 ± 0.06	0.51 ± 0.01	<.01
Luminal index	34.1 ± 3.6	34.9 ± 2.2	.33

Intimal ratio, Intimal area/(intimal + medial areas); *luminal index,* luminal diameter/(intimal + medial thickness).
Values are mean ± standard error of the mean (n = 5 per group).
Statistical differences between tube-supported vein grafts and control vein grafts were compared with unpaired Mann-Whitney test.

stress was increased by 4.8-fold in the tube-supported vein grafts as compared with the control grafts (Table I). The decrease in wall tension was expected because the pressure was not different, but the internal radius was reduced by 1.7-fold in the tube-supported vein grafts as compared with the control grafts (1.63 ± 0.06 mm vs 2.69 ± 0.09 mm, respectively; $P < .0001$). Similarly, the increase in shear stress was anticipated because the flow was not significantly changed and the shear stress is inversely proportional to the third power of the internal radius. The wall tensile stress was reduced 2.4-fold in the tube-supported vein grafts as compared with the control vein grafts (53.7 vs 131×10^6 $dyne \cdot cm^{-2}$, respectively; $P < .0001$).

Tyrosine phosphorylation. We recently showed that tyrosine phosphorylation is increased in vein grafts (control) as compared with ungrafted jugular veins and that this increase was inhibited by tyrphostin AG-51, a tyrosine kinase inhibitor.[18] In this study, the Western blot test showed a 15-fold reduction ($P < .001$) in phosphorylated tyrosine residues in the wall extracts of tube-supported vein grafts at day 3 when compared with control grafts (Fig 1). Phosphorylated tyrosine residues were detected in approximately 113 kDa proteins in tube-supported vein grafts (Fig 1*A*). In control vein grafts (Fig 1*A*), however, in addition to the greater amount of phosphorylated tyrosine residues in approximately 113 kDa proteins, phosphorylated tyrosine residues were also present in proteins with molecular weights just above 82 kDa and of 200 kDa.

Morphology. Externally supporting the vein grafts with the bioprosthetic collagen tube reduced the internal (luminal) diameter of vein grafts at day 28 by 39% as compared with the control vein grafts (3.27 ± 0.13 mm vs 5.39 ± 0.18 mm; $P < .0001$). The thickness of the intima was decreased by 45% (46 ± 2 μm vs 84 ± 5 μm; $P < .0001$), and the media by 20% (63 ± 8 μm vs 79 ± 4 μm; $P < .05$) in the tube-supported vein grafts as compared with the control grafts, respectively (Fig 2*A*). Both the intimal areas and the medial areas were also reduced, by 66% and 49%, respectively (Table II). As a result of the greater reduction in intimal dimension relative to the reduction in the media, the intimal ratio was decreased by 10% (Table II). However, the luminal index was maintained constant with or without tube support, although the luminal area was reduced by 63% (Table II; Fig 2*B*).

Scanning electron microscopy results showed a confluent endothelial lining with distinct cell borders in both the tube-supported vein grafts (Fig 3*A*) and the control vein grafts (Fig 3*B*). The endothelial cells were unaltered and flattened in the tube-supported vein grafts as compared with the more cuboidal and bulging endothelial cells in the control vein grafts. The vein grafts with tube support had less subendothelial edema and less debris (Fig 4*A*) than did the control

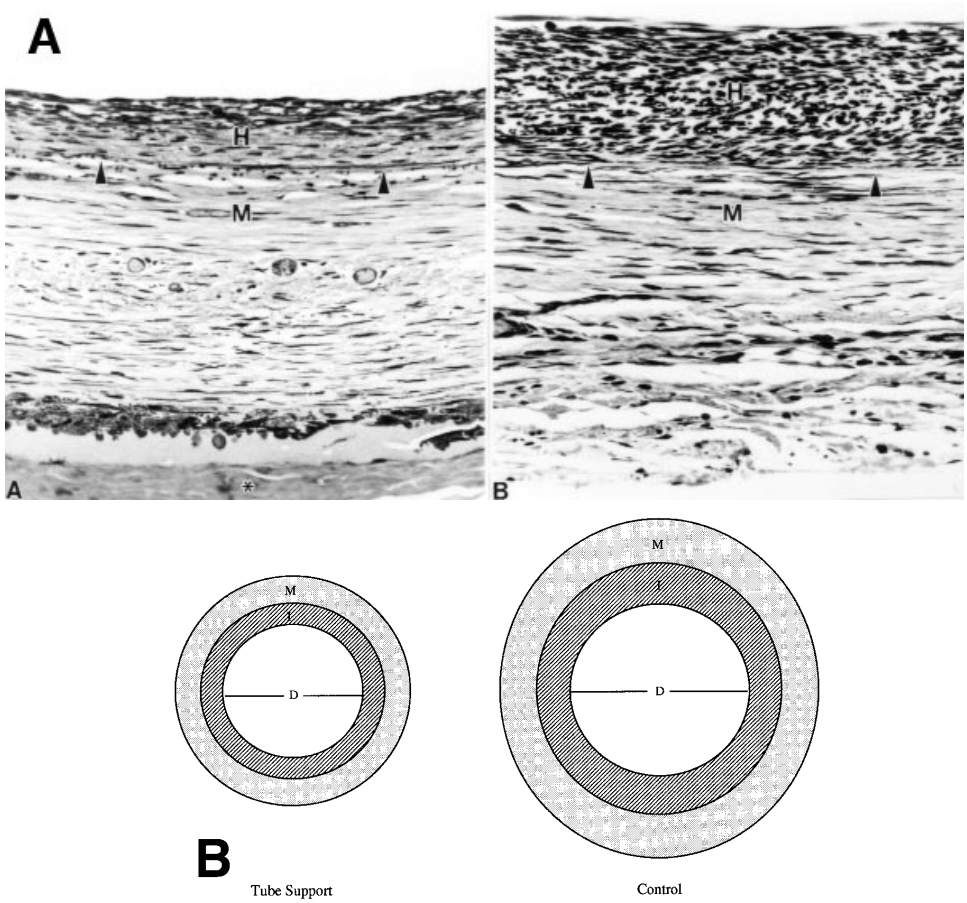

Fig 2. A, Representative composite photomicrograph of cross-sections from tube-supported vein graft *(A)* and control vein graft *(B)*. At 28 days, intimal thickness was decreased by 45% in tube-supported vein grafts as compared with controls (46 ± 2 μm vs 84 ± 5 μm, respectively; $P < .0001$). Thickness of the media also was decreased, although to a lesser extent (by 20%) with tube support as compared with no tube support (63 ± 8 μm vs 79 ± 4 μm, respectively; $P < .05$). *Top* is the lumen. *Arrowheads* delineate demarcation between intima and media. Original magnification, ×250. *H,* Intimal hyperplasia; *M,* media; *, collagen tube. **B,** Schematic diagram shows thickness of media *(M)* and intima *(I)* in relation to luminal diameter *(D)* in tube-supported vein graft as compared with control vein graft. Luminal index = luminal diameter ÷ (intimal + medial thicknesses). Note smaller diameter and lesser intimal and medial thicknesses in tube-supported vein graft as compared with control vein graft (not to scale).

Table III. Vasomotor responses of vein grafts at day 28

Parameter	*Tube-supported grafts*	*Control grafts*	*P value*
Norepinephrine	5.96 ± 0.07	5.97 ± 0.06	.91
Serotonin	6.39 ± 0.11	6.28 ± 0.07	.22
Bradykinin	6.32 ± 0.08	5.60 ± 0.09	<.001
Acetylcholine	3.92 ± 0.22	No response	
Nitroprusside	6.46 ± 0.12	6.73 ± 0.19	.25

The concentration for the half maximal response (EC_{50}) was calculated with logistic analysis, and the sensitivity was defined as $-\log_{10}(EC_{50})$. In each vein graft, the sensitivity was determined for each vessel ring (four rings per vein graft), and the mean was taken as the value for that vein graft.
Values shown are mean ± standard error of the mean for the group (n = 5 per group).
Statistical differences between tube-supported vein grafts and control vein grafts were compared with the unpaired Student *t* test.

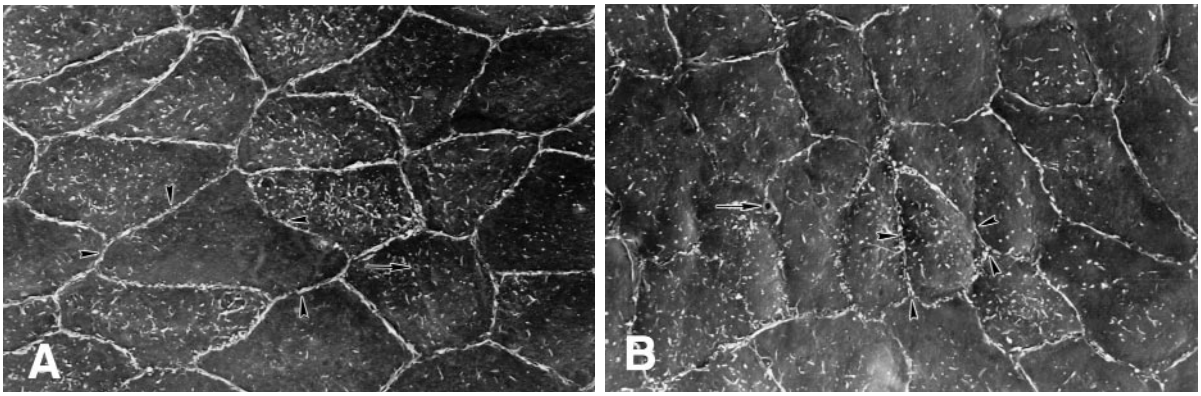

Fig 3. Scanning electron micrograph of representative tube-supported vein graft at day 28 **(A)** and control vein graft **(B)**. Endothelial lining is confluent and cell borders are well defined *(arrowheads)* in both vein grafts. Note unaltered and flattened shape of endothelial cells in tube-supported vein graft. In contrast, endothelial cells are cuboidal and slightly bulging in control vein graft. Arrows point to stomata. Original magnification, ×1250.

grafts (Fig 4*B*), as seen with transmission electron microscopy. In addition, the intimal smooth muscle cells were orderly and circular in orientation and elongated in shape with tube support (Fig 4*A*), whereas the intimal smooth muscle cells in the control vein grafts were disorganized and round-like (Fig 4*B*).

Vasomotor function. Tube-supported vein grafts showed similar responses to KCl as compared with controls (force, 300 ± 46 mg vs 280 ± 47 mg, respectively; P = ns). The sensitivities of the tube-supported vein grafts in response to norepinehrine and serotonin were not significantly different than that of the control grafts (Table III). The tube-supported vein grafts were, however, more sensitive to bradykinin than were the control grafts (Table III). The maximal contractile forces generated in response to all three agonists (norepinephrine, serotonin, and bradykinin), expressed as standardized contractile ratios, were not significantly altered with the external tube support of the vein grafts (Fig 5).

As previously reported,[17] the control vein grafts did not relax in response to acetylcholine (Fig 6*A*). In contrast, 10 of the 20 rings from tube-supported vein grafts showed dose-dependent relaxation in response to acetylcholine, with a maximal relaxation to 64% of precontracted tension (Fig 6*A*), albeit with a low sensitivity (Table III). Of the five tube-supported vein grafts studied, only one had no response to acetylcholine in all rings. In response to nitroprusside, the sensitivity (Table III) and maximal relaxation (Fig 6*B*) were similar in the vein grafts with or without tube support.

DISCUSSION

Vein grafts implanted in the arterial circulation predictably develop wall thickening, with smooth muscle cell hyperplasia and deposition of extracelullar matrix in the intima and the media, an adaptive process that has been referred to as "arterialization." In 50% of the implanted vein grafts, however, this process becomes pathologic, usually as a result of intimal hyperplastic lesions that cause either focal stenosis or promote accelerated atherosclerosis.[1] Until a significant improvement in the long-term outcome of vein grafts is achieved, the search for better wall remodeling will continue. This study shows that by reducing wall tension and increasing shear stress with an external tube support, the tyrosine kinase signaling and subsequent wall remodeling responses are effectively modulated in experimental vein grafts.

Hemodynamic forces are known to play an important role in the regulation of the cells that compose the blood vessel wall. In particular, the effects of shear stress on endothelial cells have been studied extensively in vitro.[6,8] Several shear stress–inducible endothelial genes have been identified in vitro, including PDGF-AA, PDGF-BB, basic fibroblast growth factor, and nitric oxide synthase, all of which have been implicated in wound remodeling.[8] The transformation of biomechanical (hemodynamic) stimuli into biologic responses usually begins with the activation of protein kinases and protein-to-protein interactions that lead to gene transcription (or inhibition thereof). Takahashi and

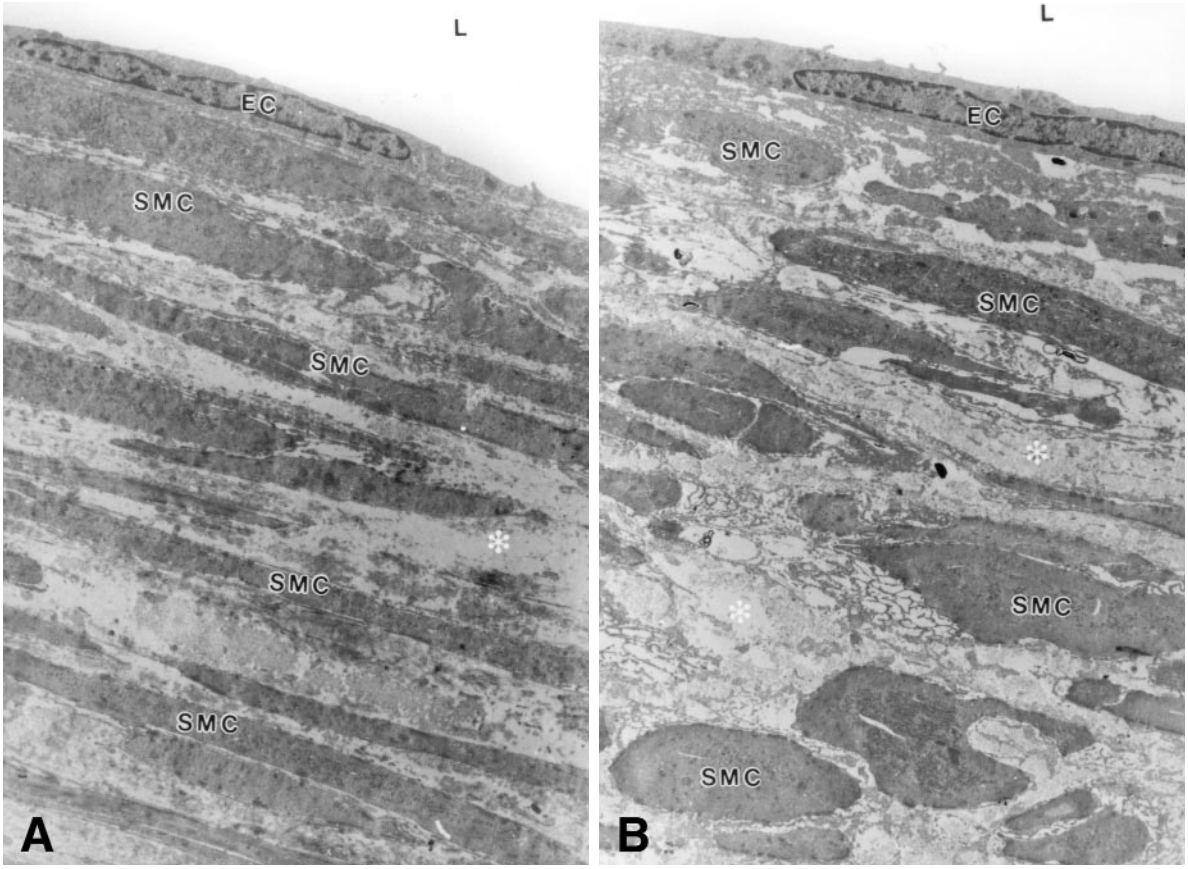

Fig 4. Transmission electron micrograph of representative cross-section of tube-supported vein graft at day 28 **(A)** and control vein graft **(B)**. Endothelial cells *(EC)* are well defined in both vein grafts. Intimal smooth muscle cells *(SMC)* are elongated and organized in several layers in tube-supported vein graft as compared with more rounded-like and disorganized smooth muscle cells in control vein graft. Also, there is more subendothelial edema *(*)* in control graft than in tube-supported vein graft. Original magnification, ×4500. *L,* Lumen.

Berk[9] have demonstrated that shear stress can activate extracellular signal-regulated kinase via a tyrosine kinase-dependent pathway in cultured human umbilical vein endothelial cells. The hemodynamic factors in vivo are complex. However, the relative importance of each factor has been identified in animal models.[3,19]

Our results showed that protein tyrosine kinase activity is markedly reduced in vein grafts with reduced wall tension and increased shear stress, both of which are the consequences of the tube support. The identity of the tyrosine phosphorylated proteins (of approximately 82, 113, and 200 kDa) remains to be further defined. However, we postulate that the decreased tyrosine kinase activity in the tube-supported vein grafts may, in part, be associated with the reduced expression or activation of the receptors for growth factors, such as PDGF, fibroblast growth factor, and epidermal growth factor. The receptors for these growth factors have intrinsic protein tyrosine kinases, which range from 110 to 190 kDa in molecular weight.[20-22] Kraiss et al[23] have shown that an abrupt reduction in both blood flow and shear stress is associated with increased PDGF-AA messenger RNA and protein expression in baboon prosthetic grafts. In parallel, Mehta et al[11] have recently demontrated a significant decrease in PDGF-BB protein with external stenting of vein grafts in the pig model. Although wall tension and shear stress were not assessed in the study of Mehta et al,[11] their external stent model likely produced hemodynamic effects that were similar to the results of our tube support model—that is, reduced wall tension and increased shear stress.

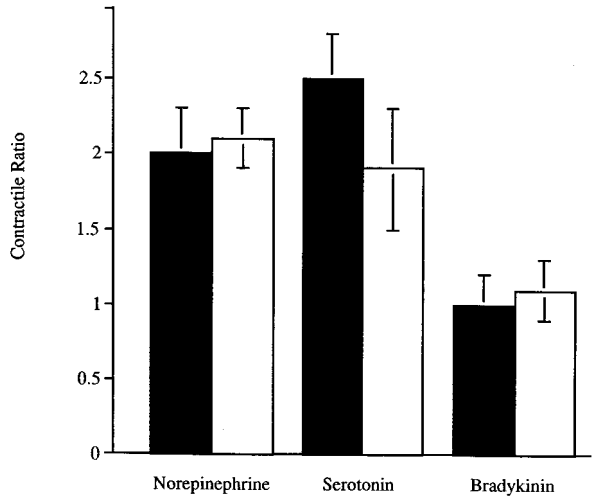

Fig 5. Maximal contractile responses of tube-supported *(solid bar)* and control *(open bar)* vein grafts to norepinephrine, serotonin, and bradykinin. Both groups of vein grafts generated similar contractile forces (tension) in response to all three agonists. Contractile ratio = actual maximal contraction to agonist (in mg) ÷ maximal contraction to 60 mmol/L KCl (in mg). Values are mean ± standard error of mean (n = 5 per group). Unpaired Student *t* test was used to compare results of two groups.

A multitude of hemodynamic factors are known to influence wall thickening in vein grafts. Schwartz et al[5] have shown that "myointimal" (which refers to both intima and media) thickening correlates most strongly with wall tension in rabbit vein grafts. On the other hand, Dobrin[3] has demonstrated that intimal thickening correlates best with low flow velocity (a determinant of shear stress) and that medial thickening is a better correlate of deformation in the circumferential direction (a determinant of wall tension). In this study, we found a greater reduction in intimal thickening than in medial thickening, which could be correlated with the larger increase in shear stress and the smaller decrease in wall tension, respectively, which supports the results from Dobrin.[3]

The tube support used in our model differs from most of the stents used in other models of vein grafts[4,11,12,24] in that it is composed of collagen, which was processed from porcine intestinal tracts. Because of its biologic characteristics, the bioprosthetic collagen tube would be incorporated into the host's tissues, remodeled, and eventually reabsorbed. Degradable biomaterials would be void of the unwanted long-term risks that are associated with prosthetic grafts. Zweep et al[13] have used a biodegradable prosthesis to support rabbit vein grafts and have shown less disruption of the

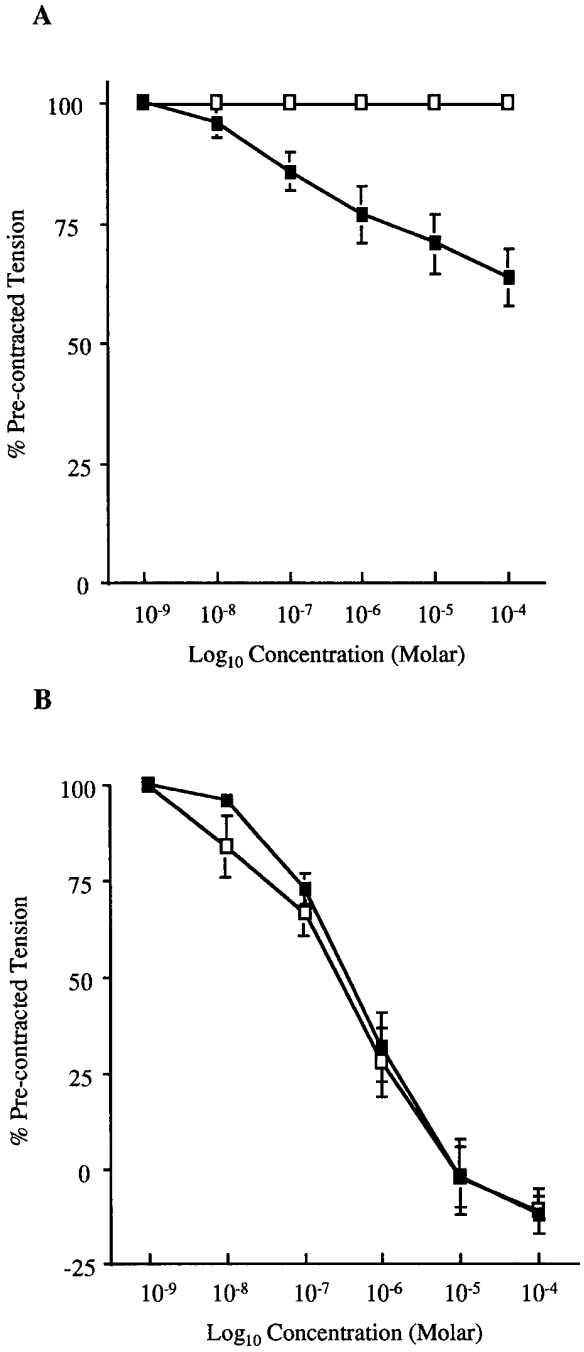

Fig 6. Dose-response curves for tube-supported *(solid bar)* and control *(open bar)* vein grafts to acetylcholine **(A)** and nitroprusside **(B)**. Control vein grafts had no response to acetylcholine (10^{-9} to 10^{-4} mol/L). Tube-supported vein grafts, however, showed dose-dependent relaxation to acetylcholine with maximal relaxation of 64% of precontracted tension. Both tube-supported and control vein grafts responded to nitroprusside in dose-dependent manner with maximal relaxation of greater than 100% of precontracted tension. Values are mean ± standard error of mean (n = 5 per group).

media, less edema, and less inflammation in the tube-supported vein grafts. On the other hand, Okadome et al[25] have reported similar ultrastructural differences in smooth muscle cell morphology and also less endothelial ultrastructural alterations in a dog model of vein grafts that were exposed to low versus high shear. Our findings of unaltered endothelial configuration, less wall edema, and a better organization of smooth muscle cells with tube support of vein grafts would be consistent with these prior studies.[13,25]

In this study, we report, for the first time, the complete preservation of smooth muscle cell function and the recovery of endothelial-dependent relaxation with tube support of vein grafts. Despite the significant reduction in wall thickness, the tube-supported vein grafts generated similar contractile forces in response to KCl and all three of the contractile agonists that were tested (norepinephrine, serotonin, and bradykinin). Moreover, the increased sensitivity to bradykinin in the tube-supported vein grafts is consistent with a similar increase that was observed in vein grafts treated with tyrphostin, a tyrosine kinase inhibitor.[18] Prior work from our laboratory has shown that the rabbit jugular veins, which were ungrafted, have a higher sensitivity to bradykinin and a lower basal protein tyrosine kinase activity as compared with the vein grafts, which were untreated[17,18]; furthermore, after 2 weeks of exposure to the arterial circulation, the vein grafts can regain the higher sensitivity level to bradykinin when reimplanted into the venous system.[17] Taken together, these findings could implicate protein tyrosine kinases in the regulation of bradykinin receptors.

That relaxation to nitroprusside, an endothelium-independent agonist, is preserved in tube-supported vein grafts is of no surprise—previous studies have described this response in control vein grafts, which were untreated. However, the recovery of endothelium-dependent relaxation to acetylcholine in 50% of the vessel rings with tube support would indicate that endothelial function was in effect modulated. Increased shear stress has been shown to stimulate increased production of nitric oxide in vitro,[6] which may explain in part the relaxation to acetylcholine in the tube-supported vein grafts. Systemic supplementation with L-arginine, the nitric oxide precursor, has also been shown to preserve endothelial-dependent relaxation of vein grafts to acetylcholine.[26] Improved endothelial function also has been reported by Onohara et al[27] with increased prostacyclin production in vein grafts exposed to high shear stress. Alternatively, the preserved endothelial function in vein grafts may be attributed to the lesser stretch injury with tube support. All in all, endothelial cells are known to have a regulatory role in smooth muscle cell proliferation and migration, in addition to its role in mechanotransduction and vasomotor responses.[1,8,20] We therefore postulate that improved endothelial function with tube support may reduce the release of mitogenic and chemoattractant signals, such as PDGF.

Although we have shown that the tube support of vein grafts produces potentially beneficial wall remodeling and vasomotor responses at 28 days, the interpretation of these results must be cautioned. First, Fillinger et al[28] have described some of the differences between animal and human vein grafts, and the interspecies comparison should be done with circumspection. Second, the reduction in the luminal diameter of the tube-supported vein grafts may be undesirable if it were flow-restrictive. We chose 4 mm as the diameter of the collagen tubes because this approximated the average diameter of the control vein grafts. Nevertheless, the measured flow was unimpeded with our tube support model, and the luminal index, a measure of wall remodeling, was similar with or without the tube support. Other studies have suggested that vein grafts adapt to the arterial circulation reaching an equilibrium, at which point the ratio of the radius (or luminal diameter) to wall thickness is maintained.[28,29] Thus, unless this equilibrium is disturbed, the luminal index should remain unchanged with or without tube support. Last, the long-term in situ fate of the bioprosthetic collagen tubes ought to be determined in future studies.

In summary, the results of our study show that supporting vein grafts with an external bioprosthetic tube optimizes the hemodynamic perturbations and effectively modulates the subsequent signaling, functional, and hyperplastic responses. We conclude that this simple strategy deserves further experimentation and clinical consideration.

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REFERENCES

1. Bourassa MG. Long-term vein graft patency. Curr Opin Cardiol 1994;9:685-91.
2. Moore WS, Rutherford RB. Infra-inguinal vascular graft failure. Semin Vasc Surg 1991;3:1-76.
3. Dobrin PB. Mechanical factors associated with the develop-

ment of intimal and medial thickening in vein grafts subjected to arterial pressure. Hypertension 1995;26:38-43.
4. Kohler TR, Kirkman TR, Clowes AW. The effect of rigid external support on vein graft adaptation to the arterial circulation. J Vasc Surg 1989;9:277-85.
5. Schwartz LB, O'Donohoe MK, Purut CM, Mikat EM, Hagen P-O, McCann RL. Myointimal thickening in experimental vein grafts is dependent on wall tension. J Vasc Surg 1992;15:176-86.
6. Takahashi M, Ishida T, Traub O, Corson MA, Berk BC. Mechanotransduction in endothelial cells: temporal signaling events in response to shear stress. J Vasc Res 1997;34:212-9.
7. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. N Engl J Med 1994;330:1431-8.
8. Resnick N, Gimbrone MA. Hemodynamic forces are complex regulators of endothelial gene expression. FASEB J 1995;9:874-82.
9. Takahashi M, Berk BC. Mitogen-activated protein kinase (ERK1/2) activation by shear stress and adhesion in endothelial cells. J Clin Invest 1996;98:2623-31.
10. Golledge JG, Turner RJ, Harley SL, Springall DR, Powell JT. Circumferential deformation and shear stress induce differential responses in saphenous vein endothelium exposed to arterial flow. J Clin Invest 1997;99:2719-26.
11. Mehta D, George SJ, Jeremy JY, Izzat MB, Southgate KM, Bryan AJ, et al. External stenting reduces long-term medial and neointimal thickening and platelet derived growth factor expression in a pig model of arteriovenous bypass grafting. Nat Med 1998;4:235-9.
12. Barra JA, Volant A, Leroy JP, Braesco J, Airiau J, Boschat J, et al. Constrictive perivenous mesh prosthesis for preservation of vein integrity. J Thorac Cardiovasc Surg 1986;92:330-6.
13. Zweep H-P, Satoh S, van der Lei B, Hinrichs WLJ, Dijk F, Feijen J, et al. Autologous vein supported with a biodegradable prosthesis for arterial grafting. Ann Thorac Surg 1993;55:427-33.
14. Angelini GD, Izzat MB, Bryan AJ, Newby AC. External stenting reduces early medial and neointimal thickening in a pig model of arteriovenous bypass grafting. J Thorac Cardiovasc Surg 1996;112:79-84.
15. Davies MG, Klyachkin ML, Dalen H, Massey M, Svendsen E, Hagen P-O. The integrity of experimental vein graft endothelium: implications on the etiology of early graft failure. Eur J Vasc Surg 1993;7:156-65.
16. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. Washington: National Academy Press, 1996.
17. Davies MG, Klyachkin ML, Dalen H, Svendsen E, Hagen P-O. Regression of intimal hyperplasia with restoration of EDRF-mediated relaxation in experimental vein grafts. Surgery 1993;114:258-71.
18. Huynh TTT, Davies MG, Barber L, Svendsen E, Hagen P-O. Local inhibition of tyrosine kinase activity markedly attenuates the development of intimal hyperplasia in experimental vein grafts. J Surg Res 1998;77:104-11.
19. Dobrin PB, Littooy FN, Endean ED. Mechanical factors predisposing to intimal hyperplasia and medial thickening in autogenous vein grafts. Surgery 1989;105:393-400.
20. Newby AC, George SJ. Proposed roles for growth factors in mediating smooth muscle proliferation in vascular pathologies. Cardiovasc Res 1993;27:1173-83.
21. Panek RL, Dahring TK, Olszewski BJ, Keiser JA. PDGF receptor protein tyrosine kinase expression in the balloon-injured rat carotid artery. Arterioslcer Thromb Vasc Biol 1997;17:1283-8.
22. Daub H, Weiss FU, Wallasch C, Ullrich A. Role of transcription of the EGF receptor signalling by G-protein-coupled receptors. Nature 1996;379:557-60.
23. Kraiss LW, Geary RL, Mattson EJR, Vergel S, Au YPT, Clowes AW. Acute reductions in blood flow and shear stress induce platelet-derived growth factor-A expression in baboon prosthetic grafts. Circ Res 1996;79:45-53.
24. Batellier J, Wassef M, Merval R, Duriez M, Tedgui A. Protection from atherosclerosis in vein grafts by a rigid external support. Arterioscler Thromb 1993;13:379-84.
25. Okadome K, Yukizane T, Shinsuke M, Sugimachi K. Ultrastructural evidence of the effects of shear stress variation on intimal thickening in dogs with arterially transplanted autologous vein grafts. J Cardiovasc Surg 1990;31:719-26.
26. Davies MG, Kim JH, Makhoul RG, Dalen H, Svendsen E, Hagen P-O. Reduction of intimal hyperplasia and preservation of Nitric Oxide (NO)-mediated relaxation by the NO precursor: L- arginine. Surgery 1994;116:557-68.
27. Onahara T, Okadome K, Ishii T, Yamamura S, Komori K, Sugimachi K. The reversibility of impaired prostacyclin production of the vein graft. J Surg Res 1993;55:344-50.
28. Fillinger MF, Cronenwett JL, Besso S, Walsh DB, Zwolak RM. Vein adaptation to the hemodynamic environment of infrainguinal grafts. J Vasc Surg 1994;19:970-9.
29. Zwolak RM, Adams MC, Clowes AW. Kinetics of vein graft hyperplasia: association with tangential stress. J Vasc Surg 1987;5:126-36.

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