Original Article

Promoting Vasa Vasorum Neovascularization of Vein Grafts Extenuates Hypoxia of the Wall and Its Subsequent Influence on Intimal Hyperplasia

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Abstract

Background: The autologous saphenous vein is the most common conduit for coronary artery bypass grafting, but the vein graft disease will occur. This study used Matrigel basement membrane matrix with many different growth factors to promote vasa vasorum neovascularization and extenuate the hypoxia to improve remodeling.

Methods: This study observed the hypoxia and thickness of the vein grafts at different times. Normal veins and vein grafts with 15 min of ischemia one day postoperatively were harvested in the neck of rabbits. Paired vein grafts with 15 min ischemia bilaterally (control vs. Matrigel basement membrane matrix) were performed and harvested at 2, 6, and 12 weeks postoperatively. The rabbits were randomly divided into four postoperative groups (six rabbits in each group): Group 1, one day postoperatively; Group 2, 2 weeks postoperatively; Group 3, 6 weeks postoperatively; and Group 4, 12 weeks postoperatively. The dimensions of vessel wall were captured, and the mean thicknesses of intima, media, and adventitia were measured. The hypoxia-inducible factor (HIF)-1α and HIF-2α labeling indices of intima, media, and adventitia were also measured.

Results: In Group 1, the labeling index of HIF-1α was high in the normal vein and decreased significantly in the vein graft one day postoperatively (intima: 80 ± 3% vs. 12 ± 1%, $P = 0.01$; media: 67 ± 5% vs. 11 ± 1%, $P = 0.01$; adventitia: 40 ± 10% vs. 7 ± 2%, $P = 0.03$). The labeling index of HIF-2α had similar trend as HIF-1α (intima: 80 ± 10% vs. 10 ± 5%, $P = 0.02$; media: 60 ± 14% vs. 12 ± 2%, $P = 0.01$; adventitia: 45 ± 20% vs. 10 ± 4%, $P = 0.03$). Compared with the control vein grafts, vein grafts with Matrigel basement membrane matrix had lower labeling indices of HIF-1α and HIF-2α in media and adventitia at Group 2 (HIF-1α: 34 ± 5% vs. 20 ± 4%, $P = 0.04$ for media; 23 ± 3% vs. 11 ± 2%, $P = 0.03$ for adventitia; HIF-2α: 37 ± 6% vs. 21 ± 4%, $P = 0.03$ for media; 24 ± 4% vs. 13 ± 2%, $P = 0.04$ for adventitia) and Group 3 (HIF-1α: 33 ± 4% vs. 7 ± 2%, $P = 0.04$ for media; 13 ± 3% vs. 3 ± 1%, $P = 0.02$ for adventitia; HIF-2α: 27 ± 4% vs. 12 ± 3%, $P = 0.02$ for media; 19 ± 2% vs. 6 ± 1%, $P = 0.02$ for adventitia). There were no differences in mean thickness of intima, media, and adventitia between bilateral vein grafts at 2, 6, and 12 weeks postoperatively.

Conclusions: This study indicated that promoting vasa vasorum neovascularization of vein grafts extenuated hypoxia, but did not influence the intimal hyperplasia of the wall.

Key words: Hypoxia; Hypoxia-inducible Factor; Intimal Hyperplasia; Vein Graft

Introduction

The autologous saphenous vein is the most common conduit for coronary artery bypass grafting, despite the increased use of arterial grafts in cardiac surgery. After grafting, this vein is subjected to immediate increase in flow, resulting in longitudinal wall shear stress, circumferential deformation, and pulsatile stress. This can cause intimal hyperplasia and then graft atheroma, resulting in stenosis and occlusion. Approximately 60% of vein grafts remained patent 10 years after surgery, of which only 50% were free of significant stenosis.[1]

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After grafting, hypoxia of the vein graft's wall upregulated the expression of hypoxia-inducible factor (HIF),[2]

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vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP), which might worsen the vein graft disease.[3,4] George *et al*.[5] found that vein grafts with macroporous external stents had thinner intima than those with microporous external stents, which suggested that vasa vasorum neovascularization of vein grafts might play an important role in the remodeling of vein grafts. This study used Matrigel basement membrane matrix with many different growth factors to promote vasa vasorum neovascularization and extenuate the hypoxia to improve remodeling.

Methods

Animals and experimental groups

New Zealand white rabbits weighing between 2.5 kg and 3.0 kg were provided and raised by the Laboratory Animal Center of Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. The protocol for animal experiments was approved by the Committee of Ethics on Animal Experiments at the School of Medicine, Shanghai Jiao Tong University, based on the Guidelines for Animal Experiments. Twenty-four rabbits were randomly divided into four postoperative groups (six rabbits in each group): Group 1, one day postoperatively; Group 2, 2 weeks postoperatively; Group 3, 6 weeks postoperatively; and Group 4, 12 weeks postoperatively.

Vein graft surgery

Anesthesia was induced in the rabbits with intravenous pentobarbital sodium (15–30 mg/kg, depending on the response of the rabbit to the drug), allowing spontaneous ventilation throughout the procedure. In addition, heparin sodium (250 U/kg) and penicillin (400 kU) were administered intravenously before creating a skin incision. In each animal, a longitudinal incision was made in the neck over the region of the internal jugular vein. The internal jugular vein and the common carotid artery were dissected using the "no-touch" technique, and the side branches were ligated with 5-0 silk sutures.

A 1-cm segment of the common carotid artery between two vascular clamps was removed. Polyvinyl chloride cuffs with 1-mm inner diameter were fixed to each end of the artery, around which the artery was everted and ligated. A 2-cm portion of the internal jugular vein was removed and rinsed with saline. Subsequently, the vein was sleeved over the cuffs and ligated. When the entire ischemic time was 15 min, the vascular clamps were removed, pulsations and turbulent blood flow within the vein indicated successful grafting.

Vein grafts with 15-min ischemia, as we called,[6] were performed in one side in Group 1 and both sides in Groups 2, 3, and 4. In one side of Groups 2, 3, and 4, the liquid Matrigel basement membrane matrix (BD Biosciences, San Jose, CA, USA) was withdrawn with a 1-ml injector and dripped down the vein graft slowly, and then changed into hyaline gelatin around the vein graft with its temperature rising [Figure 1]. Postoperatively, the rabbits were housed individually at 20°C and fed a normal diet with free access to water. Clopidogrel was administered to each rabbit the day before surgery (6 mg/kg) and daily after surgery for 2 weeks (3 mg/kg).

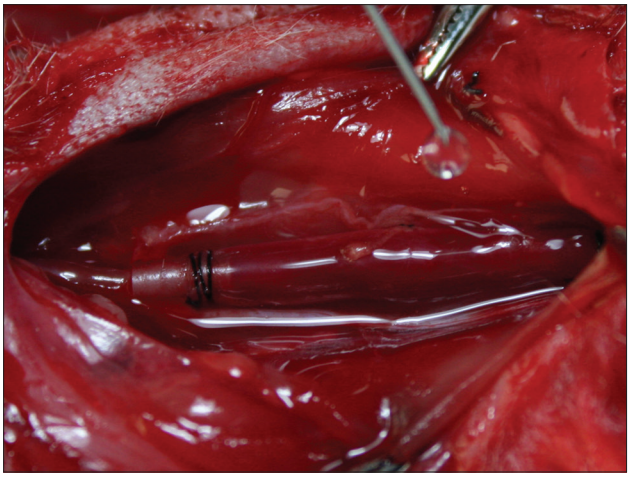

Figure 1: Vein graft with Matrigel basement membrane matrix. The liquid Matrigel basement membrane matrix was withdrawn with a 1-ml injector and dripped down the vein graft slowly, and then changed into hyaline gelatin around the vein graft with its temperature rising.

Vein grafts and the contralateral internal jugular vein were harvested one day postoperatively in Group 1. Postoperatively, vein grafts (control) and contralateral vein grafts with Matrigel basement membrane matrix were harvested at 2 weeks in Group 2, 6 weeks in Group 3, and 12 weeks in Group 4.

Grafts were rinsed in saline, and the vascular lumen was perfused with 10% formalin at a pressure of 100 mmHg, simulating the systolic pressure for 12 h. Each of the grafts was divided into three equal parts. Each part was dehydrated, cleared, and paraffin embedded. Approximately 5-μm transverse sections were taken from three points in each graft, mounted onto glass slides, and stained with hematoxylin/eosin and Ponceau red/Victoria blue.

Morphometric analysis

The dimensions of vessel wall were captured under an LW200T light microscope (Shanghai Cewei Photoelectric Technology Co. Ltd., Shanghai, China) with a color video camera head (DH-HV3103UCUSB; Daheng Group, Inc., Beijing, China) and measured with YRMV image-analysis software (Shanghai Yinrui Information Technology Co. Ltd., Shanghai, China). The mean intimal, medial, and adventitial thickness measurements were derived by measuring the areas and perimeters of the borders between the intimal and medial sections of each graft.

Immunohistochemistry analysis

In brief, 5-μm thick sections were cut from all paraffin-embedded tissue samples, and then placed onto slides, dewaxed, and hydrated in graded alcohols. After microwave pretreatment, sections were incubated with rabbit serum (BD Biosciences, San Jose, CA, USA) for 30 min, followed by 2 h incubation with primary

antibodies, mouse anti-human HIF-1α monoclonal antibody (1:100, Thermo Fisher Scientific, Waltham, MA, USA), and mouse anti-human HIF-2α monoclonal antibody (1:100, Abcam, Cambridge, MA, USA). Subsequently, sections were treated with EnVision system (HRP-R/M, DAKO, Glostrup, Denmark) for 30 min and 0.05% 3,3-diaminobenzidine for 10 min. A light counter stain with hematoxylin/eosin (30 s) was applied to permit visualization of morphology.

In each tissue section, the total number of cells and the number of HIF-1α- and HIF-2α-positive cells were counted in the intima, media, and adventitia in six microscopic fields of view with a ×40 objective. The labeling index was calculated as the percentage of positive nuclei.

Statistical analysis

All data were analyzed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and were expressed as mean ± standard deviation (SD). A comparison between bilateral veins in each group was performed using the paired Student's *t*-test. A $P < 0.05$ was considered statistically significant.

Results

Morphometric analysis

All grafts remained patent after operation, and no cases of grossly visible thrombus or mural thrombus occurred. Two and six weeks postoperatively, the control vein grafts had microvessels filled with red blood cells (RBCs) in the adventitia; the contralateral vein grafts had bigger microvessels and many blood sinuses filled with RBC in the media and adventitia [Figure 2]. At 12 weeks, the blood sinuses had almost disappeared.

There were no significant differences in mean thickness of intima (Group 2: 39.1 ± 5.8 μm vs. 40.3 ± 6.5 μm, $P = 0.43$; Group 3: 48.3 ± 7.9 μm vs. 49.6 ± 8.9 μm, $P = 0.60$; Group 4: 53.6 ± 10.5 μm vs. 51.0 ± 11.9 μm, $P = 0.79$), media (Group 2: 49.3 ± 6.1 μm vs. 48.6 ± 7.7 μm, $P = 0.72$; Group 3: 51.3 ± 6.2 μm vs. 47.6 ± 8.3 μm, $P = 0.77$; Group 4: 58.8 ± 12.9 μm vs. 53.8 ± 12.0 μm, $P = 0.54$), and adventitia (Group 2: 290.5 ± 44.0 μm vs. 300.1 ± 63.3 μm, $P = 0.57$; Group 3: 312.3 ± 54.1 μm vs. 287.3 ± 71.4 μm, $P = 0.51$; Group 4: 302.5 ± 88.3 μm vs. 320.8 ± 76.1 μm, $P = 0.67$) between control vein grafts and contralateral vein grafts with Matrigel basement membrane matrix in Groups 2, 3, and 4 [Figure 3].

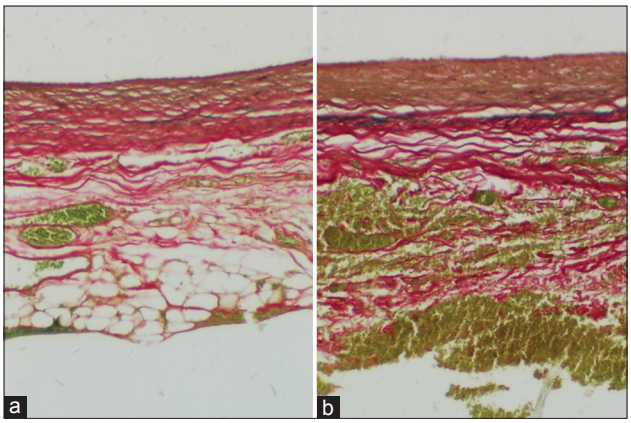

Figure 2: Vein grafts 6 weeks postoperatively: Control vein graft (a) and vein graft with Matrigel basement membrane matrix (b, Ponceau red/Victoria blue staining, original magnification ×200). Elastic fiber was stained blue-green, collagen fiber was stained red, and red blood cells were stained green.

Immunohistochemistry analysis

In Group 1, the labeling index of HIF-1α was high in the normal vein and decreased significantly in the vein graft one day postoperatively (intima: 80 ± 3% vs. 12 ± 1%, $P = 0.01$; media: 67 ± 5% vs. 11 ± 1%, $P = 0.01$; adventitia: 40 ± 10% vs. 7 ± 2%, $P = 0.03$). The labeling index of HIF-2α had similar trend as HIF-1α (intima: 80 ± 10% vs. 10 ± 5%, $P = 0.02$; media: 60 ± 14% vs. 12 ± 2%, $P = 0.01$; adventitia: 45 ± 20% vs. 10 ± 4%, $P = 0.03$; Figure 4).

In Group 2, the HIF-1α and HIF-2α labeling indices of control vein grafts were higher than those of vein grafts with Matrigel basement membrane matrix in the media and adventitia (HIF-1α: 34 ± 5% vs. 20 ± 4%, $P = 0.04$ for media; 23 ± 3% vs. 11 ± 2%, $P = 0.03$ for adventitia; HIF-2α: 37 ± 6% vs. 21 ± 4%, $P = 0.03$ for media; 24 ± 4% vs. 13 ± 2%, $P = 0.04$ for adventitia), but there were no significant differences in the HIF-1α and HIF-2α labeling indices of intima between bilateral vein grafts (HIF-1α: 20 ± 4% vs. 15 ± 3%, $P = 0.41$; HIF-2α: 25 ± 4% vs. 20 ± 2%, $P = 0.23$). In Group 3, the HIF-1α and HIF-2α labeling indices of control vein grafts were higher than those of vein grafts with Matrigel basement membrane matrix in the intima, media, and adventitia (HIF-1α: 27 ± 5% vs. 4 ± 1%, $P = 0.02$ for intima; 33 ± 4% vs. 7 ± 2%, $P = 0.04$ for media; 13 ± 3% vs. 3 ± 1%, $P = 0.02$ for adventitia; HIF-2α: 15 ± 3% vs. 7 ± 2%, $P = 0.04$ for intima; 27 ± 4% vs. 12 ± 3%, $P = 0.02$ for media; 19 ± 2% vs. 6 ± 1%, $P = 0.02$ for adventitia). In Group 4, the HIF-1α and HIF-2α labeling indices showed no statistically significant difference between control vein grafts and vein grafts with Matrigel basement membrane matrix (HIF-1α: 24 ± 3% vs. 22 ± 4%, $P = 0.33$ for intima; 36 ± 8% vs. 29 ± 6%, $P = 0.44$ for media; 43 ± 9% vs. 41 ± 11%, $P = 0.36$ for adventitia; HIF-2α: 29 ± 5% vs. 27 ± 4%, $P = 0.45$ for intima; 44 ± 7% vs. 34 ± 8%, $P = 0.21$ for media; 43 ± 10% vs. 46 ± 13%, $P = 0.61$ for adventitia; Figure 5).

Discussion

The long-term patency of autologous saphenous vein grafts has been unsatisfactory.[1] With abandonment of conventional techniques and the popularizing of the "no-touch" technique in the harvesting, the patency has been improved. Hayward *et al*.[7] found that the patency of saphenous vein grafts was similar to that of radial artery grafts during a mean follow-up of 5.5 years. In another study, the "no-touch" saphenous vein grafts showed a significantly higher patency rate than the radial artery grafts during a mean follow-up of 3 years.[8] Thus, these studies could infer that the further improvement in vein graft patency might offer patients more benefits.

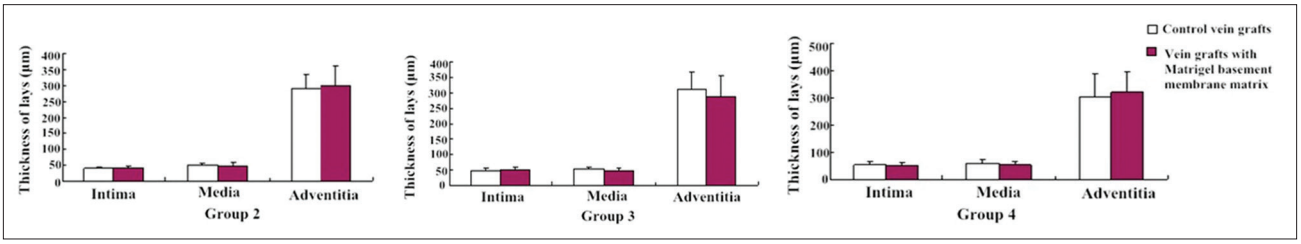

Figure 3: The intima, media, and adventitia thickness of control vein grafts and contralateral vein grafts with Matrigel basement membrane matrix in Groups 2, 3, and 4.

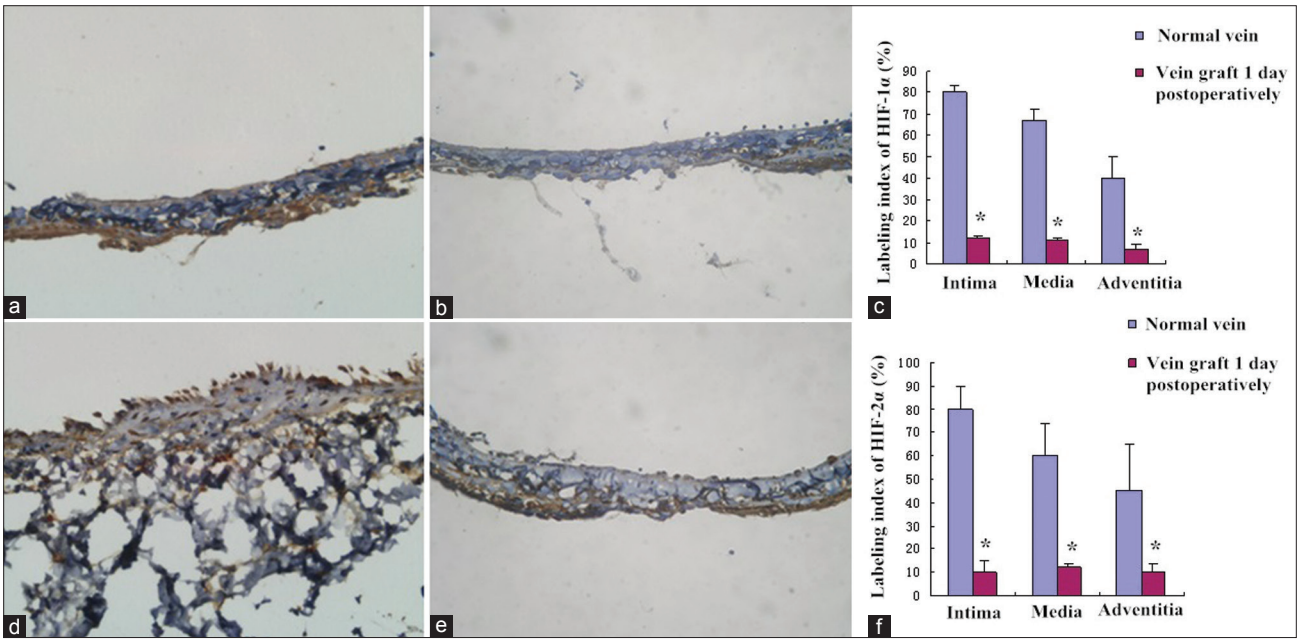

Figure 4: Labeling indices' changes of hypoxia-inducible factor-1α (a–c) and hypoxia-inducible factor-2α (d–f) in the normal vein and vein graft 1 day postoperatively in Group 1 (original magnification ×400). *$P < 0.05$, versus normal vein.

The vein graft may suffer from ischemia and hypoxia once being removed. Previously, we had found that a 90-min ischemia before vein grafting could cause serious endothelial cell loss, but did not increase early intimal hyperplasia.[6] This study aimed to investigate the effect of hypoxia postoperatively on hyperplasia of the wall.

It has been reported that HIF-1 regulated target genes to adapt to hypoxia in mammals and humans.[9,10] It regulated over 70 genes, whose products included VEGF and its receptor, transforming growth factor (TGF), platelet-derived growth factor (PDGF), MMP, extracellular matrices and their regulatory enzymes, endothelin, and nitric oxide (NO), which are closely related to hyperplasia of the vein graft.[11] HIF-1α is the functional subunit synthesized continuously, hydrolyzed quickly, and hardly detected under oxygen-rich conditions.[9] However, heat shock protein 90, reactive oxygen species, nuclear factor-κB, interferon-γ, and interleukin-4 upregulated the expression of HIF-1α, and NO and P53 downregulated its expression.[12] Thus, in this study, we detected HIF-1α and HIF-2α at the same time, because they increased significantly 30 min after hypoxia and peaked at 2–4 h, and then decreased to normal levels 30 min after hypoxia disappearing, as reported by Wiesener *et al*.[2] When the two markers were increased at the same time, hypoxia could be confirmed.

In our study, we found that the labeling indices of HIF-1α and HIF-2α were high in normal veins, which indicated that the normal veins were under hypoxia naturally. The labeling indices of HIF-1α and HIF-2α decreased sharply one day postoperatively because plenty of oxygen could diffuse into the whole wall through the thin layers. However, the increases of HIF-1α and HIF-2α labeling indices 2 weeks postoperatively indicated that the walls of the vein grafts were under hypoxia again. This was consistent with the increasing distance of oxygen diffusion and consumption caused by hyperplasia of vein grafts. To mitigate the effect of hypoxia on hyperplasia, we tried to improve the oxygen supply of vein grafts by promoting vasa vasorum neovascularization.

It is well known that neovascularization needs different kinds of growth factors work together.[13] Growth factors such as PDGF and basic fibroblast growth factor (bFGF) had dose-dependent effects. They promoted intimal repair in low doses and stimulated hyperplasia of smooth muscle cells (SMCs) in large doses.[14] However, the optimal use

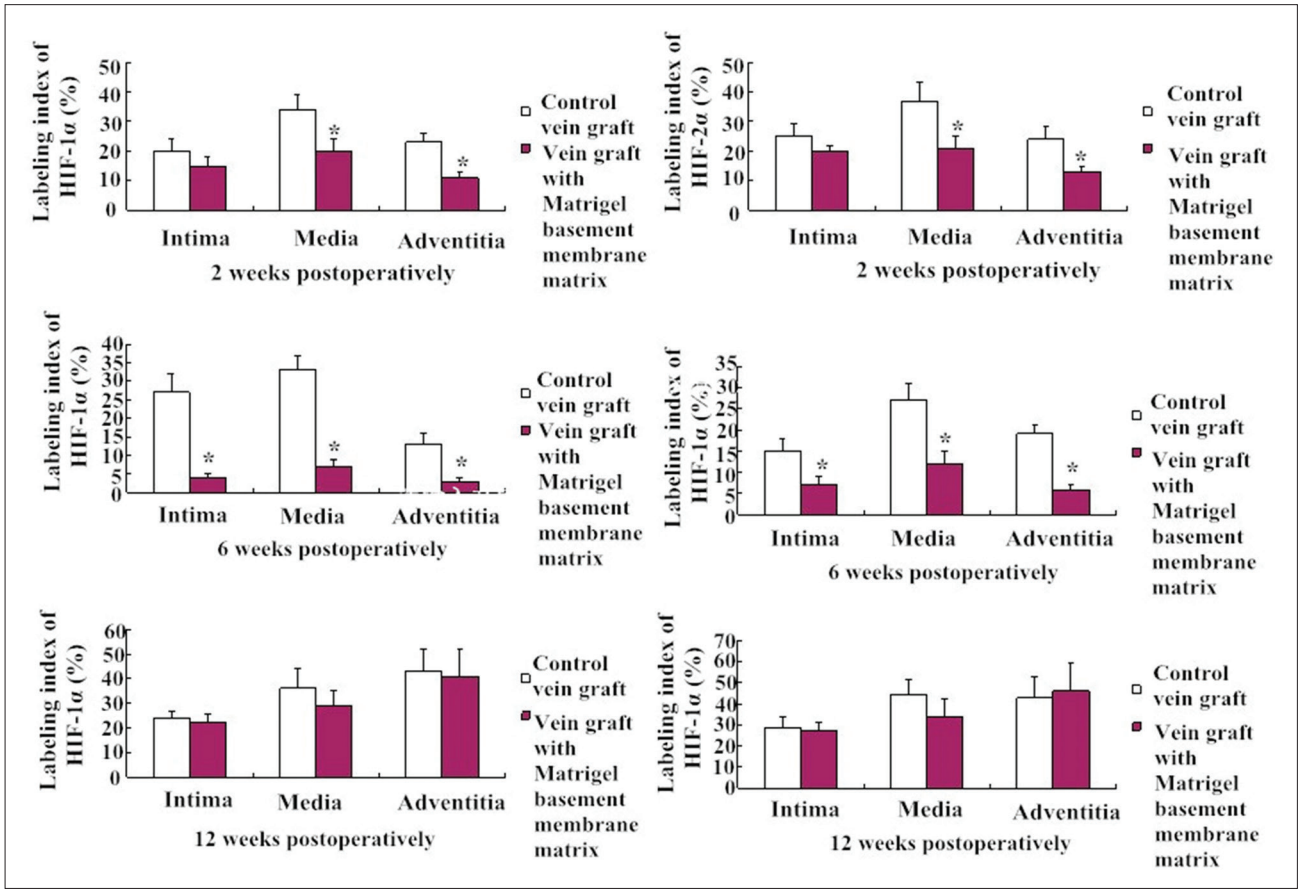

Figure 5: Labeling indices' changes of hypoxia-inducible factor-1α and hypoxia-inducible factor-2α of bilateral vein grafts in Groups 2, 3, and 4. *$P < 0.05$ versus control vein grafts.

and the appropriate concentrations of the growth factors were hard to define. In this study, we selected Matrigel basement membrane matrix extracted from tumors of Engelbreth-Holm-Swarm rats, containing laminin, collagen Type IV, heparin sulfate proteoglycans, and different growth factors, such as PDGF, bFGF, and TGF-β. The proportion and concentration of the components were suitable for neovascularization. Endothelial cells developed into capillaries 6 to 12 h after being spread on it.[15] New vessels with RBCs were found in the basement membrane 2–3 days after it was injected under the skin of the rats.[16] In addition, it changed into gelatin around the vein graft with its temperature rising and provided an undisturbed environment.

Obviously in this study, the media and adventitia of vein grafts with Matrigel basement membrane matrix had a large number of microvessels and blood sinuses filled with RBCs at 2 and 6 weeks postoperatively. Counting the number of vessels only was not appropriate because it did not identify small and large arterioles and venules, let alone the number of blood sinuses. Comparison of hypoxia of the layers may identify the degree of neovascularization better. The lower labeling indices of HIF-1α and HIF-2α indicated better oxygen supply of the wall in vein grafts with Matrigel basement membrane matrix compared to the control vein grafts at 2 and 6 weeks postoperatively. However, there were no significant differences in mean thickness of intima, media, and adventitia between bilateral vein grafts at 2, 6, and 12 weeks postoperatively. As a result, improving oxygen supply of the wall did not extenuate the hyperplasia.

In an open literature, Haraguchi *et al*.[17] found that the controlled release of bFGF from a gelatin hydrogel sheet could reduce the hyperplasia of the intima in a rat vein graft model. They attributed this to the improved repair of the intima and neovascularization of adventitia by bFGF and the increased thickness and strength of adventitia by the hydrogen sheet. In this study, Matrigel basement membrane matrix improved neovascularization significantly and formed many blood sinuses, which has never been described previously. However, it did not reduce hyperplasia of the intima.

In another literature, Wan *et al*.[18] researched the effect of oxygen supply of the whole body on the intimal hyperplasia in arteriovenous fistula model. They found that supplemental oxygen decreased the levels of plasma VEGF and inhibited HIF-1α and VEGF signaling in local blood vessel to reduce SMC proliferation. However, the research focused on the short-term effect and lacked the pathological research. More importantly, the systemic responses to oxygen supply are very complicate; the reduction of local SMC proliferation might be affected by many factors, such as growth factors.

This study only showed the extenuated hypoxia of the wall, without extenuated intimal hyperplasia of vein graft.

In conclusion, based on the experimental cases, this study found that promoting vasa vasorum neovascularization of vein grafts extenuated hypoxia of the wall, but did not influence the intimal hyperplasia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Motwani JG, Topol EJ. Aortocoronary saphenous vein graft disease: Pathogenesis, predisposition, and prevention. Circulation 1998;97:916-31. doi: 10.1161/01.CIR.97.9.916.
2. Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, *et al*. Induction of endothelial PAS domain protein-1 by hypoxia: Characterization and comparison with hypoxia-inducible factor-1alpha. Blood 1998;92:2260-8.
3. Jeremy JY, Shukla N, Muzaffar S, Handley A, Angelini GD. Reactive oxygen species, vascular disease and cardiovascular surgery. Curr Vasc Pharmacol 2004;2:229-36. doi: 10.2174/1570161043385691.
4. Jeremy JY, Yim AP, Wan S, Angelini GD. Oxidative stress, nitric oxide, and vascular disease. J Card Surg 2002;17:324-7. doi: 10.1111/j.1540-8191.2001.tb01151.x.
5. George SJ, Izzat MB, Gadsdon P, Johnson JL, Yim AP, Wan S, *et al.* Macro-porosity is necessary for the reduction of neointimal and medial thickening by external stenting of porcine saphenous vein bypass grafts. Atherosclerosis 2001;155:329-36. doi: 10.1016/S0021-9150(00)00588-8.
6. Zou R, Sun M, Lu Z, Guo Q. Influence of ischemia before vein grafting on early hyperplasia of the graft and the dynamic changes of the intima after grafting. J Cardiothorac Surg 2012;7:90. doi: 10.1186/1749-8090-7-90.
7. Hayward PA, Gordon IR, Hare DL, Matalanis G, Horrigan ML, Rosalion A, *et al.* Comparable patencies of the radial artery and right internal thoracic artery or saphenous vein beyond 5 years: Results from the radial artery patency and clinical outcomes trial. J Thorac Cardiovasc Surg 2010;139:60-5. doi: 10.1016/j.jtcvs.2009.09.043.
8. Dreifaldt M, Mannion JD, Bodin L, Olsson H, Zagozdzon L, Souza D. The no-touch saphenous vein as the preferred second conduit for coronary artery bypass grafting. Ann Thorac Surg 2013;96:105-11. doi: 10.1016/j.athoracsur.2013.01.102.
9. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995;92:5510-4.
10. Semenza GL. Hypoxia-inducible factor 1: Master regulator of O2 homeostasis. Curr Opin Genet Dev 1998;8:588-94. doi: 10.1016/S0959-437X(98)80016-6.
11. Semenza GL. Hydroxylation of HIF-1: Oxygen sensing at the molecular level. Physiology (Bethesda) 2004;19:176-82. doi: 10.1152/physiol.00001.2004.
12. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 2010;40:294-309. doi: 10.1016/j.molcel.2010.09.022.
13. Conway EM, Collen D, Carmeliet P. Molecular mechanisms of blood vessel growth. Cardiovasc Res 2001;49:507-21. doi: 10.1016/S0008-6363(00)00281-9.
14. Segev A, Aviezer D, Safran M, Gross Z, Yayon A. Inhibition of vascular smooth muscle cell proliferation by a novel fibroblast growth factor receptor antagonist. Cardiovasc Res 2002;53:232-41. doi: 10.1016/S0008-6363(01)00447-3.
15. Kubota Y, Kleinman HK, Martin GR, Lawley TJ. Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. J Cell Biol 1988;107:1589-98. doi: 10.1083/jcb.107.4.1589.
16. Passaniti A, Taylor RM, Pili R, Guo Y, Long PV, Haney JA, *et al.* A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. Lab Invest 1992;67:519-28.
17. Haraguchi T, Okada K, Tabata Y, Maniwa Y, Hayashi Y, Okita Y. Controlled release of basic fibroblast growth factor from gelatin hydrogel sheet improves structural and physiological properties of vein graft in rat. Arterioscler Thromb Vasc Biol 2007;27:548-55. doi: 10.1161/01.ATV.0000254811.11741.2b.
18. Wan J, Lata C, Santilli A, Green D, Roy S, Santilli S. Supplemental oxygen reverses hypoxia-induced smooth muscle cell proliferation by modulating HIF-alpha and VEGF levels in a rabbit arteriovenous fistula model. Ann Vasc Surg 2014;28:725-36. doi: 10.1016/j.avsg.2013.10.007.