Endothelial preservation of the minimally manipulated saphenous vein composite graft: Histologic and immunohistochemical study

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Objective: We evaluated the efficacy of minimally manipulative surgical strategies to harvest the saphenous vein for use in a Y-composite graft based on the left internal thoracic artery in terms of preservation of endothelial structure and function.

Methods: Twenty patients who underwent off-pump coronary revascularization using the saphenous vein in a Y-composite graft based on the left internal thoracic artery were studied. The saphenous vein was harvested from each patient with minimal manipulation. An excess saphenous vein segment was removed before dilatation (control group), and a second segment was removed after dilation performed using a pressure-sensing syringe (conventional group). A third segment was obtained from a Y-composite vein graft dilated by flow and pressure from the left internal thoracic artery (composite group). Hematoxylin–eosin staining and immunohistochemistry using antibodies against CD31, CD34, von Willebrand factor, and endothelial nitric oxide synthase were performed. A generalized estimating equation was adopted for statistical analysis.

Results: Histologic and immunohistochemical studies revealed better endothelial preservation in the composite and control groups than in the conventional group ($P < .01$ in each). The composite group saphenous vein showed a lower grade of endothelial integrity than the control group saphenous vein based on hematoxylin–eosin staining, CD34 immunohistochemistry, and nitric oxide synthase staining ($P < .001$ in each).

Conclusions: Harvesting of the saphenous vein using minimal manipulation for use in a Y-composite graft based on the left internal thoracic artery preserved endothelial structure and function when compared with manually dilated saphenous vein harvesting. (J Thorac Cardiovasc Surg 2012;144:690-6)

The saphenous vein (SV) is limited in its use as a bypass conduit in coronary artery bypass grafting (CABG) when compared with arterial grafts because of structural and functional differences between veins and arteries.¹ Lower long-term graft patency rates and worse clinical outcomes have been reported after CABG performed with SV grafts compared with CABG performed with arterial grafts, such as the internal thoracic artery (ITA) and right gastroepiploic artery.²⁻⁴ However, the SV is still a widely used conduit of choice because of its numerous advantages, including ease of access and manipulation, sufficient length for grafting, and short harvesting time.

A variety of efforts to overcome poor long-term patency of the SV grafts have been made.⁵⁻¹⁰ The “no-touch”

technique, in which manipulation during harvesting and anastomosis of the SV is minimized, was one of these efforts.¹¹ A novel “no-touch” technique was recently introduced that demonstrated an improved patency rate.¹⁰ In addition to the choice of harvesting technique, previous reports have also suggested that the surgical strategy of using the ascending aorta as a proximal anastomotic site for a bypass graft may have a causal effect on poor long-term patency rates of SV grafts.^{1,12,13} Using the SV as part of a composite graft theoretically could provide an advantage in that the SV would be well preserved as a result of less exposure to pressure and circulatory stress.¹³ However, only a few studies using the SV in a composite graft have been conducted.^{14,15} One previous retrospective study demonstrated improved patency rates of SV composite grafts at 1 year, compared with an earlier report of poor patency rates for SV aortocoronary grafts.^{3,15}

The aim of this study was to evaluate the benefits of minimally manipulative surgical techniques for harvesting the SV and using it to create a Y-composite graft based on the in situ left ITA in terms of preservation of venous endothelial structure and function.

MATERIALS AND METHODS

The study protocol was approved by the institutional review board (approval no. H-0803-024-237), and informed consent was obtained from all study patients.

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Abbreviations and Acronyms

- CABG = coronary artery bypass grafting
- eNOS = endothelial nitric oxide synthase
- ITA = internal thoracic artery
- SV = saphenous vein
- vWF = von Willebrand factor

Patient Characteristics

From February 2009 to December 2009, 78 patients underwent primary isolated off-pump CABG using the SV as part of a Y-composite graft based on the in situ left ITA. Twenty of those patients, in whom the harvested SV from a lower limb was long enough to obtain approximately 30 mm of excess SV segment after revascularization was completed, were enrolled in the present study for histologic evaluation of the SV portion of the graft. Mean patient age at the time of surgery was 61.0 ± 7.6 years. Preoperative demographic data of the 20 study patients were similar to those of the other 58 patients who underwent off-pump CABG using SV Y-composite grafts during the study period, with the exception of a younger mean age of the study patients (Table 1).

Operative Technique

The basic surgical procedures and principles of off-pump CABG and harvesting technique of the SV have been described.^{15,16} The left ITA was harvested using a skeletonization technique. The left ITA was then immersed in a 10-mL syringe filled with warm diluted papaverine saline solution (1 mg/mL) to dilate pharmacologically until use. We did not use intraluminal injection of papaverine solution. The SV was harvested as a second graft and anastomosed to the side of the in situ left ITA to construct a Y composite graft. The SV from a lower leg was chosen for use in grafting as opposed to the upper leg SV so that the possibility of size mismatch with native coronary arteries or ITA was decreased. Saphenous vein harvest was initiated after systemic heparinization during harvest of the left ITA and was performed using an atraumatic, open technique, so that manipulation with micro-forceps was limited to the perivascular tissue and never included the full thickness of the venous wall. Side branches were divided after metal clipping. To prevent inadvertent endothelial injury, clips were applied at least 1 mm apart from branch origins. Tension on the SV was minimized during harvest, and manual intraluminal dilatation was avoided. Immediately after the SV was harvested, it was used to construct a Y-composite graft without any pharmacologic treatment. After the Y-composite graft was constructed, the distal end of the SV was clamped with an atraumatic bulldog (Scanlan International Inc, St Paul, Minn) and left to be dilated spontaneously by the native flow and pressure of the left ITA.

The left anterior descending coronary artery territory was revascularized first. A sequential anastomotic technique using each Y-composite graft side-arm was almost always used for complete revascularization when more than 2 coronary arterial anastomoses were needed. Patients were given an initial dose of heparin (1.5 mg/kg) and periodic supplemental doses to maintain an activated clotting time of more than 300 seconds. All patients received aspirin therapy until the day of surgery and resumed it as soon as possible after surgery, usually 1 day postoperatively.

Preparation of the Saphenous Vein for Histologic Evaluation

Of the 16 to 18 cm of SV harvested from a lower leg for grafting, approximately 30 mm of excess SV near the knee level were used for this study. A 5-mm segment of the 30 mm was removed before dilation (control group). A 20-mm segment of the 30 mm was dilated by manual injection of papaverine (1 mg/mL) saline solution with a pressure-sensing

TABLE 1. Preoperative characteristics and risk factors of the study patients and nonstudy patients

	Study patients (n = 20)	Non-study patients (n = 58)	P value
Age (y)	61.0 $\pm$ 7.6	66.2 $\pm$ 9.4	.029
Female	6 (30.0%)	10 (17.2%)	.334
Risk factors, n (%)			
Smoking	10 (50.0%)	25 (43.1%)	.593
Hypertension	15 (75.0%)	42 (72.4%)	.822
Diabetes mellitus	7 (35.0%)	29 (50.0%)	.246
Dyslipidemia	3 (15.0%)	7 (12.1%)	.711
Overweight (BMI $\geq$ 25)	10 (50.0%)	22 (37.9%)	.334
History of stroke	1 (5.0%)	5 (8.6%)	>.999
Chronic renal failure	0 (0.0%)	2 (3.4%)	>.999
LVEF < 35%	1 (5.0%)	10 (17.2%)	.272
Unstable angina	14 (70.0%)	35 (60.3%)	.441
Left main disease	11 (55.0%)	26 (44.8%)	.432
3-vessel disease	12 (60.0%)	45 (77.6%)	.126

BMI, Body mass index (kg/m²); LVEF, left ventricular ejection fraction.

syringe (DLP Inc, Grand Rapids, Mich) connected to a manometer (conventional group). Mean pressure applied for manual dilation of the 20 mm segment was 271 ± 53 mm Hg in the conventional group. After a Y-composite graft was constructed, an atraumatic clamp was applied at the distal end of the SV graft segment, leaving the SV portion of the graft exposed to the native blood flow and pressure of the in situ left ITA for dilation during the distal anastomoses. The average systolic, diastolic, and mean blood pressures during distal anastomoses were 108 ± 9 mm Hg, 59 ± 6 mm Hg, and 74 ± 5 mm Hg, respectively. Immediately before performing the far distal anastomosis, 5 mm of the distal segment of the SV graft that had been dilated by the native ITA pressure was removed and prepared for histologic evaluation (composite group). The distal end of the SV graft was exposed to the native ITA flow and pressure for an average of 83 ± 32 minutes.

All samples were fixed with 10% formalin solution, embedded in paraffin, and cut into 5 μ m-thin sections. Hematoxylin–eosin staining was performed for evaluation of the SV endothelium. Immunohistochemistry was performed using mouse monoclonal antibodies against human endothelial glycoprotein CD31 (1:25, Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), CD34 (1:10, Cell Signaling Technology, Inc, Boston, Mass), von Willebrand factor (vWF 1:200, Dako, DK-2600 Glostrup, Denmark), and endothelial nitric oxide synthase (eNOS 1:50, R&D Systems, Inc, Minneapolis, Minn). Endogenous peroxidase activity of the sections was blocked by 10-minute preincubation within 3% hydrogen peroxide in methanol. Sections were then incubated in 5% normal goat serum in Tris buffer solution at room temperature for 10 minutes to block background staining. They were then incubated in mouse monoclonal antibodies against CD31, CD34, and vWF for 30 minutes to evaluate structural integrity and eNOS to evaluate endothelial function. The sections were rinsed, and secondary antibody conjugated with horseradish peroxidase was applied for 30 minutes. After staining with diaminobenzidine chromogen for 10 minutes and counterstaining with Mayer’s hematoxylin for 15 seconds, the sections were prepared for microscopic examination. The amount of positive staining was graded from 1 to 10, based on the percentage of positive staining of the lumen length (Table 2).

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation, median and ranges, or proportions. Comparisons between the 2 groups were made using the chi-square test and Fisher exact test for categoric variables and Student *t* test for continuous variables. Generalized estimating equation analysis

TABLE 2. Semiquantitative grading criteria of endothelial staining

Grade	% of luminal length
1	0–10
2	10–20
3	20–30
4	30–40
5	40–50
6	50–60
7	60–70
8	70–80
9	80–90
10	90–100

was used to compare the degree of stain among the 3 groups. Adjusted *P* value using the Hochberg method was calculated for each staining method. A *P* value of <.05 was considered statistically significant.

RESULTS

Generalized estimating equation analysis revealed significant differences in the grades of staining between the groups and between the staining methods (*P* < .001 and *P* = .003, respectively). Because there was a significant interaction between the groups and staining methods (*P* = .037), *P* values of differences in the grades of staining between groups were calculated for each staining method and adjusted using the Hochberg correction method.

Histologic Study

Hematoxylin–eosin stain revealed a higher grade of well-preserved endothelial cells in the composite group than in the conventional group (grades 7.4 ± 2.5 vs 5.0 ± 2.0 , *P* < .001). However, the composite group grade was lower than the control group grade (grades 7.4 ± 2.5 vs 9.2 ± 0.8 , *P* < .001) (Figure 1).

Immunohistochemical Study

There were no significant differences in the grades of immunohistochemistry using CD31 antibody between the control and composite groups (9.0 ± 1.5 and 9.0 ± 1.5 , respectively; *P* = .653). The grades of immunohistochemistry using CD31 antibody in both the control and composite groups were significantly higher than in the conventional group (6.9 ± 2.5), suggesting poorly preserved endothelium in the conventional group (*P* < .001). Immunohistochemistry using vWF antibody revealed better preserved endothelium in both the control and composite groups than in the conventional group (control, composite, and conventional groups; grades 8.4 ± 1.9 , 8.2 ± 2.0 , and 6.7 ± 2.7 , respectively; *P* < .001) (Figures 2 and 3). Immunohistochemistry using CD34 antibody revealed similar results to those seen by hematoxylin–eosin staining. Although endothelial integrity in the composite group was preserved better than in the conventional group (*P* < .001), it was preserved worse than in the control group (*P* < .001) (Figure 4).

FIGURE 1. Hematoxylin–eosin staining of luminal endothelium of the SV in the control (A), conventional (B), and composite groups (C) (×200). Note the defects in staining (black arrows), particularly in the conventional group.

Endothelial function evaluated with immunohistochemical stain against eNOS showed similar results with CD34 staining; eNOS in the composite group was preserved better than in the conventional group (*P* < .001), but was less preserved than in the control group (*P* < .001) (Figure 5) (Table 3).

FIGURE 2. Luminal endothelium of the SV in the control (A), conventional (B), and composite groups (C). CD31 was stained as a brown color (A, B: $\times 200$; C: $\times 100$). Note the defects in staining (black arrows), particularly in the conventional group.

DISCUSSION

The present study revealed that surgical strategies to harvest the SV with a minimally manipulative surgical technique for use in a Y-composite graft based on the in situ left ITA were beneficial in preserving endothelial structure and function of the SV portion of the graft.

FIGURE 3. Luminal endothelium of the SV in the control (A), conventional (B), and composite groups (C). vWF was stained as a brown color ($\times 100$). Note the defects in staining (black arrows), particularly in the conventional group.

The SV is a widely used conduit that offers advantages of ease of access and manipulation, sufficient length, and short harvesting time. However, poorer long-term patency rates and clinical outcomes have been reported after CABG performed using SV grafts compared with those after CABG

FIGURE 4. Luminal endothelium of the SV in the control (A), conventional (B), and composite groups (C). CD34 was stained as a brown color (A: $\times 200$; B, C: $\times 100$). Note the defects in staining (*black arrows*), particularly in the conventional group.

FIGURE 5. Luminal endothelium of the SV in the control (A), conventional (B), and composite groups (C). eNOS was stained as a brown color ($\times 100$). Note the defects in staining (*black arrows*), particularly in the conventional group.

with arterial grafts, such as the ITAs and right gastroepiploic artery.²⁻⁴ Major limitations in using the SV as a bypass graft originate from structural and functional differences between veins and arteries.¹ Chronic endothelial damage and

dysfunction stimulate migration and proliferation of smooth muscle cells into the intima, a key event in the development of atherosclerosis and graft failure of the SV.¹⁷ Methods used to improve patency of SV grafts included pharmacologic

TABLE 3. Group comparisons

Stain	Control group	Conventional group	Composite group
Hematoxylin–eosin	9.2 ± 0.8*	5.0 ± 2.0	7.4 ± 2.5*,‡
CD31	9.0 ± 1.5*	6.9 ± 2.5	9.0 ± 1.5*
vWF	8.4 ± 1.9*	6.7 ± 2.7	8.2 ± 2.0‡
CD34	7.8 ± 1.7*	2.6 ± 2.0	5.0 ± 3.0*,‡
eNOS	9.5 ± 0.9*	3.6 ± 2.1	6.5 ± 3.1*,‡

Comparison of luminal staining of native SVs (control group), SV grafts harvested using a conventional method (conventional group), and those harvested with a minimal manipulation surgical technique and anastomosed to the left ITA (composite group). *Significant difference compared with the conventional group ($P < .001$). †Significant difference compared with the control group ($P < .001$). ‡Significant difference compared with the conventional group ($P = .003$).

relaxation of the SV after harvest and strict medical therapy, including administration of antiplatelet and lipid-lowering agents after surgery.^{18,19} Further efforts to improve patency of the SV have continued, including external support using stents, intraoperative gene transfer, and improvement in harvesting technique.⁵⁻¹⁰

In addition to the harvesting technique, previous reports have suggested that the surgical strategy of using the ascending aorta for the proximal anastomotic site of a bypass graft may have an effect on poor long-term patency rates of the SV graft.^{1,12,13} Theoretic advantages of using the SV as part of a composite graft over an aortocoronary bypass graft are 1) the SV graft anastomosed to the left ITA may be exposed to less pressure trauma than a graft anastomosed to the ascending aorta, and 2) the risk of atherosclerotic disease is reduced by the constant exposure of the SV graft to nitric oxide produced from the left ITA.^{12,13} In a previous study performed by our group, similar patency rates of SV composite grafts and arterial composite grafts were demonstrated.¹⁵ Early and 1-year patency rates of SV composite grafts were 97.1% (132/136) and 92.1% (105/114), respectively, which were not significantly different from those of arterial composite grafts. On the contrary, we had demonstrated poor patency rates of the SV when used as part of aortocoronary grafts (95.9% [116/121] and 76.0% [92/121] early and at 1 year, respectively).³

Our surgical strategies to preserve the SV from endothelial injury included changing harvesting technique and using the SV as part of a Y-composite graft. We harvested the SV after systemic heparinization to prevent microthrombus formation on the venous endothelium during harvest. Manipulation and tension were minimized, and dilatation using a pressure-sensing syringe was avoided because mean pressure applied for manual dilation with a pressure-sensing syringe was higher than expected as presented in this study. Instead, the SV graft was connected to the side of the in situ left ITA to be dilated spontaneously by the native flow and pressure.¹⁵

Of numerous endothelial markers, CD31, CD34, and vWF are the most commonly used markers for evaluating

vascular endothelial structural integrity. However, limited data are available on the immunohistochemical expression pattern in normal endothelium. Previous studies have reported that CD31 was strongly and homogeneously expressed in pulmonary vascular endothelial tissue.²⁰⁻²² Conversely, CD34 and vWF were more heterogeneous in distribution; CD34 was expressed more strongly in capillaries and arteries, and vWF was expressed mainly in venous endothelia. Another study reported that the expression of endothelial cell markers CD31, CD34, and vWF in the vascular tree was heterogeneous, with specific patterns for individual vessel types and different anatomic compartments of the same organ.²⁰ In the present study, histologic examination was performed with multiple markers, including hematoxylin–eosin staining and immunohistochemistry performed using monoclonal antibodies against CD31, CD34, and vWF to overcome the limitations of different expressions of endothelial markers. Antibodies against eNOS were used for evaluation of endothelial function. The SV grafts that were harvested using a minimally manipulative surgical technique and exposed to the native flow of the ITA (composite group) showed better preservation of endothelial integrity and function compared with those treated with a conventional method (conventional group). These results were in agreement with recent studies demonstrating histologic differences between conventionally harvested SVs and those harvested using a no-touch surgical technique.^{23,24} In previous studies, however, the SVs were prepared for histologic evaluation immediately after harvest. The possible detrimental effect of direct aortic pressure on the SV endothelium was not evaluated.

Study Limitations

There are several limitations to the present study that must be recognized. First, the SV was exposed to the native flow for only a short time. Second, we did not compare our results with SVs used as aortocoronary grafts because our surgical strategy was to avoid aortic manipulation, if possible. During the study period, only 3 patients underwent isolated CABG using the SV for aortocoronary bypass. Third, correlation of long-term angiographic patency with histologic results in a large patient population might be necessary to demonstrate that the preserved SV endothelium has any effect on long-term patency rates of SV composite grafts. Fourth, we evaluated only structure and function of the endothelial layer, and did not examine conduit behavior in an isolated bath to test function of both endothelium and smooth muscle.

References

1. Cox JL, Chiasson DA, Gotlieb AI. Stranger in a strange land: the pathogenesis of saphenous vein graft stenosis with emphasis on structural and functional differences between veins and arteries. *Prog Cardiovasc Dis*. 1991;34:45-68.

2. Cameron A, Davis KB, Green G, Schaff HV. Coronary bypass surgery with internal-thoracic-artery grafts - effects on survival over a 15-year period. *N Engl J Med*. 1996;334:216-20.
3. Kim K-B, Cho KR, Jeong DS. Midterm angiographic follow-up after off-pump coronary artery bypass: serial comparison using early, 1-year, and 5-year postoperative angiograms. *J Thorac Cardiovasc Surg*. 2008;135:300-7.
4. FitzGibbon GM, Kafka HP, Leach AJ, Keon WJ, Hooper GD, Burton JR. Coronary bypass graft fate and patient outcome: angiographic follow-up of 5,065 grafts related to survival and reoperation in 1,388 patients during 25 years. *J Am Coll Cardiol*. 1996;28:616-26.
5. Wan S, Yim AP, Johnson JL, Shukla N, Angelini GD, Smith FC, et al. The endothelin 1A receptor antagonist BSF 302146 is a potent inhibitor of neointimal and medial thickening in porcine saphenous vein-carotid artery interposition grafts. *J Thorac Cardiovasc Surg*. 2004;127:1317-22.
6. Wan S, Shukla N, Angelini GD, Yim APC, Johnson JL, Jeremy JY. Nitric oxide-donating aspirin (NCX 4016) inhibits neointimal thickening in a pig model of saphenous vein-carotid artery interposition grafting: a comparison with aspirin and morpholinodnonimine (SIN-1). *J Thorac Cardiovasc Surg*. 2007;134:1033-9.
7. Krejca M, Skarysz J, Szmaga P, Plewka D, Nowaczyk G, Plewka A, et al. A new outside stent - does it prevent vein graft intimal proliferation? *Eur J Cardiothorac Surg*. 2002;22:898-903.
8. Petrofski JA, Hata JA, Gehring TR, Hanish SI, Williams ML, Thompson RB, et al. Gene delivery to aortocoronary saphenous vein grafts in a large animal model of intimal hyperplasia. *J Thorac Cardiovasc Surg*. 2004;127:27-33.
9. PREVENT IV investigators. Efficacy and safety of endofoligide an E2F transcription factor decoy, for prevention of vein graft failure following coronary artery bypass graft surgery: PREVENT IV: a randomized controlled trial. *JAMA*. 2005;294:2446-54.
10. Souza DS, Johansson B, Bojö L, Karlsson R, Geijer H, Filbey D, et al. Harvesting the saphenous vein with surrounding tissue for CABG provides long-term graft patency comparable to the left internal thoracic artery: results of a randomized longitudinal trial. *J Thorac Cardiovasc Surg*. 2006;132:373-8.
11. Gottlob R. The preservation of the venous endothelium by 'dissection without touching' and atraumatic technique of vascular anastomosis. The importance for arterial and venous surgery. *Minerva Chir*. 1977;32:693-700.
12. Tedoriya T, Kawasuji M, Sakakibara N, Ueyama K, Watanabe Y. Pressure characteristics in arterial grafts for coronary bypass surgery. *Cardiovasc Surg*. 1995;3:381-5.
13. Filho JG, Leitão MC, Forte AJ, Filho HG, Silva AA, Bastos ES, et al. Flow analysis of left internal thoracic artery in myocardial revascularization surgery using Y graft. *Tex Heart Inst J*. 2006;44:430-6.
14. Gaudino M, Alessandrini F, Pragliola C, Luciani N, Trani C, Burzotta F, et al. Composite Y internal thoracic artery-saphenous vein grafts: short-term angiographic results and vasoreactive profile. *J Thorac Cardiovasc Surg*. 2004;127:1139-44.
15. Hwang HY, Kim JS, Kim K-B. Angiographic equivalency of off-pump saphenous vein and arterial composite grafts at one year. *Ann Thorac Surg*. 2010;90:516-21.
16. Kim K-B, Cho KR, Chang WI, Lim C, Ham BM, Kim YL. Bilateral skeletonized internal thoracic artery graftings in off-pump coronary artery bypass: early result of Y versus in situ grafts. *Ann Thorac Surg*. 2002;74:S1371-6.
17. Ratliff NB, Myles JL. Rapidly progressive atherosclerosis in aortocoronary saphenous vein grafts. Possible immune-mediated disease. *Arch Pathol Lab Med*. 1989;113:772-6.
18. Roubos N, Rosenfeldt FL, Richards SM, Conyers RAJ, Davis BB. Improved preservation of saphenous vein grafts by the use of glyceryl trinitrate-verapamil solution during harvesting. *Circulation*. 1995;92(Suppl 2):II31-6.
19. Goldman S, Copeland J, Moritz T, Henderson W, Zadina K, Ovitt T, et al. Saphenous vein graft patency 1 year after coronary artery bypass surgery and effects of antiplatelet therapy: results of a Veterans Administration Cooperative Study. *Circulation*. 1989;80:1190-7.
20. Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *J Histochem Cytochem*. 2006;54:385-95.
21. Kawanami O, Jin E, Ghazizadeh M, Fujiwara M, Jiang L, Nagashima M, et al. Heterogeneous distribution of thrombomodulin and von Willebrand factor in endothelial cells in the human pulmonary microvessels. *J Nippon Med Sch*. 2000;67:118-25.
22. Müller AM, Hermanns MI, Skrzynski C, Nesslinger M, Müller KM, Kirpatrick J. Expression of the endothelial markers PECAM-1, vWF and CD34 in vivo and in vitro. *Exp Mol Pathol*. 2002;72:221-9.
23. Dries D, Mohammad SF, Woodward SC, Nesson RM. The influence of harvest technique on endothelial preservation in saphenous veins. *J Surg Res*. 1992;52:219-25.
24. Rueda F, Souza D, Lima RC, Menezes A, Johansson B, Dashwood M, et al. Novel no-touch technique of harvesting the saphenous vein for coronary artery bypass grafting. *Arq Bras Cardiol*. 2008;90:356-62.