

# Comparison of Conventional and No-Touch Techniques in Harvesting Saphenous Vein for Coronary Artery Bypass Grafting in View of Endothelial Damage

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## ABSTRACT

**Background:** Optimization of saphenous vein patency for myocardial revascularization.

**Objective:** The goal of this study was to present the no-touch technique of saphenous vein preparation. This technique consists of harvesting the vein with a pedicle of surrounding tissue, which protects the vein from distension pressure.

**Methods:** We performed a prospective, randomized study that compared 2 techniques for harvesting saphenous vein—conventional and no-touch in 40 patients undergoing coronary artery bypass grafting. We carried out a morphologic study of the endothelium with the aid of light and transmission electron microscopy and an immunohistochemical assessment to identify adenosine, inducible nitric oxide synthase (iNOS), and vascular endothelial growth factor (VEGF) in the vein wall.

**Results:** The integrity of endothelial cell and all vascular layers was maintained better with the no-touch technique than with the conventional procedure. The immunohistochemical assessment revealed that adenosine receptor, iNOS, and VEGF immunoexpression levels were normal or lower in the no-touch group than in the conventional-harvest group, as shown by the staining densities in all layers of the vein wall.

**Conclusion:** Endothelial integrity and adenosine, iNOS, and VEGF immunoreactivities were better preserved when the no-touch technique was used for vein graft harvesting. The mechanical protection provided by the cushion of surrounding tissue in the no-touch group and the vaso-relaxation and thromboresistant activities of nitric oxide may be responsible for the reduction in vasospasms and the improved patency rate.

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## INTRODUCTION

Atherosclerotic coronary artery disease is the most important cause of morbidity and mortality in developed countries. The annual mortality of coronary heart disease is greater than 1 million people worldwide [Raja 2004]. Heart surgery has made important progress, thanks to technological developments in medicine, as well as the discovery of heparin, the use of extracorporeal circulation (ECC) systems, and the developments in pharmacology and anesthesia. Since Favallora's first use of a harvested saphenous vein graft for coronary artery bypass in 1967, coronary artery surgery has become widely performed [Raja 2004]. The success of coronary artery bypass surgery depends on graft patency in all vascular interventions. Twenty-five percent of grafts are occluded by the fifth year following the surgery, and 35% are occluded by the 10th year [Fulton 1997]. Notwithstanding these facts, saphenous vein grafts become occluded for different reasons, such as inflation of the vein with high pressure during preparation, which causes endothelial and media damage to the vein graft [Cambria 1985]. Endothelial damage has been determined to lead to graft thrombosis by causing thrombus accumulation in the early period after surgery and lipid accumulation in the late postoperative period [Boerboom 1980; Angelini 1990]. Such predisposing factors as vasoconstriction and vasospasm, hyperlipidemia, inappropriate graft-preparation technique, and inappropriate anastomosis technique are also closely related to surgical techniques responsible for the complications that can occur in the early period following coronary bypass surgery [Thatte 2001].

In this study, we analyzed the potential for damage caused by mechanical trauma to the intima and media layer at the cellular level by using light microscopy and transmission electron microscopy to compare human saphenous veins prepared by the conventional method and human saphenous veins prepared with a “no touch” technique.

## METHODS

We received ethics committee approval for this study. The study population consisted of 40 patients between the ages of 40 and 70 years who underwent aorta coronary artery bypass surgery between January 2010 and May 2010 at the

cardiovascular surgery clinic. The ejection fractions of these patients were 40%. We used a proximal section of almost 4 cm from the saphenous vein planned for grafting.

### Conventional Harvesting Technique

Saphenous veins from 20 patients were harvested with the conventional technique. The vein was laid completely bare and cannulated by making the skin incision along the trace of the saphenous vein. The saphenous vein and its subbranches were dissected with care taken to avoid trauma and were separated from the surrounding tissues. The subbranches were clipped, and the vein was prepared by inflating to a maximum distension pressure of 100 mm Hg. After administering normothermic heparin saline solution (5000 U heparin in 300 mL 0.9% NaCl) to the patient for ECC, we made measurements with a transducer. We then dissected an approximately 4-cm piece from the proximal section without trauma. The saphenous vein section was then divided into 2 equal parts, one for electron microscopy and the other for light microscopy.

### No-Touch Harvesting Technique

The same surgeon harvested saphenous veins from 20 patients with the no-touch technique following heparinization for ECC. The saphenous vein was harvested at the proximal section of the medial malleolus. The vein was laid completely bare by making an incision along the trace of the saphenous vein. The vein was prepared by clipping its subbranches without inflating the vein and by cannulating the vein with its surrounding tissues. For light microscopy and electron microscopy examinations, we then dissected an approximately 4-cm piece from the proximal section without trauma.

### Light Microscopy Examination

Samples of saphenous vein tissue were harvested from the 2 groups of patients, and 5- $\mu$ m sections were cut with a cryomicrotome. The sections were preserved within petri dishes in cryoprotectant solution. We then carried out immunohistochemistry measurements with antibodies against inducible nitric oxide synthase (iNOS) (1/250 dilution of rabbit anti-iNOS; Life Technologies/Zymed Laboratories, Grand Island, NY, USA), adenosine A2b receptor (1/10 dilution of rabbit anti-adenosine A2b receptor; Millipore/Chemicon, Billerica, MA, USA), and vascular endothelial growth factor (VEGF) (1/50 dilution of mouse monoclonal antibody; Santa Cruz Biotechnology, Dallas, TX, USA). We used the free-floating method and examined the immunoreactivities of the specimens by light microscopy (BX50F-3 microscope; Olympus, Tokyo, Japan).

### Electron Microscopy Examination

We fixed 0.5-mm samples of saphenous vein tissue in a 2.5% phosphate-buffered glutaraldehyde solution at 4C for 24 hours and then performed a postfixation treatment in 1% phosphate-buffered osmium tetroxide for 1 hour. The samples were dehydrated by passing them through an ethyl alcohol series and were then embedded in EPON 812. Samples were polymerized overnight in an incubator at 70C. Thin

sections (400-600 Å) were cut with an LKB ultramicrotome and stained with uranyl acetate and lead citrate. Finally, sections were evaluated with a JEOL JEM-1011 transmission electron microscope (JEOL USA, Peabody, MA, USA).

### Statistical Analyses

All statistical analyses were carried out with SPSS for Windows (version 15.0; IBM/SPSS, Chicago, IL, USA). The Kolmogorov-Smirnov test was used to test for normality of continuous variables. Data for continuous variables were analyzed with the Mann-Whitney U test or the independent-samples Student t test. Data for categorical variables were analyzed with the 2 test or the Fisher exact test. A 2-tailed *P* value <.05 was considered statistically significant.

## RESULTS

The 2 groups showed no significant differences with respect to demographic features (sex, age, height, weight, and body surface area (*P* > .05) (Table). We evaluated comorbidities (hypertension, diabetes mellitus, chronic obstructive pulmonary disease, and smoking) and found no significant differences (*P* > .05). A systolic pressure >140 mm Hg and a diastolic pressure >90 mm Hg were accepted as indicating hypertension [Guessous 2012]. The 2 groups also showed no significant differences with respect to left ventricular ejection fraction and cholesterol profile (*P* > .05).

Demographic Features of the Patients\*

Characteristic	No-Touch Group (n = 20)	Conventional-Harvest Group (n = 20)	<i>P</i>
Male/female sex, n	16/4	17/3	.687
Age, y	61.3 $\pm$ 6.0	62.6 $\pm$ 7.1	.554
Height, cm	171.4 $\pm$ 8.6	169.4 $\pm$ 6.4	.400
Weight, kg	81.1 $\pm$ 17.2	79.5 $\pm$ 10.3	.725
Ejection fraction, %	53.2 $\pm$ 6.6	53.4 $\pm$ 6.3	.942
Hypertension, n (%)	16 (80)	18 (90)	.389
Diabetes mellitus, n (%)	7 (35)	8 (40)	.752
Total cholesterol, mg/dL	186.8 $\pm$ 37.3	190.7 $\pm$ 48.7	.778
HDL, mg/dL	43.6 $\pm$ 11.2	43.8 $\pm$ 11.6	.956
LDL, mg/dL	120.1 $\pm$ 35.3	121.9 $\pm$ 43.7	.884
COPD, n (%)	3 (15)	2 (10)	.643
Smoking, n (%)	10 (50)	9 (45)	.759

\*Data are presented as the mean  $\pm$  SD or as a number (percentage). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; COPD, chronic obstructive pulmonary disease.

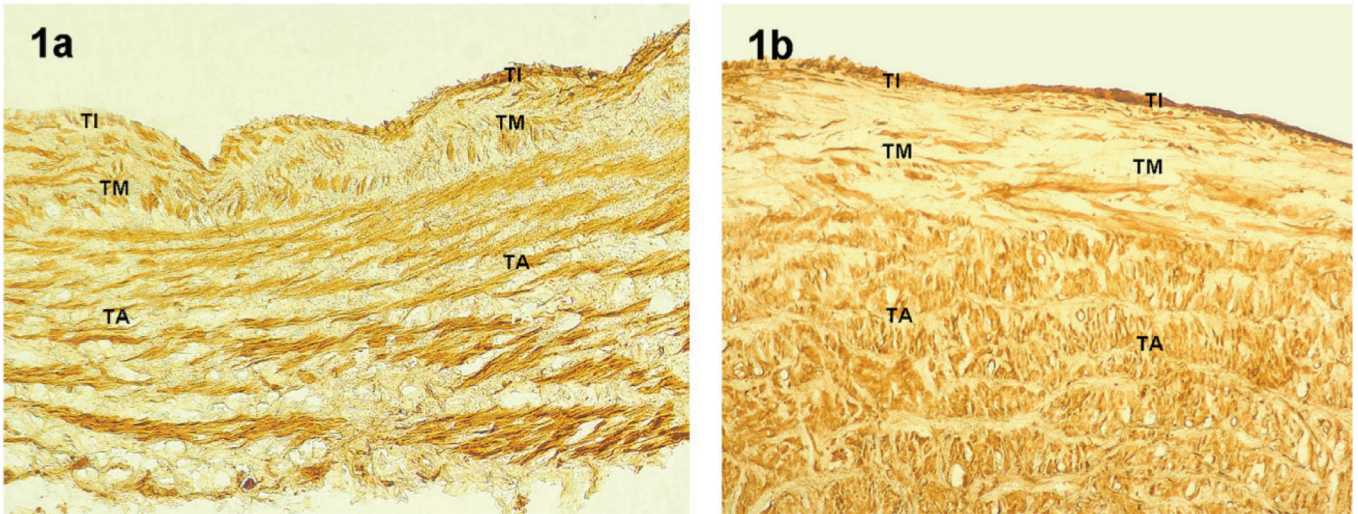


Figure 1. A and B, The 2 groups had similar intensities in adenosine receptor immunoreactivity. Endothelial cells in the tunica intima (TI), smooth muscle cells in the tunica media (TN) and tunica adventitia (TA), and the vaso vasorum were positive for immunoreactivity (original magnification  $\times 100$ ).

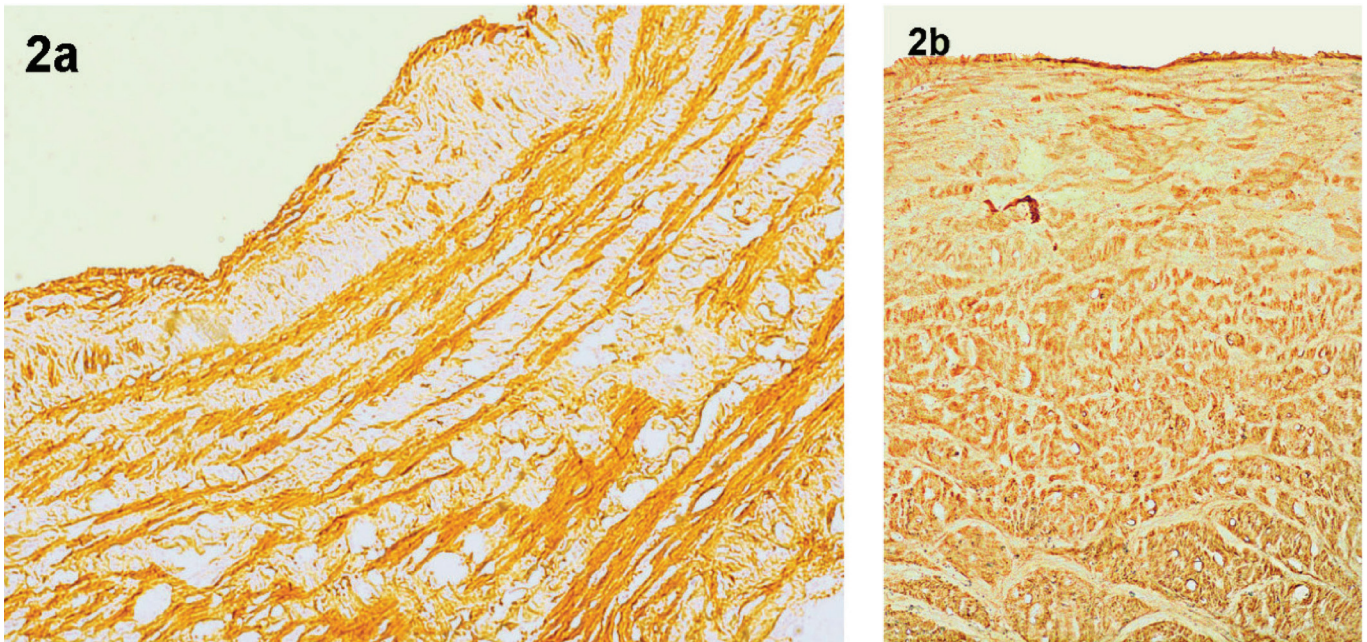


Figure 2. Expression of inducible nitric oxide synthase immunoreactivity was increased in the conventional-harvest group (A) compared with the no-touch group (B). Immunoreactivity was especially present in smooth muscle cells located in the tunica media (TM) and the tunica adventitia (TA). In the control group, detachment of the connective tissue was apparent in the TM and the TA. Vessel integrity was better preserved in the no-touch group (original magnification  $\times 100$ ).

### Light Microscopy Evaluation

Adenosine receptor immunostaining of saphenous vein revealed strong adenosine receptor immunoreactivity (++++) in all wall layers (tunica intima, tunica media, tunica adventitia) in both the conventional-harvest group and the no-touch group (Figure 1). Immunostaining densities were similar in the 2 groups. Immunoreactivity was observed in endothelial cells in the tunica intima, in smooth muscle cells in the tunica media, and in the tunica adventitia.

iNOS immunostaining was observed in different vessel sections in the conventional-harvest and no-touch groups. iNOS immunoreactivity was more evident in the conventional-harvest group (+++) than in the no-touch group (++) (Figure 2). Tissue detachment from the wall of the tunica media and the tunica adventitia was evident in the conventional-harvest group. Morphologic structure was better preserved in the no-touch group.

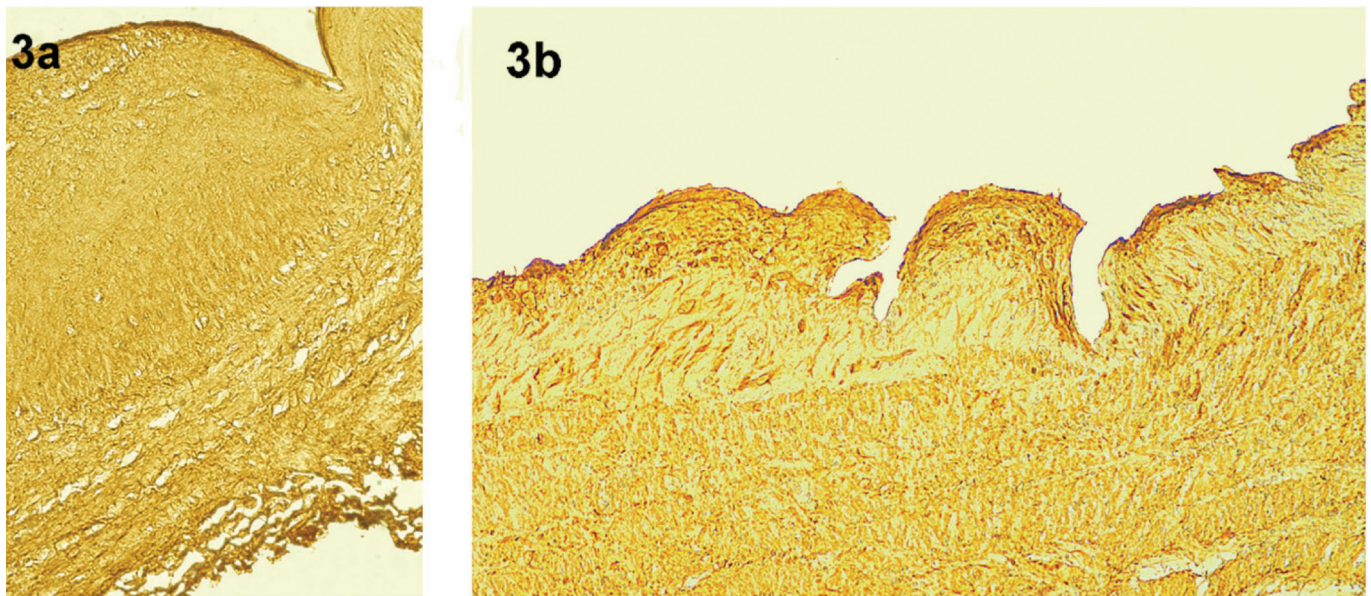


Figure 3. Vascular endothelial growth factor (VEGF) immunostaining was apparent in all layers of the 2 groups. The conventional-harvest group (A) showed strong VEGF immunostaining and detachment of the connective tissue in the tunica media and the tunica adventitia, compared with the no-touch group (B). Vessel integrity was better preserved in the no-touch group (original magnification  $\times 100$ ).

VEGF immunocytochemical staining was observed in the 3 layers in both the conventional-harvest and no-touch groups. VEGF immunoreactivity was more evident in the conventional-harvest group (++++) than in the no-touch group (++) (Figure 3). Tissue detachment was evident in the wall of the tunica media and tunica adventitia in the conventional-harvest group, and morphological structure was better preserved in the no-touch group.

#### *Electron Microscopy Evaluation*

The electron microscopy evaluation revealed distortion in the morphologic integrity of endothelial cells in the conventional-harvest group, as well as some cell separations and fractionations (Figure 4A). We also observed grade 4 splitting from the basal membrane (Figure 4B). The deterioration in the integrity of the vessel wall and the tearing in the vessel wall are remarkable (Figure 4C). Endothelial cell morphology was preserved in the saphenous vein sections obtained with the no-touch technique. The nucleus and cytoplasm of endothelial cells were evident, and their appearance was preserved within the normal limits of the original squamous epithelium (Figure 5A). Endothelial cells were distinctly attached to the basal membrane (grade 1). Moderate contraction was observed in smooth muscle cells in the intima layer (Figure 5B). The vasa vasorum was evident in the adventitia layer (Figure 5C). Our analysis indicated that, overall, saphenous vein layers in the conventional-harvest group exhibited discontinuity and detachment of the vascular wall (Figure 5D). The 2 groups were determined to be significantly different with respect to the grade of cell damage ( $P < .001$ ).

## DISCUSSION

Saphenous vein is the graft material most frequently used for coronary artery bypass operations worldwide. Vein grafts are reportedly exposed to histopathologic changes, including decreases in the contractile phenotype and the proliferation of smooth muscle [Kockx 1992]. Mural ischemia occurs because of the loss of vasa vasorum, and changes in the tension and pressure of the vessel wall play a role in this process [Karayannacos 1980]. Supportive tissue around the saphenous vein graft maintains the perfusion of saphenous vein tissue. The vasa vasorum, which has connections with supportive tissues, provides perfusion. The macroscopic mass of supportive tissue is less in the distal part of the leg; however, scraping all of the tissues from the saphenous vein would damage perfusion of vein tissue. Consequently, we preserved the surrounding tissue as much as possible for all saphenous vein graft segments [Rueda 2008]. Moreover, spasm occurs during preparation of the graft generally and in the postoperative period rarely [Ramos 1976]. Structural changes occurring during preparation can be prevented by priming in various pharmacologic solutions, and spasm can be avoided by inflating veins at a particular pressure with these solutions [He 1993]. The main goal of the no-touch technique is not to touch the saphenous vein graft during harvesting. One of the main causes of spasm is manipulation of the saphenous vein graft; therefore, the application of antispasmodic agents was unnecessary with the no-touch technique.

In coronary bypass surgery, the excessive distension applied during preparation of a saphenous vein graft to relieve spasm can cause both degenerative changes in the vein

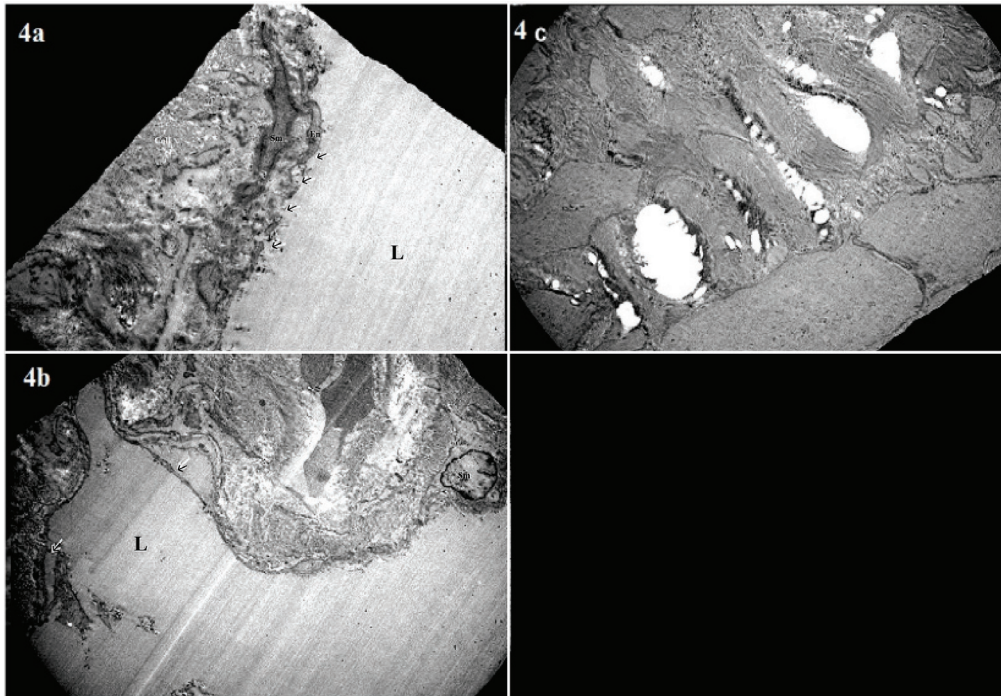


Figure 4. A, Transmission electron microscopy images for the conventional-harvest group demonstrated larger areas of endothelium with both denudation and discontinuity of endothelial cells (arrows). B, Detachment of the basal membrane from endothelial cells was apparent (arrows). Because the saphenous vein was harvested without the perivascular nerves (axons) and the adventitial layer, the smooth muscle cells in the subintimal layer were in the relaxed position. C, In all the saphenous vein sections, the layers of the vascular wall indicated discontinuity and detachment (original magnification  $\times 100$ ). L indicates lumen; En, endothelium; Sm, smooth muscle cell.

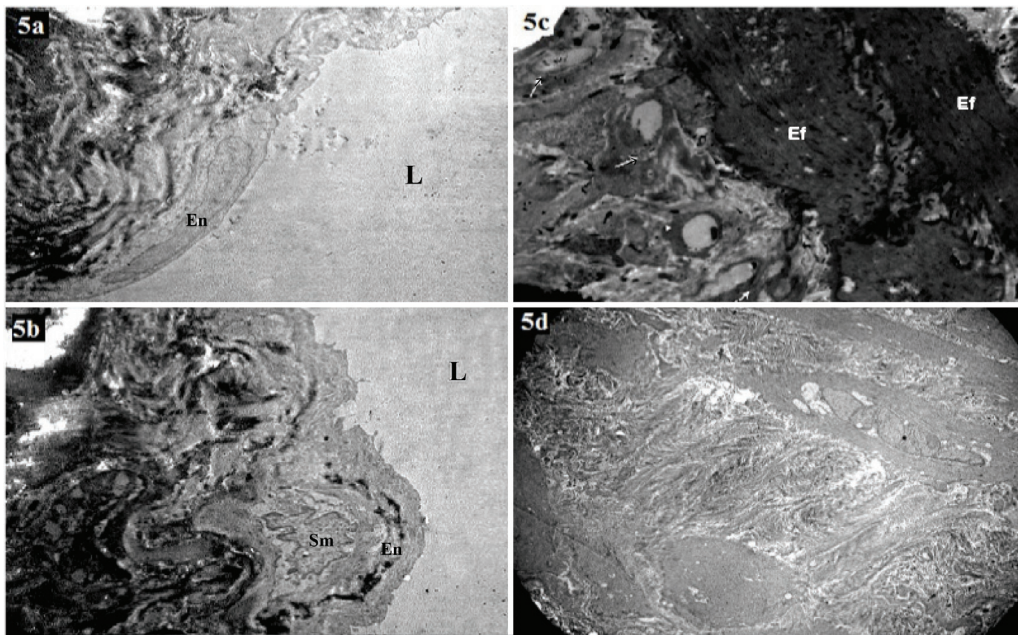


Figure 5. Transmission electron microscopy images of the no-touch group. The images of the sections demonstrated intact endothelium. A, Endothelial cells showed prominent microvilli and improved continuity compared with the conventional-harvest group. B, Smooth muscle cells in the subintimal layer were in the contraction state. C, Because this process of harvesting the saphenous vein retained the perivascular nerves (axons) and the adventitial layer, the vasa vasorum in the adventitial layer was maintained (arrows). D, The layers of the vascular wall indicated better vessel integrity and continuity for all saphenous vein sections (original magnifications  $\times 5000$ ,  $\times 5000$ ,  $\times 5000$ , and  $\times 2500$  for A-D, respectively). L indicates lumen; En, endothelium; Sm, smooth muscle cells; Ef, elastic fibers.

wall and endothelial damage. In addition, the trauma that probably occurs during vein cannulation and the scraping of tissues around the vein can cause dissection of the vessel and some dehiscence and deterioration of the connective tissues in the tunica media and tunica adventitia. Endothelial damage during graft preparation is an important cause of early and late graft failure. The loss of endothelium may lead to an acute but reversible temporary inflammatory cell reaction and to edema in the intima and media. Fibrin and thrombus accumulate on the intimal surface. For 4 to 6 weeks following surgery, the proliferation of smooth muscles, fibroblasts, and endothelial cells produce intima thickening. The endothelial damage triggers thrombus, along with platelet and fibrin accumulation. At the same time, platelet-produced growth factor causes proliferation of smooth muscle cells in the tissue between the intima and media, leading to stenosis of the lumen. These processes lead to increased lipid accumulation on the vein wall over the long term and accelerate graft atherosclerosis [He 1993; Gollidge 1997]. These pathologic changes might also occur with arterial tension. In studies conducted in 1999 and 2008, however, Souza et al [1999] compared 3 methods for harvesting the saphenous vein: the conventional method, an intermediate method (scraping away surrounding tissues and cannulating without inflating), and the no-touch method (harvesting the vein along with peripheral supporting tissue). These studies demonstrated that arterial pressure on the saphenous graft wall does not cause such inflammatory changes [Souza 1999; Rueda 2008]. Furthermore, these investigators argued that the graft endothelium is best protected by using the no-touch technique, which harvests the saphenous vein along with peripheral supporting tissues without touching the vein, and maintained that endothelium integrity is fully preserved with this method. The potential for damaging the saphenous vein and simultaneously producing numbness and torpidity at the operation site during harvesting has been thought to be a drawback of the no-touch technique. Therefore, it is necessary to take care not to harm the saphenous nerve during the procedure. Saphenous nerve damage was not detected in either group in this study. The literature indicates that intimal damage can occur when a pressure >100 mm Hg is applied [Gundry 1980; Hausmann 1996; Karabulut 1998]. Although we monitored the mechanical-distension level while we harvested saphenous veins with the no-touch method and kept the pressure at 100 mm Hg during the study, the pressure is not measured routinely, and the degree of barotrauma varies from surgeon to surgeon. In addition, we preferred clipping subbranches of the saphenous vein. This approach is technically easier and less damaging to the saphenous vein than ligation with silk thread [Sanisoglu 2011].

Our study using immunohistochemical staining to evaluate the effects of all these variables on the saphenous vein has revealed that the immunoreactivities or expression of adenosine receptor, iNOS, and VEGF molecules released from the vessel wall were significantly higher in the conventional-harvest group. We believe that the increase in expression of these factors (as reflected as increases in staining density) activated one of the protection mechanisms of smooth muscle cells and

endothelial cells when they respond to the interventions performed on the saphenous vein during conventional harvesting and that these responses include increased synthesis of adenosine, iNOS, and VEGF molecules.

We have presented immunohistochemical-staining results showing that the immunoreactivities or synthesis of adenosine receptor, iNOS, and VEGF molecules (as demonstrated by staining density) produced during saphenous vein harvesting in the no-touch group are at normal levels or lower than when the conventional-harvest method is used.

Our electron microscopy observations show that the interventions performed during conventional saphenous vein harvesting (such as those used in the conventional-harvest group) caused morphologic damage to endothelial cells. This damage included tears and breaks in the vessel wall. Contractions in endothelial and smooth muscle cells were not observed, because of vasodilatation caused by the increase in the synthesis of adenosine, iNOS, and VEGF released from the vessel wall. Such changes could also be due to damage to the neural network on the vessel wall. We found that endothelial cells preserved their normal morphology and structure in the no-touch group, the integrity of the vessel wall was maintained, and both smooth muscle cells and endothelial cells retained their moderate contractile capacity. In addition, the vasa vasorum was maintained in the adventitia layer, owing to harvesting the saphenous vein with its surrounding tissue, or pedicle. The retention of the stability and integrity of the vessel wall, signs of normal cellular contraction, and the lack of cellular damage demonstrated that the no-touch technique did not cause mechanical or pressure damage to the vessel wall. Our light microscopy and electron microscopy evaluations showed that use of the no-touch technique for saphenous vein harvesting is better than the conventional-harvest method in terms of protecting the morphologic structure of the vessel wall.

Questions have arisen because of the significant histologic differences we observed between the 2 methods we evaluated in our study: (1) Does systemic arterial hypertension eliminate the histologic advantages of grafts harvested with the no-touch technique? (2) How much do the histologic results reflect on clinical outcomes? Systemic arterial pressure is different from the pressure applied to the saphenous vein during harvesting with the conventional technique. Arterial pressure is intermittent, and the pressure applied to the saphenous vein is not. For most patients, the mean arterial pressure is <100 mm Hg in the intensive care unit after anesthesia induction and surgery. Our observations of the general patient population following operations are that the systolic blood pressure is between 90 and 120 mm Hg, the diastolic pressure is between 65 and 90 mm Hg, and the mean arterial pressure is <100 mm Hg. These values can be due to the effects of therapy with antihypertensive medications. Moreover, another cause of mechanical trauma (rather than barotrauma) is inter-layer separation and dehiscence (especially on the media and adventitia) caused by the pulling occurring during the scraping of tissues around the saphenous vein.

The best method for investigating the effects of the between-group histologic differences on clinical outcomes is to perform coronary angiography in long-term follow-up

evaluations. Rueda and colleagues divided saphenous vein harvesting methods into 3 groups (no-touch, conventional-harvest, intermediate). They concluded from their results for coronary angiography examinations performed approximately 18 months after surgery that the rate of graft patency would be higher in the no-touch group [Souza 2001].

## CONCLUSIONS

The light microscopy and electron microscopy imaging data we obtained in this study have shown that saphenous vein grafts harvested with the no-touch method for coronary bypass surgery are less prone to mechanical trauma than saphenous vein grafts prepared with conventional-harvest method. Therefore, we believe that saphenous vein grafts can be harvested with greater quality and at a lower risk for thrombus formation occurring in the early postoperative period. We also expect the subsequent risk of subendothelial fibromuscular hyperplasia and atherosclerosis formation in the late period to be decreased, leading to higher patency rates.

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