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A Comparative Study of Endothelial Cell Injury During Open and Endoscopic Saphenectomy: An Electron Microscopic Evaluation

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ABSTRACT

Background: The role of damaged endothelium in early graft occlusion has been extensively demonstrated. Seventy percent of early graft occlusions in coronary artery bypass were caused by thrombi overlying areas of endothelial loss. With the increased use of endoscopic vein harvesting, it becomes important to study the extent of endothelial damage by light and electron microscopy. In this study, we compared the degree of endothelial damage inflicted by the open and endoscopic techniques of vein harvesting using light, scanning, and transmission electron microscope.

Material and Methods: Ninety samples of saphenous veins from 45 patients prepared for coronary artery bypass grafting (CABG) utilizing both endoscopic and standard open incision techniques were examined using light, scanning, and transmission electron microscopy. These vein samples were prepared in Plasma-lyte solution (Baxter) in combination with or without papaverine, at two distending pressures of 100 or 300mmHg and at temperatures of either 4°C or 28°C in eight subgroups and one control group. The pathological alterations in the saphenous veins were graded either based on a scoring system (0 = none, 1 = < 10%, 2 = 10-25%, 3 = 25-50%, 4 = > 50%) to assess the degree of damage inflicted by these two different types of saphenectomies or by electron microscopic observed abnormalities, including endothelial cell (EC) separation, EC detachment, basement membrane (BM) exposure, collagen exposure, and EC edema.

Results: Using cross-tabulation and Chi-square statistical analysis, we found that the differences in the degree of endothelial damage using either of the techniques is not statistically significant ($P > 0.05$).

Conclusion: Our findings indicate that endoscopic and open saphenectomies are technically comparable as far as structural damage is concerned, rendering the endoscopic technique of vein handling the preferred method for CABG.

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INTRODUCTION

Saphenous vein (SV) bypass grafting remains routine in most cardiac practices despite increased use of arterial conduits. The long SV that is used as a conduit for coronary revascularization is most commonly harvested by means of a "long cut" over the length of the vein in the thigh or leg. This usually provides an adequate vein for subsequent coronary artery revascularization [DeLaria 1981].

Although harvesting SVs using a longitudinal or skin bridging technique infrequently results in major morbidity (sepsis or limb amputation), minor wound complications such as dehiscence, excessive drainage, delayed healing, hematoma, cellulitis, lymphangitis, and superficial or deep infections occur in 1% to 24% of patients [Allen 1998]. These complications may require prolonged surgical management, including hematoma drainage, debridement of necrotic tissue, and repeated surgical dressings or even

Table 1. The scale of the endothelial damage in both techniques of saphenous vein harvesting

Scale of damage to endothelium	Endoscopic technique (Number)%	Open technique (Number)%
0	(10) 22.22%	(9) 20.00%
1	(8) 17.78%	(10) 22.22%
2	(8) 17.78%	(7) 15.56%
3 & 4	(19) 42.22%	(19) 42.22%

skin grafting [Meldrum-Hanna 1986]. Specific predictors of an unacceptable surgical result include female sex, diabetes mellitus, peripheral vascular disease, obesity, and anemia [Utley 1989].

The most important complication of SV bypass grafting is loss of graft patency. Early occlusion is thought to be due to thrombus formation [Lawrie 1976]. The occlusion rate of SV grafts in the first year is reported to range from 10% to 26% [FitzGibbon 1986]. It is also known that endothelial injury is a causal and initial stage in the development of atherosclerosis [Bjork 1981]. By 10 years, 50% of the grafts are occluded and of the rest of the grafts that were patent, 50% showed marked atherosclerotic change [Grondin 1984].

The integrity of the endothelial lining is affected by many factors, among them technique of harvesting and preservation solution. Meticulous preservation of the endothelial lining of vein grafts harvested during vascular operations is undoubtedly an important factor in determining patency rates following bypass procedures. Intact, confluent endothelium serves as an electrical, mechanical, and physiological barrier between flowing blood and the subendothelium. Destruction of the endothelial lining of the vein graft prior to graft implantation results in a more thrombogenic graft, which essentially becomes a collagen-lined tube [Cunningham 1981]. Disruption of this barrier may also result in occlusive medial smooth muscle cell proliferation and intimal migration. In addition, normal endothelium produces prostacyclin [Allen 1998] and exhibits plasminogen-dependent fibrinolytic activity [Todd 1972, Logerfo 1981].

Table 2. The electron microscope abnormality in both techniques of saphenous vein harvesting, odds ratio, Chi-square, and p-value

EM abnormality	Endoscopic (no.)%	Open (no.)%	Odds Ratio	Chi-square	p-value
EC separation	(16) 35.56%	(13) 28.89%	1.23	0.458	0.499
EC detachment	(18) 40%	(14) 31.11%	1.29	0.776	0.378
BM exposure	(12) 26.66%	(10) 22.22%	1.2	0.241	0.624
Collagen exposure	(10) 22.22%	(10) 22.22%	1.0	0.00	1.0
EC edema	(8) 17.77%	(5) 11.11%	1.6	0.809	0.368

EM = electron microscopy, EC = endothelial cell, BM = basement membrane

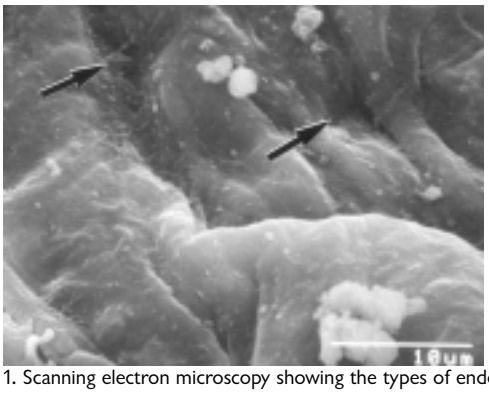

Figure 1. Scanning electron microscopy showing the types of endothelial cell injury (endothelial cell separation) of saphenous vein specimen

Because aorto-coronary bypass grafting is the most common cardiac surgical intervention in the United States, any technique that could reduce the complication rate would have a dramatic impact on the health care delivery system [Cable 1998]. With the introduction of endoscopic saphenous vein harvesting, complications are much less common compared with the open technique [Utley 1989, Cable 1998].

The purpose of this study was to examine multiple indicators of endothelial cell (EC) damage using the electron microscope (EM), and to conduct a comparison between the endoscopic and open techniques of SV harvesting with respect to the presence of each indicator.

MATERIALS AND METHODS

Vein Retrieval

This study was done at Maimonides Medical Center from November 1998 to May 1999. Saphenous veins were harvested from 45 patients undergoing CABG. The veins were harvested and handled under sterile conditions according to the operating room protocol at the Center. In the traditional technique of open (no-touch) vein harvest-

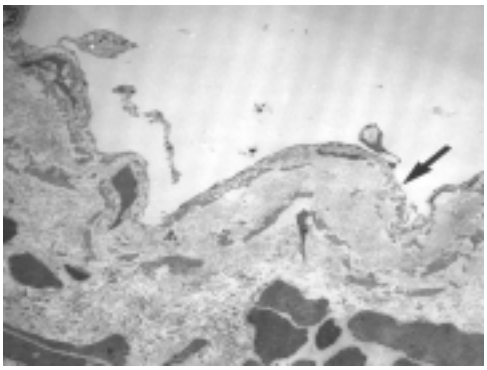

Figure 2. Transmission electron microscopy showing the types of endothelial cell injury (endothelial cell separation) of saphenous vein specimen

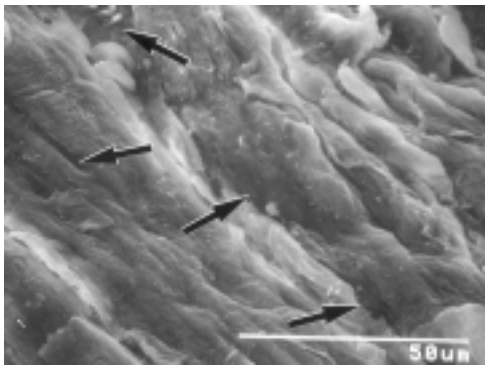

Figure 3. Scanning electron microscopy showing the types of endothelial cell injury (endothelial cell loss) of saphenous vein specimen

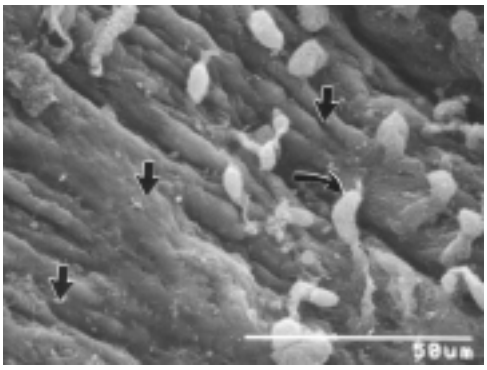

Figure 5. Scanning electron microscopy showing exposed basement membrane and collagen of the harvested saphenous vein

ing, the greater saphenous vein was exposed and harvested under direct vision through a long continuous skin incision, with the patient's leg in a "frog-leg" position. In the endoscopic technique, a small incision was made four fingers' breadth posterior to the proximal margin of the patella, the greater saphenous vein was identified, dissected both cranially and distally under endoscopic visualization through the incision, and then stripped and retrieved. The standard instrumentation used in the procedure is commercially known as the Endo-Path (Ethicon Endo-Surgery, Inc., Cincinnati, OH). It is comprised of a subcutaneous dissector, retractor, and modified vein stripper. In addition, standard endoscopic devices, including a television monitor, light source, fiber-optic camera, and a 5-mm lens, were used.

In our study, we sampled two vein segments from the same leg of each patient using the two harvesting techniques. This was done by excising a five centimeter segment of the thigh portion of the saphenous vein, cranially dissected and retrieved endoscopically, as the endoscopic sample. The sample was clipped distally for orientation of blood flow direction. At the same time, the vein segment remaining at the site of the incision, about seven centimeters in length, was excised as the direct or open sample.

Vein Preparation

Vein samples were incubated in 10 ml Iscove's Modified Dulbecco's Medium (IMDM) with 200mL penicillin-streptomycin during transport to the laboratory.

In the laboratory, each vein sample was flushed, cannulated, and injected with Plasma-lyte solution (Baxter), and the branches were ligated with 3.0 silk sutures. Each vein was then divided into two pieces using a sterile scalpel (no. 15 blade). The proximal piece was used for endothelial cell culture (results published elsewhere), and the distal one for microscopic analysis (light and electron microscopy, both scanning and transmission).

Procedure for Light and Electron Microscopic Evaluation of Endothelial Cells

Transmission Electron Microscopic Preparation: Description

The vessels were submerged in fixative containing 3% gluteraldehyde with a 0.2M sodium cacodylate buffer at pH 7.4. We received and washed the vessels in a 0.2M cacodylate buffer. Each vessel was then dissected into a single cross-section at the middle of the vessel, which was designated for embedding in epoxy perpendicular to the plane of the lumen for transmission electron microscopy

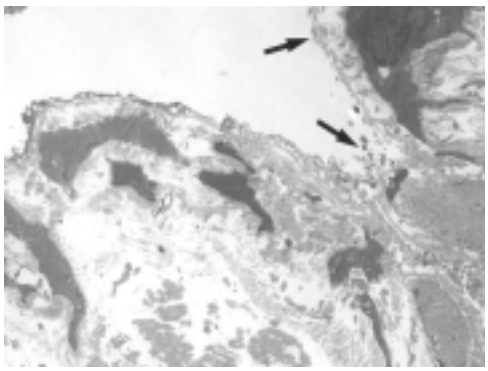

Figure 4. Transmission electron microscopy showing the types of endothelial cell injury (endothelial cell loss) of saphenous vein specimen

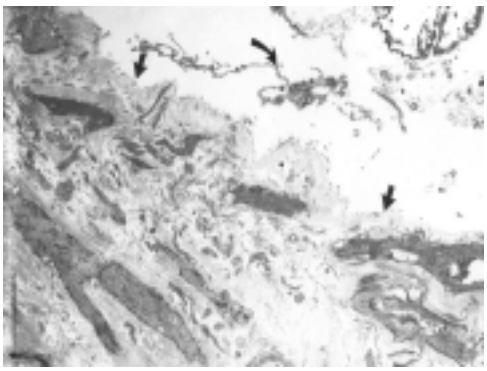

Figure 6. Transmission electron microscopy showing exposed basement membrane and collagen of the harvested saphenous vein

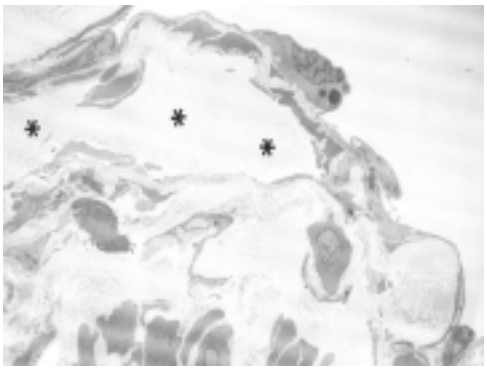

Figure 7. Transmission electron microscopy showing intimal edema of the harvested saphenous vein

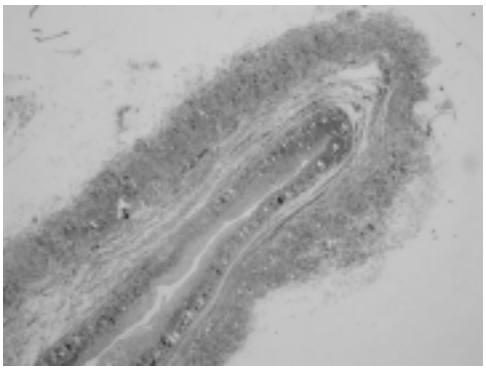

Figure 9. Light microscopy showing intimal and medial tear of the harvested saphenous vein

(TEM) of thin sections. The remaining two segments were bisected longitudinally to expose the luminal surface of the vessel. These specimens were designated for scanning electron microscopy (SEM). All the vessels were treated for one hour with 1% osmium tetroxide, dehydrated in graded steps of ethanol, and only the TEM specimens were processed through propylene oxide and embedded in Embed 812. Representative areas for ultrathin sections were chosen by light microscopy from one-micrometer plastic sections stained with methylene blue and azure II. Ultrathin sections were stained with uranyl acetate and lead citrate. The vessels were observed with a JEM 100CX TEM and assessed by criteria described below.

Scanning Electron Microscopic Preparation: Description

The saphenous vein sections were treated the same as for TEM but removed after dehydration in 100% ethanol. They were then placed in the critical point drier where the alcohol was exchanged for liquid CO_2. The CO_2 was removed at the critical temperature and pressure. The specimens were removed, mounted on aluminum stubs with silver paint and lightly coated with gold palladium. The specimens were viewed with an S-530 Hitachi SEM.

The normal light and ultrastructure of the saphenous vessels were assessed. By light microscopy the endothelium forms a continuous layer of attenuated cells except where the nuclei are present. The surfaces of the cells have occasional short microvilli. There are many randomly arranged pinocytotic vesicles at the basal and apical plasma membranes. The cytoplasm of the cells also contains a fair amount of granular endoplasmic reticulum, some mitochondria, Weibel-Palade bodies, and numerous intermediate microfilaments. Adjacent cells contact one another and form leak-tight junctions and small desmosomes at the lumen. At the basal aspect the cells rest on a continuous basement membrane. There were occasional fibroblasts amidst a collagen matrix between the basement membrane (intima) and the layer of smooth muscle cells (media).

Injury was assessed by both light and electron microscopy. At the light level, the vessel was assessed for injury by increasing degree with endothelial cell separation as the mildest degree of injury then endothelial edema, endothelial cell loss, basement membrane exposure, edema, detachment, collagen exposure, intimal edema, intimal and, finally, medial tear. Scanning electron microscopy made it possible to observe much larger areas of the lumen surface than could be seen with single cross-sections through the vessel. As a result, it was possible to define the injury as being focal rather than exten-

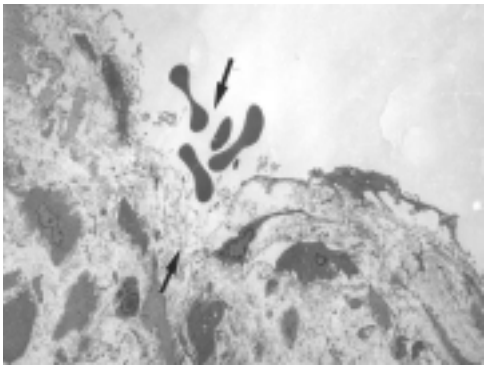

Figure 8. Transmission electron microscopy showing intimal and medial tear of the harvested saphenous vein

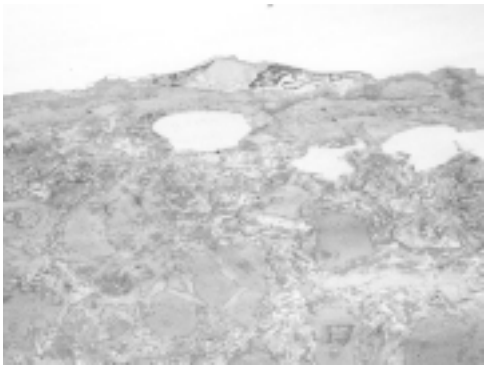

Figure 10. Transmission electron microscopy showing intimal hyperplasia of the harvested saphenous vein

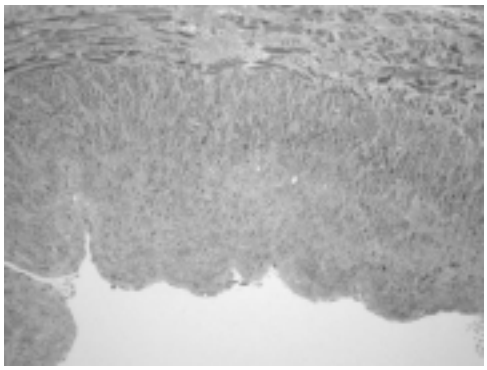

Figure 11. Light microscopy showing intimal hyperplasia of the harvested saphenous vein

sive, an observation that would not be accurate by light or transmission electron microscopy. Since it is difficult to see the subtle difference of only slight endothelial separation, lifting, edema and basement membrane damage, transmission electron microscopy of thin cross-sections of vessels were performed.

Solutions

The chemical characteristics of the Plasma-lyte solution were as follows: each 100 ml contained 526 mg sodium chloride USP, 502 mg sodium gluconate USP, 368 mg sodium acetate trihydrate USP, 37 mg potassium chloride USP, 30 mg magnesium chloride USP (sodium = 140 mEq/L, potassium = 5 mEq/L, magnesium = 3 mEq/L, chloride = 98 mEq/L, acetate = 27 mEq/L, gluconate = 23 mEq/L). The pH of the solution was adjusted with sodium hydroxide to 7.4 (6.5 – 8.0) and the osmolarity was 294 mOsmol/L.

The type and composition of the solutions used in our experiments were chosen based upon cardiac surgical practice recommendations at Maimonides Medical Center and recent surveys of North America Cardiac Surgery Centers, as well as upon recipes suggested in the current literature.

Institutional Approval and Patient Consent

The Institutional Review Board at Maimonides Medical Center approved the study, and consent was obtained from each patient included in the study.

Statistical Analysis

Statistical analysis was performed using the SAS system (© 1989-1996 SAS Institute Inc., Cary, NC). Data was analyzed using the Chi-square test for comparison of categorical outcomes.

RESULTS

The pathological alterations in the human saphenous vein were graded by a scoring system depending on the degree of damage inflicted during the vein preparation, which was evaluated by two different pathologists blindly. The results were as follows:

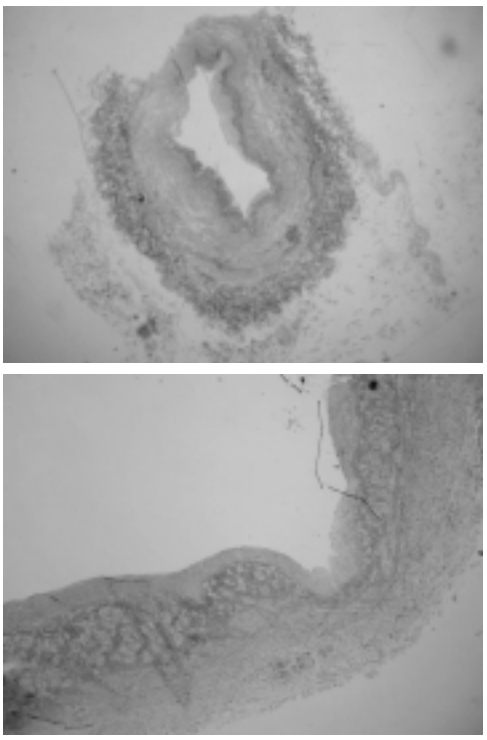

Figure 12. Light microscopic examination showing a cross-section of saphenous vein harvested by endoscopic technique (A) compared to saphenous vein harvested by open technique (B)

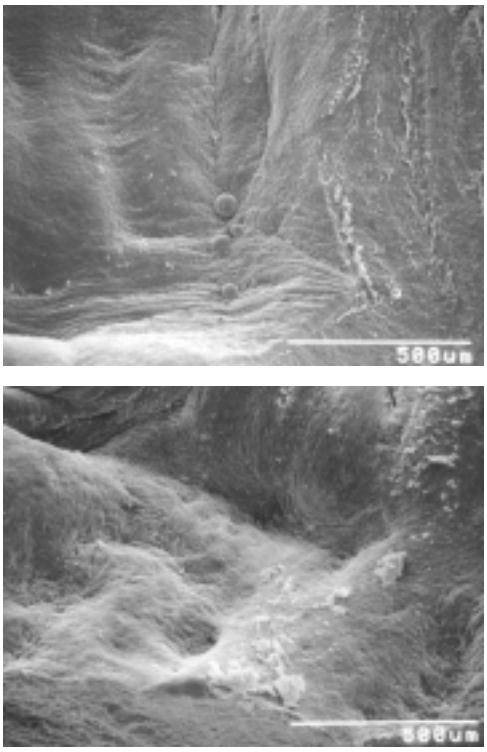

Figure 13. Scanning electron microscopic examination (low power) showing a cross-section of saphenous vein harvested by endoscopic technique (A) compared to saphenous vein harvested by open technique (B)

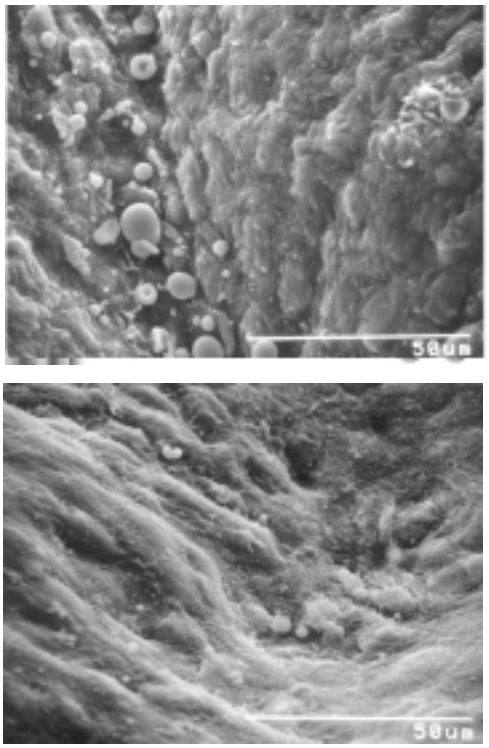

Figure 14. Scanning electron microscopic examination (medium power) showing a cross-section of saphenous vein harvested by endoscopic technique (A) compared to saphenous vein harvested by open technique (B)

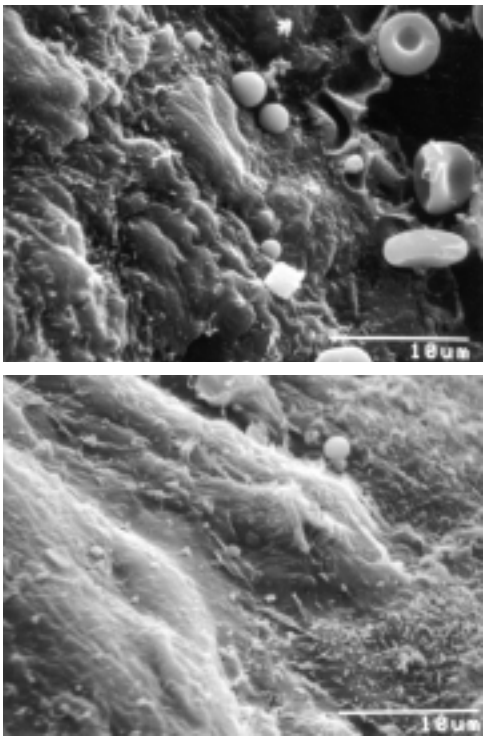

Figure 15. Scanning electron microscopic examination (high power) showing a cross-section of saphenous vein harvested by endoscopic technique (A) compared to saphenous vein harvested by open technique (B)

0 = none
1 = < 10%
2 = 10-25%
3 = 25-50%
4 = > 50%

Comparing both techniques using cross-tabulation and the Chi-square test, we found that there is no statistically significant difference in the damage of vein preparations. These criteria and the percent of damage were examined using both light and electron microscopy (Table 1, ◉).

At the same time each EM abnormality was individually analyzed and compared across the harvesting techniques (Table 2, ◉). Endothelial cell (EC) separation was present in 35.56% of the examined endoscopic samples compared with 28.89% of the open samples. The difference between the proportions of this abnormality was statistically not significant ($\chi^2 = 0.458$, $p = 0.499$). Similarly, no statistically significant difference was found between the endoscopic and open techniques with respect to the presence of EC detachment (40.00% vs. 31.11%, $\chi^2 = 0.776$, $p = 0.378$), basement membrane (BM) exposure (26.66% vs. 22.22%, $\chi^2 = 0.241$, $p = 0.624$), collagen exposure (22.22% vs. 22.22%, $\chi^2 = 0.00$, $p = 1.0$), and EC edema (17.77% vs. 11.11%, $\chi^2 = 0.809$, $p = 0.368$).

Comparison of individual electron microscopic abnormalities between endoscopic and open methods of saphenous vein harvesting is shown in Table 2 (◉) and Figures 1-11 (◉).

DISCUSSION

Following the introduction of aorto-coronary bypass grafting in 1968, the use of saphenous veins as conduits for revascularization has become an established method of treatment for symptomatic coronary artery disease [Gelbfish 1986]. The reversed saphenous vein is the most commonly used conduit for myocardial revascularization. A number of investigators have attempted to determine which harvesting technique is most successful in preserving ultrastructural integrity of venous conduits placed in the aorto-coronary position. Intraoperative structural integrity relates directly to subsequent graft patency [Roberts 1984].

It has been clearly demonstrated that early graft closure or narrowing is related to the treatment of the vein during harvesting and surgical manipulation [O'Regan 1984]. Furthermore, a relationship between early endothelial damage and late graft atherosclerosis has also been postulated. It appears that early endothelial damage exposes sub-intimal tissue to the circulating blood, enhances platelet and fibrin deposition, initiates a chronic injury-repair process, and often may be followed by fibrous and myo-epithelial proliferation [Catinella 1982].

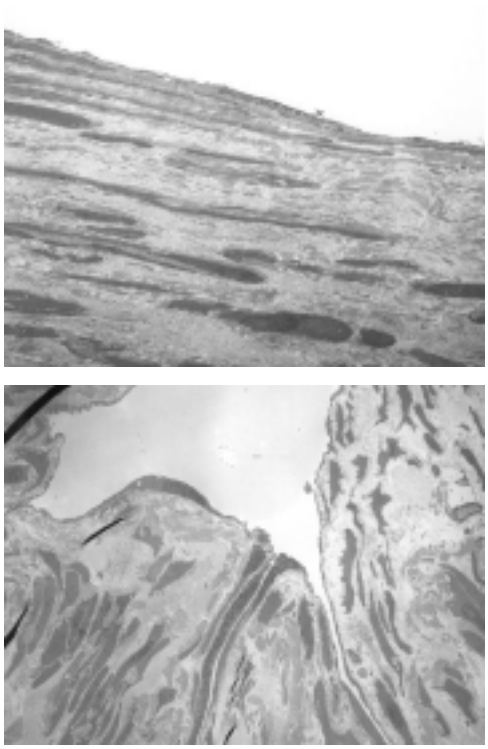

Figure 16. Transmission electron microscopic examination (low power) showing a cross-section of saphenous vein harvested by endoscopic technique (A) compared to saphenous vein harvested by open technique (B)

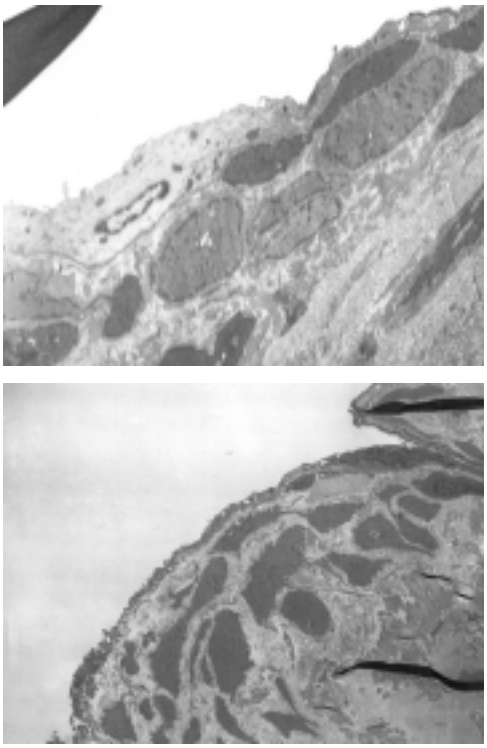

Figure 17. Transmission electron microscopic examination (high power) showing a cross-section of saphenous vein harvested by endoscopic technique (A) compared to saphenous vein harvested by open technique (B)

The major effect of endothelial damage is the exposure of highly thrombogenic subendothelial tissue to circulating platelets, primarily collagen types I and III. Exposed collagen is strongly attractive to platelets, which subsequently adhere, undergo shape change, and release thromboxane A_2, adenosine diphosphate (causing further aggregation) and platelet-derived growth factor [Gryglewski 1976, Adcock 1984].

Scanning electron microscopy is an exceptionally useful tool in studies attempting to determine the effects of various vein graft preparation techniques on vein graft ultrastructural morphology. Scanning electron microscopy permits one to observe the EC lining of much of the lumen of a vessel at one time and thus obtain an accurate overall impression of the condition of this lining [Yoder 1982, Cable 1998].

Our study is unique in that, for the first time, detailed individual parameters of endothelial cell injury to veins retrieved by the endoscopic technique are compared with those from the traditional open method of SV harvesting, using the electron microscope as a tool. The study clearly demonstrates essentially similar levels of injury resulting from surgical handling and manipulation during both techniques (see Figures 12-17, ◉).

These findings support results from earlier studies in that, despite a perceived compromise in the quality of the operative procedure and postoperative results, endoscopic harvesting remains a minimally-invasive procedure with levels of endothelial injury comparable to that in the traditional method but with less patient morbidity [Meldrum-Hanna 1986].

There are many limitations of our study that might influence interpretation of the results. The number of patients and therefore the number of vein samples taken were relatively small. Furthermore, the small counts obtained for each endothelial injury parameter might be a source of imprecise analysis. Although endothelial cell injury has been shown to correlate with vein graft patency, the long-term effect on graft patency and survival, and the contribution of other adverse factors, remain to be verified.

Acknowledgment

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