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Microscopic and ultrastructural evaluation of the saphenous vein endothelium for CABG prepared by the no touch technique

Avaliação microscópica e ultra-estrutural do endotélio de veia safena preparada pela técnica "no touch"

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Abstract

Objective: **Saphenous vein grafts (SV) used in coronary artery bypass grafting have a limited life and vein occlusion may be the final adverse effect. Efforts to develop new techniques to harvest the saphenous vein may improve the viability of the graft.**

Methods: **Twenty patients were randomly divided into two groups with the objective of evaluating the vascular endothelium. The No-touch (NT) technique consists in removing the saphenous vein with perivascular tissue. The conventional technique consists in harvesting with "in situ" removal of the perivascular tissue. The standard saphenous vein harvesting procedure used bridged incisions. Characteristics of the vein were considered. Evaluation of the endothelium was achieved by electron microscopy and histologic analysis using hematoxylin eosin staining. The Picrosirius and Masson's Trichrome methods were used to analyze subendothelial collagen.**

Results: **Electron microscopy demonstrated that the NT Group had larger non-denudated endothelial areas as well as a smaller number of degraded cells. Histological analysis showed the form and integrity of the saphenous vein layers. A larger amount of collagen fibers were identified in the NT Group.**

Conclusions: **The NT technique better preserves the saphenous vein endothelium suggesting a more viable graft in the long term.**

Descriptors: **Saphenous vein. Myocardial revascularization. Endothelium, vascular. Coronary artery bypass.**

Resumo

Objetivo: **O enxerto de veia safena (VS) utilizado em revascularização miocárdica possui uma vida útil, sendo o estágio final a oclusão do vaso. Esforços em adquirir novas técnicas de coleta da VS podem possibilitar uma viabilidade maior do enxerto.**

Métodos: **Vinte pacientes foram randomizados e divididos em dois grupos com o objetivo de avaliação do endotélio vascular. A técnica "no touch" (NT) consiste em retirar o segmento de VS com o tecido perivascular. A técnica convencional consiste em retirar a VS, com remoção "in situ" do tecido perivascular**

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e conseqüente vasoespasmo. Houve um padrão de retirada das VS com incisões longitudinais escalonadas. Características da VS foram consideradas. A avaliação do endotélio das VS foi realizada usando microscópio eletrônico (ME) pelo método de varredura e de transmissão. Cortes histológicos das VS foram corados em Hematoxilina-Eosina (HE). O colágeno subendotelial foi analisado pelos métodos de Picro-Sirius e Tricrômio de Masson.

***Resultados:* A ME evidenciou que o Grupo NT possui maiores áreas endoteliais não desnudadas, além de um menor número de células degradadas. A coloração em HE nos permitiu verificar a forma e a integridade das camadas das VS. Há um predomínio maior de fibras colágenas coradas no Grupo NT.**

***Conclusões:* A técnica NT permite uma melhor preservação endotelial da VS, sugerindo um enxerto mais viável em longo prazo.**

***Descritores:* Veia safena. Revascularização miocárdica. Endotélio vascular. Ponte de artéria coronária.**

INTRODUCTION

Atherosclerotic artery disease is the most common cause of morbidity and mortality in industrialized nations. Around the world, more than one million people die annually from coronary artery disease [1].

Coronary artery bypass grafting (CABG) is one of the most frequent procedures performed worldwide, with, over the last three decades since the first direct CABG, many advances being introduced principally in respect to the concepts concerning atherosclerosis, technology and surgical technique.

One of the key elements to long-term success of CABG is the choice of the ideal graft. Analyses, such as the state of native coronary arteries, the co-morbidities, the clinical presentation and the possibility of flow steal are all important in the choice of the graft to be employed. The most commonly used grafts are the left internal thoracic artery (LITA) (mammary); the right internal thoracic artery (RITA); the radial artery (RA); gastroepiploic artery (GA); inferior epigastric artery (IEA) and the great saphenous vein (SV) [2].

The use of the saphenous vein (SV) as a coronary graft, introduced in the 1960s, greatly changed the situation of patients with ischemic heart disease by providing a better quality of life and increasing survival. However, the limited patency of SV grafts is associated with the reoccurrence of angina [3], as well as being one of the main reasons for reoperation.

It has been demonstrated that approximately 30% of SV grafts occlude within the first postoperative year and in ten years the occlusion rate may be as high as 50% [4].

The utilization of arterial grafts has proved to have better results in terms of long-term patency, in particular the LITA utilized to revascularize the anterior interventricular artery. Despite of this, the SV continues to be widely used in CABG as the number of available adequate arterial grafts is limited, thereby restricting complete arterial revascularization.

In this setting, it has become essential to research methods to optimize the results obtained with the currently utilized grafts, in particular with the SV.

The preparation method of the vein plays an important role and the dissection technique may significantly influence the result obtained, as mechanical trauma may harm the endothelial integrity with consequent morphofunctional damage [5].

Conventionally, during SV harvesting, the perivascular tissue is removed from the vein with a consequent vasospasm, which is overcome by intentional mechanical distention of the vein. These procedures result in significant denudation of the endothelial surface [6].

Recently a new surgical technique has been described in which the SV is harvested together with the perivascular tissue by minimal handling of the vessel [7]. This technique, denominated “No-touch”, prevents vasospasms as well as eliminates the necessity of distention of the vein. When compared to the conventional method, this technique demonstrated a significant increase in the long term vascular functionality of the venous graft [8].

This study aims, by means of histological and ultrastructural studies, to reinforce the evidence that the so-called “No-touch” technique of SV harvesting is better then the traditional methods.

METHODS

After the project was approved by the Research Ethics Committee of the Federal University of Sao Paulo – Escola Paulista de Medicina (protocol nº 0984/05), twenty patients were randomized in two groups. The patients were divided in Conventional and “No-touch” Groups with ten patients in each. The selection process of the patients considered the possibility of long-term follow ups. Exclusion criteria of the study included: patients with ischemic disease who would be submitted to off-pump CABG and who presented other diseases, such as neoplasms and immunodeficiency among others, that may alter the hemodynamic pattern of the patients and mask the results.

Preparation technique of the vein

After a review of publications, a surgical technique to harvest the vein for CABG was proposed using the "No-touch" technique, which minimizes aggression.

Study groups

"No-touch" Group

The technique denominated "No-touch" consists in harvesting a SV segment from the leg with the perivascular tissue. This eliminates the handling of the intimal region around the SV so that possible molecular factors produced by that tissue might be preserved (Figure 1).

Fig. 1 - A segment of saphenous vein by the No-touch technique

Conventional Group

The conventional technique consists of the harvesting of the SV with the perivascular tissue being removed "in situ" with consequent vasospasm that is overcome by intentional mechanical distention of the vein. These procedures result in a significant denudation of the endothelial surface (Figure 2).

The SV was harvested for all patients using the bridge technique making the surgery more esthetically acceptable and reducing the risks of local infection.

Fig. 2 - A segment of saphenous vein by the conventional technique

Surgical technique

Anterograde cardioplegia (4ºC) through the coronary ostium with extracorporeal circulation and moderate hypothermia (28ºC to 30ºC) were routinely used. To prevent bleeding at the anastomoses, collateral vessels of the veins were ligated with 4.0 cotton thread. The distal anastomoses were performed first and the proximal segments were later ligated at the aorta. The internal thoracic artery was utilized for the anterior interventricular artery graft. Characteristics of the vein, such as the quality and origin, were noted.

Ultra-structural evaluation

Morphological analysis of the endothelium of the veins was achieved using electron microscopy. For this, a 0.5-cm sample of the proximal segment was collected and fixed in an appropriate reagent. The segment was immersed in a flask containing a solution of 2.5% glutaraldehyde and placed in a thermal box cooled to 4ºC. This was then taken to the Electronic Microscopy Center of UNIFESP-EPM. Here it was prepared by a specialized technician and analyzed under a scanning microscope to visualize the endothelial surface and also an immersion microscope to see the margins and cellular content.

Microscopic evaluation

Another two 0.5-cm segments (proximal and distal) of the SV were collected and immersed in a flask containing 10% formaldehyde. The two samples were taken to the Research Sector of the Pathology Department of UNIFESP-EPM and labeled. The veins were cross-sectioned and embedded in paraffin. The samples were then sectioned using microtomy and slides were prepared with hematoxylin eosin (HE), Picrosirius and Masson's Trichrome. For each paraffin block, three slides were prepared, one with each reagent.

HE identifies the general structures of the SV, categorizing vascular layers and possible cell deposits. Both Picrosirius and Masson's Trichrome are used to stain the subendothelial collagen.

The slides were assessed by a pathologist who was unaware of which group the slides belonged.

Pilot study

To optimize the sampling, surgical technique and preparation of the slides, a pilot plan was elaborated. Ten veins were collected and prepared as already described. This material permitted us to test the reagents, optimizing their use and to identify errors during the collection and preparation of the material and enabled us to inform the surgeons of the best method to remove the segments.

RESULTS

The electronic microscopic analysis of the surgical samples demonstrated that the "No-touch" Group had larger areas of endothelium without denudation, as well as a smaller number of cells degraded in the handling process (Figures 3 to 6).

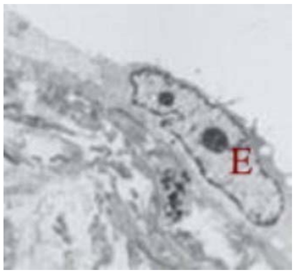

Fig. 3 - Immersion microscopy photograph demonstrating a preserved endothelial cell (E)

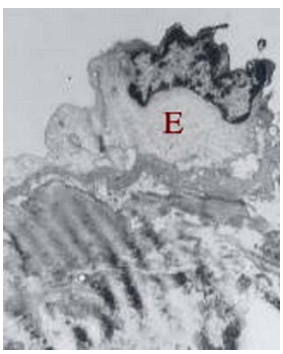

Fig. 4 - Immersion microscopy photograph demonstrating a damaged endothelial cell (E)

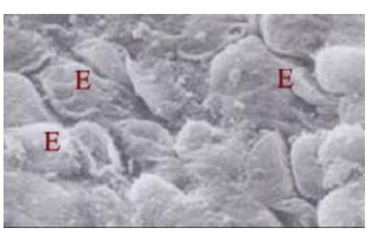

Fig. 5 - Scanning microscopy photograph demonstrating an area with preserved endothelial cells. Endothelial cell (E)

Fig. 6 - Scanning microscopy photograph demonstrating an area denudated of endothelial cells. Endothelial cell (E)

Staining with HE demonstrated the presence of all the SV layers. This is important as it excludes factors related to trauma or possible reactions with the reagent used to preserve the sample that might interfere with the vascular layers, as well as to give us an idea of the aspect of the veins. The Conventional Group is characterized by a narrower lumen and by greater injury to the intimal layer, probably due to the manual manipulation of the sample (Figures 7 & 8).

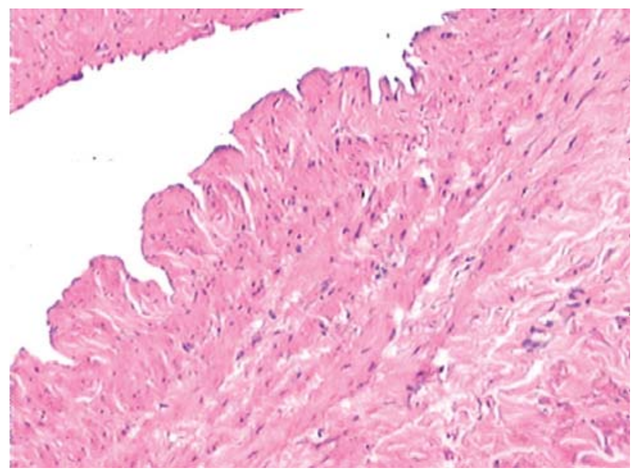

Fig. 7 - Cross-sectional slice of saphenous vein harvested using the conventional technique stained with HE. Note the split lumen, characteristic of vasospasms due to pressure distention

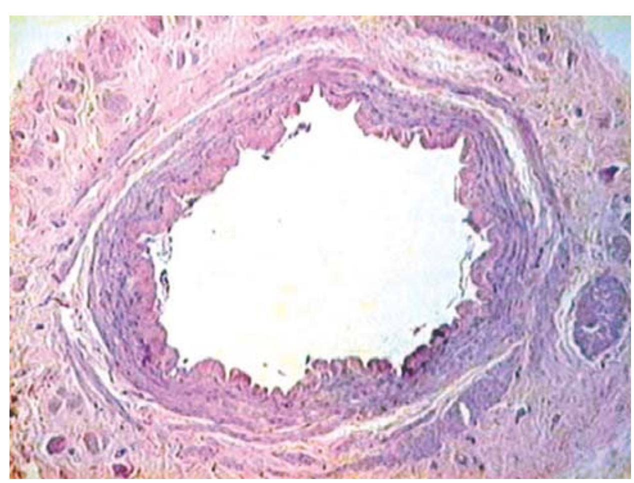

Fig. 8 - Cross-sectional slice of saphenous vein harvested using the No-touch technique stained with HE. Observe the oval format of the lumen and the continuity of the intimal layer

The analysis of the slides prepared to evaluate the subendothelial collagen showed that there is a higher predominance of stained fibers in the "No-touch" Group. This demonstrates that there is less degradation of the SV when they are not manipulated and distended at high pressure. The photographs of the slides showed more homogenous areas in the "No-touch" Group, which is a factor of preservation of the endothelial integrity (Figures 9 & 10).

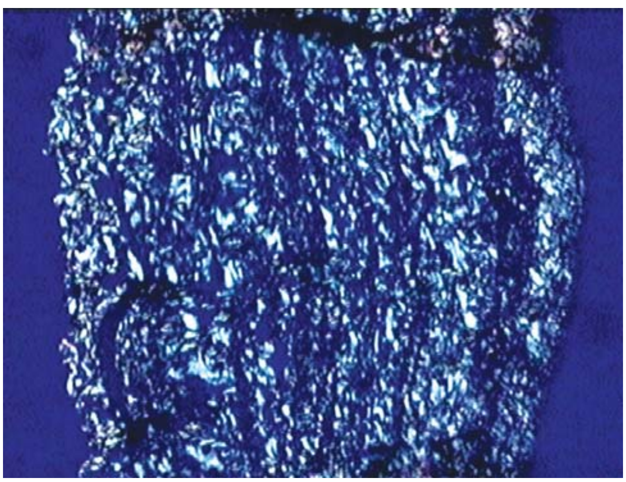

Fig. 9 - Cross-sectional slice of saphenous vein harvested using the conventional technique stained by the Masson's trichrome method and observed by contra-staining

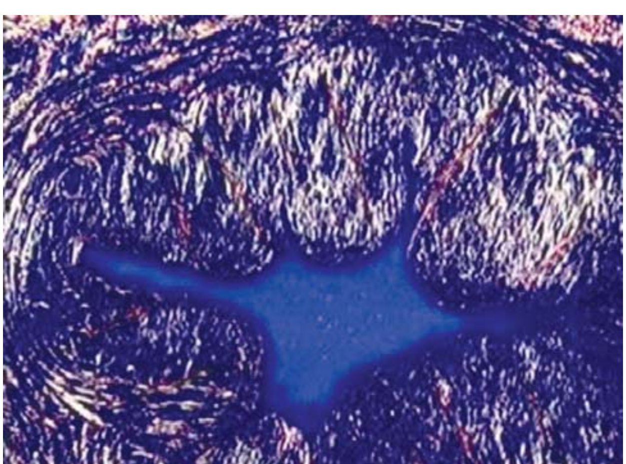

Fig. 10 - Cross-sectional slice of saphenous vein harvested using the No-touch technique stained by the Masson's trichrome method and observed by contra-staining. Observe the greater brilliance emitted by the staining in this photograph compared to Figure 9, thus demonstrating a higher number of preserved collagen fibers

DISCUSSION

The endothelial cells form a single layer that lines all the vascular system. Its structural and functional integrity constitutes a fundamental requisite for the maintenance of hemostasis of the vessel wall and of circulatory function. The vascular endothelium is a multifunctional tissue with synthetic and metabolic properties, including the maintenance of the permeability barrier of the vessel; synthesis of anticoagulation and antithrombotic molecules; synthesis of pro-thrombotic molecules; production of extracellular matrix; modulation of blood flow and cellular reactivity; and regulation of inflammation, immunity and cell growth [9].

CABG is becoming more and more common. Bypasses are more frequently achieved utilizing SV and ITA grafts. Different functional and structural properties between arteries and veins may explain the better results using arteries [10]. However it is important to note that the endothelium of the ITA is protected during the entire preparation procedure and in 90% of patients the graft remains unobstructed for 10 years [11]. On the other hand, the SV graft is not protected with the conventional preparation technique which may lead to significant endothelial lesion [12].

Proliferation of the smooth muscle in the tunica media is similar in the SV and ITA [13]. However, the proliferation is more accentuated in veins that suffer endothelial lesions during preparation. This demonstrates that the great cause of hyperplasia of the intimal layer, detected in venous grafts, may not be only justified by the intrinsic differences between arteries and veins, but by the sensitivity of the vein to trauma.

Many strategies have been utilized to prevent occlusion of veins. Advances in new therapies with pharmacological agents [14] and gene therapy [15] are becoming more important. Even so it is necessary that better surgical techniques are developed to prevent lesions of the venous wall during the operation.

The No-touch technique for SV harvest, in which the vein is removed with the pedicle of perivascular tissue, is one of the techniques that has emerged to overcome this problem [7]. Prospective studies using this technique have demonstrated that the endothelial integrity and the nitric oxide synthesis enzyme (NOS) activity were better conserved with the use of this technique for the preparation of the SV [16,17].

Nitric oxide is a potent vasodilator that is synthesized from L-arginine by NOS [18]. Another property of nitric oxide is its capacity to inhibit the activation and aggregation of platelets, leukocyte adhesion and proliferation of smooth muscle cells. In the cardiovascular system, NOS is expressed by endothelial cells, smooth muscle cells, perivascular nerve cells and platelets [19].

The vasodilatory activity and the blockage of platelet aggregation by nitric oxide are responsible for protection against spasms, as well as its high immediate patency. Additionally, the mechanical properties provided by adipose tissue around the vein may contribute to the better result of this new technique [17,20].

Using the aforementioned morphological and staining techniques, demonstrated an important difference in the preservation of veins when they are prepared by these two techniques. Handling the vessel, with abusive distention and local lesion due to the removal of the perivascular tissue, results in morphofunctional damage, with vasospasm which favors thrombosis of the vessel and its occlusion.

Endothelial aggression, observed on electron microscope photographs, makes the production of local protecting factors, such as NOS, impossible as well as making the production of kinins likely thereby favoring vasoconstriction in a situation in which there is an imbalance of enzymatic factors.

The cellular architecture was retained using the No-touch technique, as seen by undamaged collagen fibers organized around the vessel preserving an adequate tension in the vascular wall.

Although our results demonstrate that the SV has characteristics suggesting a better integrity when the vein is removed from its native vascular bed using the No-touch method, our results, until now, are characterized as observational. They do not report numeric values of intimal thickness, proportion of endothelial cells by optical field or refringency by pixel analysis of the subendothelial collagen expression. To answer these issues further studies with morphometric and immunohistochemical evaluations are necessary.

CONCLUSION

The endothelial integrity was better using the No-touch technique. Conservation of the perivascular tissue, with less pressure and manual manipulation of the SV, suggests that its structure is conserved and that local hormonal factors are preserved.

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