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ORIGINAL ARTICLE

Pedicled no-touch saphenous vein graft harvest limits vascular smooth muscle cell activation: the PATENT saphenous vein graft study[†]

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Abstract

OBJECTIVE: Neointimal hyperplasia secondary to vascular smooth muscle cell (VSMC) activation limits the long-term patency of saphenous vein grafts (SVGs). We compared markers of vascular injury and VSMC activation in SVGs harvested using the pedicled 'no-touch' (NT) vs the conventional (CON) technique.

METHODS: Patients undergoing coronary artery bypass surgery were enrolled in the PATENT SVG trial (clinicaltrials.gov NCT01488084). Patients were randomly allocated to have SVGs harvested with the NT technique from one leg and the CON method from the other. SVG segments underwent morphometry, histological and electron microscopy assessments and transcript measurements of VSMC activation and differentiation markers. Leg wound functional recovery and harvest site complications were assessed using a quality-of-life questionnaire.

RESULTS: A total of 17 patients (65.3 ± 7.3 years) were enrolled. SVGs harvested using the NT vs CON technique exhibited preserved intimal, medial and adventitial architecture. CON harvest was associated with greater medial Kruppel-like factor 4 transcript levels (0.26 ± 0.05 vs 0.11 ± 0.02, $P < 0.05$). CON samples had significantly lower medial serum response factor (0.53 ± 0.11 vs 1.44 ± 0.50, $P < 0.05$) and myocardin (0.59 ± 0.08 vs 1.33 ± 0.33, $P < 0.05$) transcript levels. MicroRNA-145, an inhibitor of VSMC activation and differentiation, was higher in the NT vs CON samples (1.84 ± 1.03 vs 0.50 ± 0.19, $P < 0.05$). Leg assessment scores were worse in the NT legs at 3 months, but similar to CON scores at 12 months.

CONCLUSIONS: SVGs harvested using the 'NT' technique exhibit an early molecular and morphological pattern consistent with decreased VSMC activation compared with CON harvesting. Functional leg recovery was similar in both groups at 12 months. Larger studies are required to corroborate these findings.

Keywords: Coronary artery bypass • Pedicled 'no touch' • Saphenous vein grafts • Smooth muscle

INTRODUCTION

Although saphenous vein grafts (SVGs) remain one of the most commonly employed conduits for coronary artery bypass graft (CABG) surgery, predisposition towards intimal hyperplasia continues to limit their long-term patency [1]. While the development of intimal hyperplasia is likely multifactorial, trauma and/or high-pressure distension at the time of SVG harvest may set the stage for aberrant remodelling and subsequent graft failure [2–5].

In 1996, Souza [6] proposed the 'no-touch' (NT) vein harvest technique. This involves harvesting a pedicled SVG with the perivascular tissue intact, without a direct contact with the vein or high-pressure distension. A single randomized controlled trial demonstrated improved short- and mid-term graft patency and less intimal hyperplasia using the NT technique compared with conventional (CON) vein harvest [4, 7].

One of the best accepted mechanisms of neointimal hyperplasia leading to SVG failure is VSMC activation, which can be triggered by both haemodynamic and non-haemodynamic factors, such as mechanical stretch and vascular injury [2, 8]. VSMC activation usually follows a well-described sequence, with early changes in the expression of key genes involved in VSMC differentiation (such as an increase in Kruppel-like factor 4 [KLF4], with reductions in myocardin and serum response factor [SRF]), which result in phenotypic modulation of VSMCs into proliferative, migratory and secretory cells capable of synthesizing extracellular matrix [9, 10]. The fate and plasticity of VSMCs is believed to be governed

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upstream by microRNA-145, which serves to regulate multiple pathways of VSMC differentiation, including but not limited to attenuating KLF4 and stimulating myocardin expression [11–13].

NT SVG harvesting is performed with an open method—we wanted to compare leg wound healing using the NT technique with the standard open harvesting method. In addition, the original NT studies excluded patients with acute coronary syndrome, insulin-dependent diabetes and elevated creatinine, who may be at a greater risk of leg wound complications [4].

The objectives of the current proposal were 2-fold, namely: (i) to evaluate SVG morphometry and markers of VSMC activation in veins harvested with the CON vs NT technique and (ii) to compare leg wound healing and functional outcomes following CON and NT SVG harvesting. We hypothesized that SVG harvest using the NT (vs CON) technique will be associated with morphological and molecular properties consistent with decreased VSMC activation, and that this may explain, in part, the improved patency rates noted in clinical trials. Secondly, we postulated that there will be no clinically important difference in the incidence and severity of SVG harvesting site complications or functional recovery by 1-year post-CABG.

MATERIALS AND METHODS

Study population and protocol

Adult patients who had a left ventricular ejection fraction of >20% and were scheduled for primary, isolated, non-emergent CABG surgery with or without cardiopulmonary bypass requiring at least two SVGs were eligible for the PATENT SVG study (clinicaltrials.gov identifier: NCT01488084). Patients underwent preoperative non-invasive duplex imaging and mapping of the long saphenous vein from both lower legs. Patients in whom one or both of the lower leg saphenous veins were deemed unusable due to severe peripheral vascular disease, vein stripping, varicose veins, previous amputation or inadequate quality on preoperative imaging were ineligible. Randomization was performed 'within' rather than 'between' patients. Saphenous veins were harvested from both lower legs; the long saphenous vein was harvested from one leg using the NT technique and from the opposite leg with the CON technique. The harvesting method was determined by random allocation, which was revealed at the time of surgery. The patient remained blinded to randomization. All patients signed a consent form approved by the institutional research ethics boards.

All saphenous veins were harvested in an open manner either by a senior trainee or by a physician who also performed the leg closures. All individuals involved in the harvesting procedures were initially trained at the Sunnybrook site. CON saphenous veins were stripped of its adventitial layer, excised and then distended using heparinized saline. CON SVGs were distended according to the usual clinical method, i.e. very brief manual distension using the least pressure necessary to relieve any visible vein spasm; the distending pressure was not directly measured but only assessed digitally. NT saphenous veins were harvested with minimal trauma with a pedicle of surrounding perivascular adipose tissue. The preoperative saphenous mapping helps prevent flaps during harvesting. Branches were doubly clipped and transected away to prevent direct handling of the adventitial region. The method has been previously described in detail [14]. The isolated vein was not manually dilated but left *in situ* covered with a moist saline-soaked sponge. Following heparinization, the graft was flushed with heparinized blood, connected to the aortic cannula and allowed to dilate when exposed to arterial pressure and checked for leaks. The vein was then placed in a small container of heparinized blood until use. All SVG samples were maintained at room temperature between harvest and use. Segments (1–2 cm) of the harvested CON and NT veins to be used for analysis were fixed prior to construction of the distal anastomosis after verifying that the vein was of sufficient length—from experience, the surgeons would routinely harvest lengths of saphenous vein that would ensure that there was a small amount of excess for the experimental samples. The length of time that the vein was *ex vivo* prior to fixation was not measured, but estimated to be 60 min maximum. Legs were closed in two layers with continuous absorbable suture and continuous subcuticular absorbable suture for the skin and/or additional staples for the skin. The same closure technique was used for both legs. Drains were not used in any of the patients. Graft flows were not measured intraoperatively.

Patients were followed in the hospital and in the clinic at 3 and 12 months postoperatively. Patients were queried for interim cardiac events and administered a questionnaire assessing leg wound healing and functional outcomes in both lower legs based on a leg discomfort score (0–33/33), a harvest site complication score (0–16/16) and a total score (0–49/49) (see Supplementary material). The severity of leg infection was characterized according to the clinical severity of infection and the treatment received.

The primary study outcomes relate to morphological integrity and markers of early VSMC activation. The secondary study outcomes relate to leg wound healing and functional recovery at 3 and 12 months.

Saphenous vein assessment

Morphology and histology. Antigen retrieval in formalin-fixed, paraffin-embedded SVG sections (5 μm) was accomplished with incubation in boiling 10 mM sodium citrate buffer (pH 6.0) for 10 min. Sections were subsequently exposed to primary antibodies directed against KLF4 (1:100; Bioss, Woburn, MA, USA), SRF (1:40; Proteintech Group, Chicago, IL, USA) or myocardin (1:50; Abcam, Cambridge, UK), then immunostained with the appropriate avidin–biotin detection system (VECTASTAIN Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA) and counterstained with Harris haematoxylin (Thermo Scientific, Waltham, MA, USA). Immunopositivity was visualized with 3,3′-diaminobenzidine (DAB Substrate Kit, Vector Laboratories) and measured in five random fields by the same blinded investigator with the ImageJ software (http://rsb.info.nih.gov/ij/).

Transmission electron microscopy. SVG samples were immersion-fixed for at least 5 h in 4% formaldehyde with 1% glutaraldehyde in 0.1 M phosphate buffer, and then post-fixed for 1 h at 4°C in 1% osmium tetroxide in 0.1 M phosphate buffer. After washing and serial dehydration in ethanol, samples were embedded in EPON 812 resin (Ted Pella, Redding, CA, USA) and polymerized at 40°C for 48 h. Ultrathin sections were cut, stained with uranyl acetate and lead citrate. Ultrastructural features were examined with a Hitachi H-7000 transmission electron microscope (Tokyo, Japan), equipped with a digital image acquisition system.

Real-time RT–PCR analyses of Kruppel-like factor 4, serum response factor and myocardin. NT samples were transferred into RNAlater (Qiagen, ON, Canada) prior to removal of the

attached pedicles. Total RNA from all SVG samples was extracted with the RNeasy plus Mini kit (Qiagen) and reverse transcribed with Omniscript Reverse Transcriptase (Qiagen). Real-time polymerase chain reaction (PCR) was performed on the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) using the SYBR Green PCR Master Mix (Applied Biosystems) coupled with the following primers: for caldesmon, forward 5′-CGTCGCAGAGA ACTTAGAAGG and reverse 5′-ATTCCTCTGGTAGGCGATTCT; for calponin, forward 5′-TCCACGCAGTTCAACAAGG and reverse 5′-AT GGAGAGGCCGGTGAGT; for KLF4, forward 5′-GCCGCTCCATTA CCAAGA and reverse 5′-TCTTCCCCTCTTTGGCTTG; for SM22a, forward 5′-GTCCGAACCCAGGCACAAGT and reverse 5′-ACCCTT GTTGGCCATGTCT; for SRF, forward 5′-CCTACCAGCTTCACCCTCAT and reverse 5′-CACTTGAATGGCCTGCACT; for myocardin, forward 5′-AAGGGCACAGGGTCTCCT and reverse 5′-GGGAGAGAAGCTT GGAGGAT; for GAPDH, forward 5′-CACCAGGGCTGCTTTTAACT CTGGTA and reverse 5′-CCTTGACGGTGCCATGGAATTGC. Gene expression data were analysed using the Pfaffl's relative expression software (REST-MSC, version 2, available from http://www.wzw.tum.de/gene-quantification/).

Laser capture microdissection and real-time RT–PCR analysis of microRNA-145. SVG tissues were flash-frozen in liquid nitrogen and stored at −80°C. Frozen sections (∼8 μm) were treated with the Applied Biosystems Arcturus HistoGene® stain before SMCs, as visualized with the PixCell IIe laser capture microdissection system (Applied Biosystems, Arcturus, Foster City, CA, USA), were isolated from the vessel media. Total RNA was extracted from captured cells with the RNAqueous-micro extraction kit (Ambion, Austin, TX, USA). DNase-I digestion was included to eliminate genomic DNA contamination. RT samples were prepared with the TaqMan® MicroRNA Reverse Transcription Kit and analysed on the StepOnePlus™ Real-Time PCR System using the TaqMan PCR Mastermix in combination with either hsa-miR-145 or RNU6 primers (all Applied Biosystems). Gene expression data were analysed by the delta–delta CT method.

Statistical analysis

Clinical data are presented as *n* (%) or mean ± standard deviation. Leg wound assessment data are presented as the median and interquartile range. Leg assessment scores were treated as a continuous variable, and the Wilcoxon Sign-Rank test was used to compare median differences in areas of leg discomfort, harvest site complications and total score between the NT and the CON technique. All other data are presented as mean ± standard error and analysed with either the Wilcoxon-matched paired test or Student's *t*-test using GraphPadInStat (GraphPad Software, Inc., La Jolla, CA, USA; version 3.05). Differences between multiple means were evaluated by one-way analysis of variance followed by the Bonferroni test.

RESULTS

Patient baseline characteristics and protocol adherence

There were 18 patients enrolled between July 2010 and January 2013. Of these, 14 had preoperative duplex studies and completed the standardized follow-up leg wound assessments at 3 months, and 13 of 14 patients completed the 12-month leg assessment (1 patient moved out of the country and did not complete the 12-month leg assessment); 3 only contributed paired SVG samples. There was one crossover, i.e. a CON SVG was harvested from the leg assigned to NT, and the NT SVG harvested from the leg assigned to CON. In this patient, the preoperative saphenous vein mapping was misleading which led to creation of a flap; the leg scores were analysed according to the intention to treat, while the vein analysis was completed according to the treatment received. There was one protocol violation—the vein was removed from one rather than both legs and vein segment samples were not obtained; this patient was excluded from the analysis. The patient characteristics for the 17 study patients are listed in Table 1. The mean age was 65.3 ± 7.3 years, 16 (94.1%) were male and 9 were diabetic (52.9%). There were no perioperative myocardial infarctions, repeat revascularizations or deaths. Discharge medications included acetylsalicylic acid or clopidogrel (94.1%), beta-blocker (71.4%) and a lipid-lowering agent (78.6%). All patients were alive and event-free at 12 months postoperatively.

Saphenous vein grafts harvested using the conventional vs no-touch technique exhibit different morphology

Light microscopic examinations indicated that although there were no differences in intima, media and adventitial thickness between the two groups of SVGs (data not shown), those from the CON group had dilated lumens with the absence of the characteristic intimal folds resulting in a markedly greater lumen diameter-to-thickness ratio (CON 1.36 ± 0.17 vs NT 0.66 ± 0.11, $P = 0.0001$)

Table 1: Patient characteristics ($n = 17$)

Male[a]	16 (94.1)
Age (years)[b]	65.3 (7.3)
CCS Angina Class III or IV[a]	6 (35.3)
Left ventricular grade ≥2[a,c]	16 (94.1)
Congestive heart failure[a]	2 (11.8)
Triple vessel disease[a]	17 (100.0)
Peripheral vascular disease[a]	1 (5.9)
Previous percutaneous coronary intervention[a]	3 (17.6)
Myocardial infarction[a]	5 (29.4)
Hypercholesterolaemia[a]	13 (76.5)
Diabetes mellitus[a]	9 (52.9)
Hypertension[a]	16 (94.1)
Renal insufficiency[a]	2 (11.8)
History of smoking[a]	14 (82.4)
Creatinine (μmol/l)[b]	86.3 (11.7)
Preoperative medical therapy[a]	
Acetylsalicylic acid	16 (94.1)
Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker	9 (52.9)
Beta-blocker	12 (70.6)
Calcium channel blocker	3 (17.6)
Clopidogrel	5 (29.4)
Lipid-lowering agent	16 (94.1)

Data are expressed as [a]*n* (%) or [b]mean (SD).
[c]Left ventricular (LV) Grade 1: left ventricular ejection fraction (LVEF) >50%; LV Grade 2: LVEF 40–49%; LV Grade 3: LVEF 30–39%; LV Grade 4: LVEF <30%.
CCS: Canadian Cardiovascular Society.

compared with SVGs harvested using the NT technique (Fig. 1). Compared with SVGs from the CON group, SVGs harvested using the NT technique notably exhibited the normal pattern of circularly arranged SMCs with an intact advential layer. Medial SMCs appeared flat and distorted in the CON harvest group (Fig. 1).

Ultrastructural analyses with transmission electron microscopy at ×4000 magnification confirmed distension in SVGs from the CON group with loss of intimal folds and stretched SMC layers (Fig. 2A). In contrast, SVGs harvested using the NT technique demonstrated intimal folding with normal SMC architecture

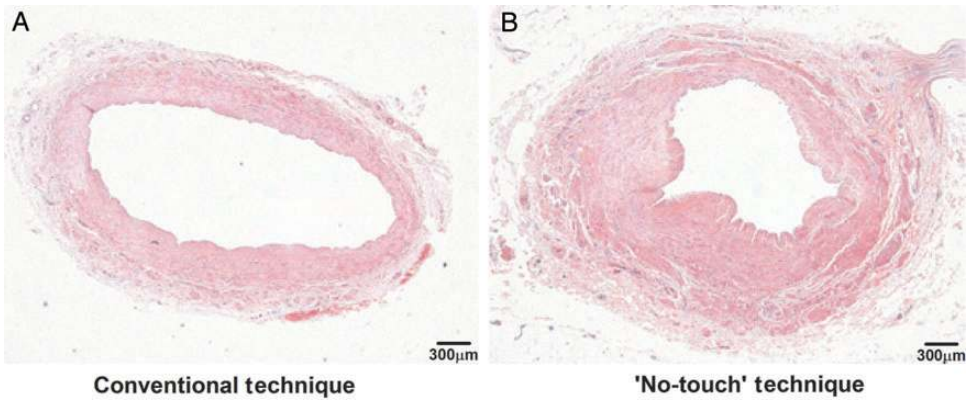

Figure 1: Light microscopic comparisons revealed that the NT vs CON harvesting technique yields SVGs with preserved intimal, medial and adventitial architecture. Representative micrographs of H&E-stained transverse SVG sections harvested using either the (**A**) CON or (**B**) NT technique. Samples shown were harvested from the same patient (*n* = 14).

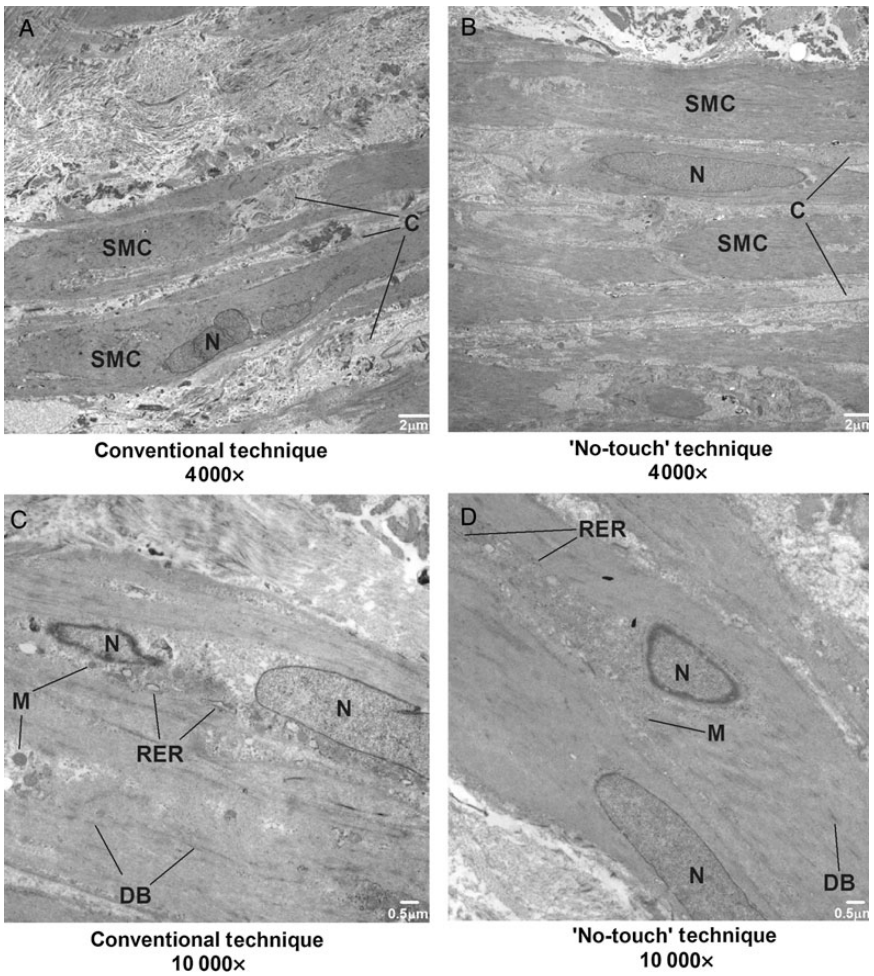

Figure 2: Ultrastructural comparisons by transmission electron microscopy revealed that the NT vs CON harvesting technique yields SVGs with preserved intimal and nuclear membrane folding. Representative micrographs of transverse SVG sections harvested using either the (**A** and **C**) CON or (**B** and **D**) NT technique. Samples shown were harvested from the same patient (*n* = 5). C: collagen; DB: dense bodies; M: mitochondria; N: nucleus; RER: rough endoplasmic reticulum.

(Fig. 2B). Images taken at the higher magnification of ×10 000 demonstrated that nuclear membrane infolding was preserved in the NT but not CON samples. Furthermore, nuclear material was condensed to the nuclear membrane in SVGs from the NT group, whereas in the CON group, the nuclear material was dispersed throughout the nucleus (Fig. 2C and D).

Aberrant vascular smooth muscle cell transcriptional activation in saphenous vein grafts harvested using the conventional vs no-touch technique

MicroRNA-145 plays a central role in the phenotypic activation of VSMCs [11–13]. It promotes myocardin binding to SRF, thus activating transcription of VSMC contractile/quiescent genes. It also represses KLF4 that promotes aberrant VSMC differentiation by inhibiting myocardin and SRF. SVGs harvested using the CON technique exhibited dramatically greater KLF4 immunostaining within the medial wall of the vessel compared with the NT group ($P < 0.05$; Fig. 3A and B). Furthermore, KLF4 expression as evaluated by real-time RT–PCR showed higher KLF4 mRNA levels in CON tissues compared with the NT group ($P < 0.05$; Fig. 3C). In conjunction with an increase in KLF4, mRNA expression of SRF and myocardin were significantly lower in the CON- vs NT-harvested SVGs ($P < 0.05$; Figs 4 and 5). In contrast, VSMC mRNA expression of contractile genes, including SM22a, calponin and caldesmon, were similar between groups (Supplementary Fig. 1).

Since CON harvest was associated with reciprocal changes in KLF4 and myocardin consistent with VSMC activation and phenotypic modulation, we sought to determine whether the expression of microRNA-145, their common upstream regulator, was different between the two groups. We observed that microRNA-145 expression was higher in SVGs harvested using the NT vs CON technique ($P < 0.05$; Fig. 6).

Leg assessment

The leg wound assessments at 3 and 12 months are presented in Table 2. The median leg discomfort score and the total score were significantly higher in the NT legs compared with the controls at 3 months. All leg assessment scores were similar and very low in both the NT and CON legs at 12 months. At 3 months, there were four leg wound harvest site infections in legs randomized to NT harvesting and none in the CON legs; at 1 year, there were no reported infections. Two of the infections were graded as severe. One of the infections occurred in the crossover patient, i.e. in the leg assigned to NT harvesting but in which the vein was harvested using the CON method and analysed according to the intention

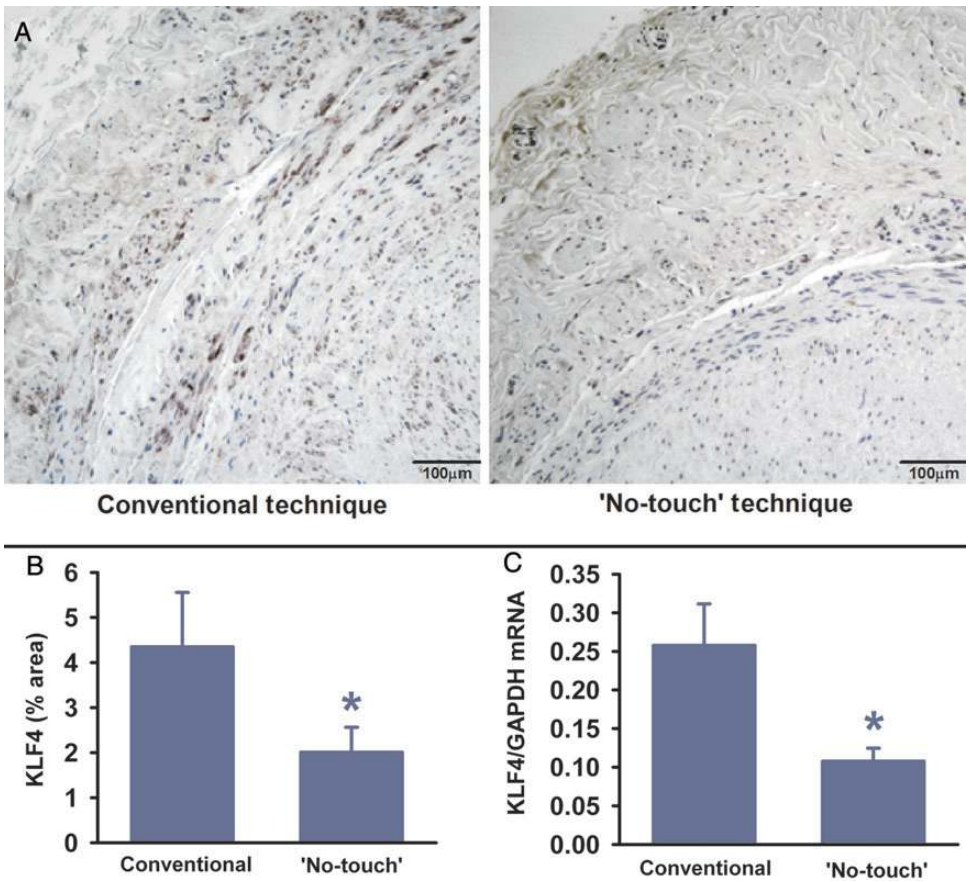

Figure 3: Immunohistochemical staining and real-time PCR analyses revealed that the NT vs CON harvesting technique yields SVGs with lower KLF4 levels. (**A**) Representative micrographs and (**B**) semi-quantitative analysis of KLF-4 staining in transverse SVG sections harvested using either the CON or NT technique. KLF4-positive staining (brown) was located in the SMC nuclei and cytoplasm ($n = 14$); *$P < 0.05$ vs the CON group. (**C**) Real-time PCR measurements of KLF4 transcripts in SVGs harvested using the NT or CON harvesting technique were normalized against the corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression ($n = 9$); *$P < 0.05$ vs the CON group.

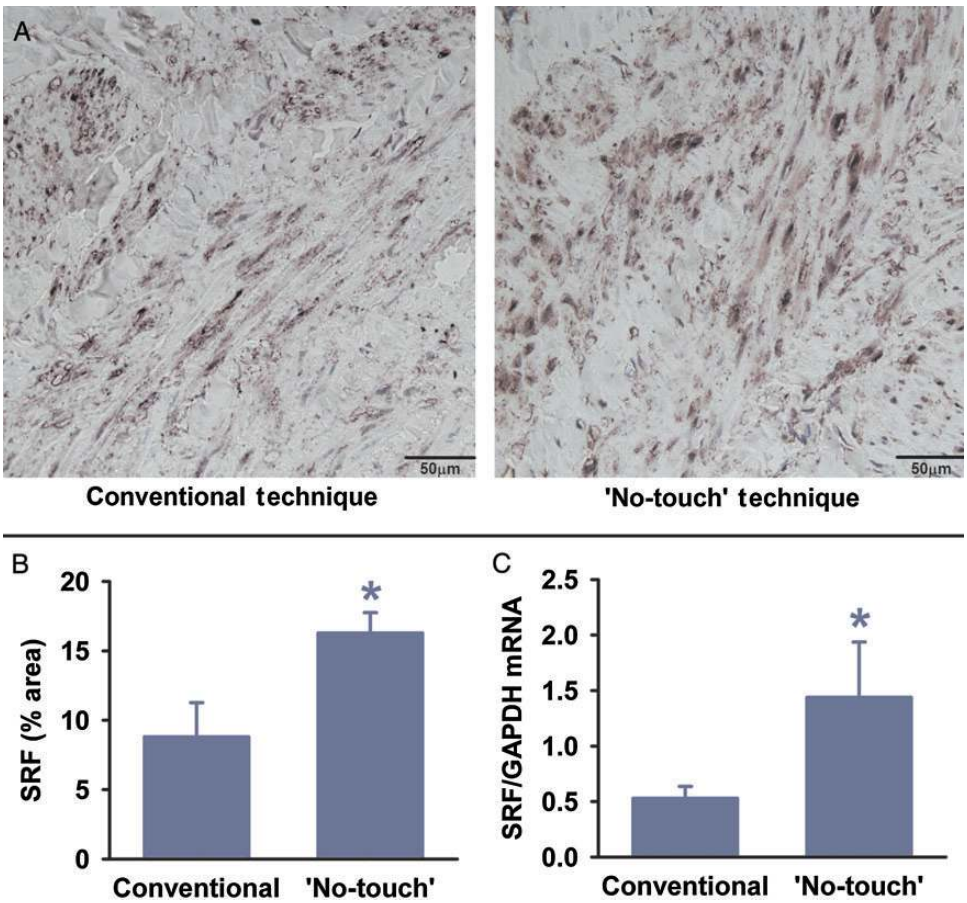

Figure 4: Immunohistochemical staining and real-time PCR analyses revealed that the NT vs CON harvesting technique yields SVGs with higher SRF levels. (**A**) Representative micrographs and (**B**) semi-quantitative analysis of SRF staining (brown) in transverse SVG sections harvested using either the CON or NT technique ($n = 14$); *$P < 0.05$ vs the CON group. (**C**) Real-time PCR measurements of SRF transcripts in SVGs harvested using the NT or CON harvesting technique were normalized against the corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression ($n = 9$); *$P < 0.05$ vs the CON group.

to treat. All infections occurred in non-insulin-dependent diabetic patients.

DISCUSSION

The primary purpose of the present study was to evaluate whether SVGs harvested using the NT vs CON technique would exhibit any early molecular differences with respect to VSMC activation—a causal mechanism of intimal hyperplasia and restenosis. We report that, in addition to preserved morphological architecture, VSMC expression of key transcriptional genes involved in aberrant differentiation and phenotypic modulation were significantly lower in the NT vs CON group. These data suggest that the harvest technique results in immediate changes in VSMC differentiation genes, which may be prime and/or accelerate SVG restenosis. Furthermore, leg healing was similar between the NT and CON techniques by 1 year after surgery.

The major differences in the NT harvest technique are (i) reduced surgical injury/trauma from vein harvesting, (ii) lack of high-pressure vein distension and (iii) preservation of perivascular adipose tissue [6]. A single-centre prospective randomized trial compared the patency rates between the NT and CON harvest groups [4, 7]. Souza *et al*. randomized 156 patients and found patency rates of 95 and 89% at 18 months and 90 and 76% at the 8.5-year follow-up in the NT and CON groups, respectively [7]. In a subsequent study from the same group, the NT vein was compared with a radial graft [15]. While a radial artery has been shown to have superior long-term patency compared with a conventionally harvested SVG [16], in this study, overall patency at 3 years of the NT SVG (94%) exceeded that of the radial artery (82%, $P = 0.01$) [15].

The underlying hypothesis is that the differences in patency noted are related to multifactorial vasculoprotective mechanisms in the NT vs CON group. Several studies have demonstrated that the NT technique is associated with improved morphological architecture, improved indices of endothelial function, endothelial nitric oxide synthase preservation and a reduction in adhesion molecule expression and neutrophil adhesion [2, 8, 17]. In addition, it has been proposed that the preservation of the surrounding perivascular tissue serves as a reservoir of NO release, in addition to a source of vasodilatory adipokines (such as leptin) [18]. Other investigators have also identified that increased distension pressure alone can lead to endothelial injury and is also neointimal hyperplasia in an organ culture model [5].

SVG neointimal hyperplasia is a multifactorial process that involves VSMC activation [19]. Vascular injury, in the form of either endothelial dysfunction, mechanical or haemodynamic stress/trauma or inflammation, promotes the switching of VSMCs from a normal, quiescent and 'contractile' state to a 'synthetic' phenotype.

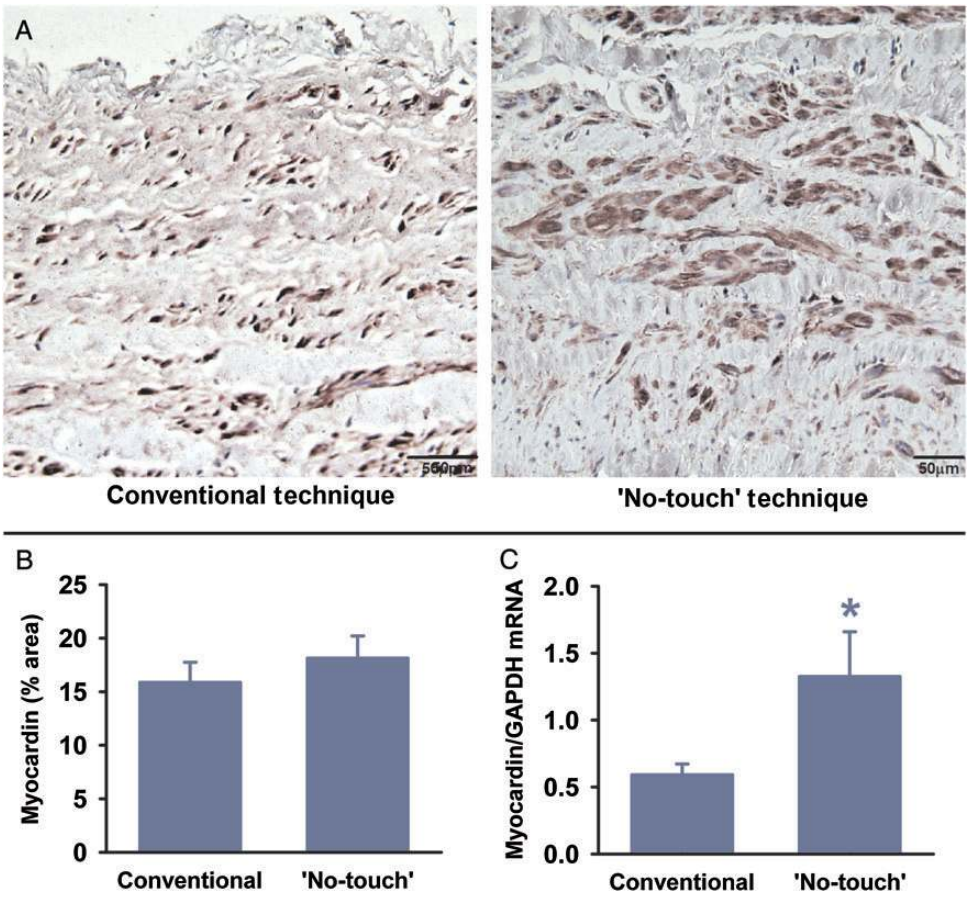

Figure 5: Immunohistochemical staining and real-time PCR analyses revealed that the NT vs CON harvesting technique yields SVGs with higher myocardin transcript levels. (**A**) Representative micrographs and (**B**) semi-quantitative analysis of myocardin staining (brown) in transverse SVG sections harvested using either the CON or NT technique were normalized against the corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression ($n = 14$). (**C**) Real-time PCR measurements of myocardin transcripts in SVGs harvested using the NT or CON harvesting technique were normalized against the corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression ($n = 9$); *$P < 0.05$ vs the CON group.

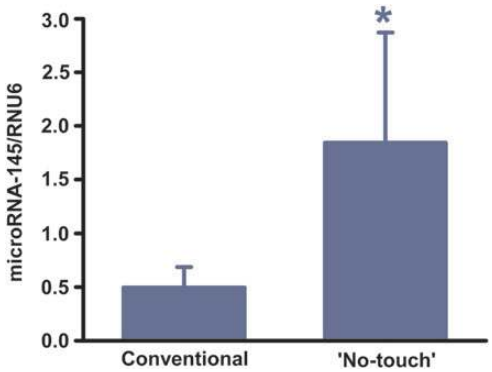

Figure 6: Real-time PCR analyses revealed that the NT vs CON harvesting technique yields SVGs with higher microRNA-145 transcript levels. SMCs, isolated from the media of SVG sections, were processed for real-time PCR analysis of microRNA-145 levels. MicroRNA-145 levels were normalized against the corresponding RNU6 expression ($n = 7$); *$P < 0.05$ vs the CON group.

These changes result in the synthesis of large quantities of extracellular matrix, proteases and cytokines, and mark the initiation of lesion formation [3]. Phenotypically modified synthetic VSMCs appear to also play an important role in defining the cellularity, necrotic core area and fibrous cap stability of plaques, and in this fashion, they modulate plaque composition and plaque rupture.

It has been suggested that several transcription factors, including SRF, myocardin, myocardin-related transcription factors and members of the KLF family, act as molecular switches regulating VSMC differentiation. Recently, the small single-stranded, non-coding microRNAs, particularly the microRNA-143/145 cluster, have been identified as novel critical regulators and molecular chaperones of VSMC differentiation and phenotypic modulation [11].

In the postnatal state, microRNA-143/145 are exclusively expressed in VSMCs with absent expression in other vascular cell types, including endothelial cells. Cordes *et al.* [11] demonstrated that microRNA-145 cooperatively targeted a network of transcriptions factors, such as myocardin, KLF4 and KLF5, to promote differentiation and repress proliferation of VSMCs. Boettger *et al.* [20] reported that mice that had the microRNA-143/145 cluster knocked out exhibited thinning of the arterial tunica media, with a reduction in the number of contractile SMCs and a concomitant increase in the number of synthetic SMCs. MicroRNA-145 has recently also been found to regulate angiotensin-I-converting enzyme, with the suggestion that a reduction in microRNA-145 expression may allow increased local/tissue production of angiotensin II, a molecule critical in the pathophysiology of atherosclerosis and adverse vascular remodelling [20]. Of note, we have recently reported that microRNA-145-based treatment strategies can limit atherosclerosis in animal models [13].

Table 2: Leg assessment

	No-touch Median (IQR)	Conventional Median (IQR)	*P*-value[a]
Three-month follow-up (*n* = 14)			
General leg discomfort (max score = 33)	2 (1–5)	0 (0–1)	0.0005
Harvest site complications (max score = 16)	0 (0–4)	0 (0)	0.13
Total score (max score = 49)	3 (1–9)	0 (0–1)	0.0005
One-year follow-up (*n* = 13)			
General leg discomfort (max score = 33)	1 (1–2)	1 (0–1)	0.13
Harvest site complications (max score = 16)	0 (0)	0 (0)	–
Total score (max score = 49)	1 (1–2)	1 (0–1)	0.13

[a]*P*-value testing for significance of differences in medians between the NT and CON technique. General leg discomfort included heaviness, weakness, stiffness, itching, tingling, heat/burning, numbness, pain, discolouration, rash and swelling. Harvest site complications included necrosis, dehiscence, wound drainage, haematoma, seroma and presence and absence of infection. Total score is the sum of general leg discomfort and harvest site complications.
IQR: interquartile range.

In the current work, we found that the molecular fingerprint of VSMC activation is primed in CON- vs NT-harvested SVGs. This is witnessed by significantly higher KLF4 expression and markedly lower myocardin transcript levels in SVGs from the CON group with less microRNA-145 expression. SVGs harvested using the NT technique not only exhibited morphological preservation of the architecture, but were also protected against these early molecular signalling events as evidenced by a greater VSMC content of microRNA-145, lower KFL4 and higher myocardin expression. Notably, SM22a, calponin and caldesmon expression were similar between the CON and NT groups, indicating that the transcription factors responsible for the phenotypic modulation of VSMC are activated differentially and occur very rapidly. It is, hence, plausible that, CON-harvested veins are primed for early and more aggressive VSMC remodelling and neointimal proliferation relative to NT-harvested veins.

Many cardiac surgical centres have chosen to use an endoscopic method for saphenous vein harvesting, as wound complications and infections are reduced compared with the standard open method [21]. We anticipated that wound complications using the NT method could be greater than the standard open method as a pedicle of tissue is excised in association with the saphenous vein. We, therefore, wanted to carefully assess leg functional recovery and harvest site infections following the NT method and used within-patient randomization as a powerful study design to explore this question. To the best of our knowledge, this is the first study to compare leg complications between the two harvesting techniques. The PATENT SVG study patients were older than the patients in the original study by Souza and also included diabetics. The 3-month functional recovery scores were higher in the NT legs with a large portion of patients reporting mild-to-severe numbness and swelling. An explanation for the paraesthesia sensation may be that the saphenous nerve is typically included in the pedicle with the NT technique, whereas the saphenous nerve is usually manipulated but preserved with CON SVG harvesting. Alternatively, there could be more saphenous neuropraxia (a potential complication after CABG) [22]. As greater swelling was reported at 3 months following pedicled harvesting (presumably due to disruption of more venous and lymphatic channels), this could have also contributed to the greater discomfort. Given that the status of both legs was similar by 1 year, these effects are likely compensated for and resolve over time. Saphenous vein harvest site infections were also seen in NT diabetic legs. Diabetes is well recognized as a risk factor for wound infection [23]. Of note, we found in this study that infections only occurred in the NT leg of diabetic patients. Whether or not this observation has any significant clinical impact, however, will need to be assessed in future investigations that comprise larger cohorts considering the importance of diabetes in the selection of surgical revascularization for multivessel disease [24]. Enhanced anti-microbial prophylaxis may be required in diabetics using the NT method [23]. Nonetheless, functional recovery of the leg wound was similar in the NT and CON legs with low scores at 1 year after surgery.

LIMITATIONS

Several limitations of this study are noted. Whether the VSMC changes are secondary to the harvest technique *per se*, the lack of distension or preservation of the perivascular adipose tissue cannot be established from our protocol. A more scientifically rigorous design would have four groups (CON vs pedicled harvesting and manual distension vs no distension). In practice, vein graft spasm is inevitable using CON harvesting and manual distension is required. Indeed, in the original trial reported by Souza *et al*. [4], there was a third intermediate study group who had CON SVG harvesting but without manual distension; this group had results that were no better than the standard CON patients (and manual distension was usually necessary). Of note, our usual care for conventionally harvested veins involves gentle and controlled dilatation only for the minimal time required for relief of the graft spasm. Also, we did not control for the amount of time that the vein segments were *ex vivo* prior to fixation. In our study, the NT vein was usually harvested prior to the CON SVG. As such, we would anticipate that any temporal differences should lead to greater activation in the NT vein, and not in the CON vein, as we observed in these experiments. Furthermore, the NT and CON SVGs were kept in different solutions *ex vivo* (heparinized blood vs normal saline). Accordingly, we can only conclude that there are substantial early differences in markers of VSMC activation between the CON and NT harvesting methods, but cannot attribute the differences to any specific aspect of the NT technique. Also, due to the limited amount of tissue available and the restrictions imposed by ethics on how much tissue we could collect, we were unable to perform all of the assays in the same sample. Finally, the sample size, although sufficient for the assessment of vascular biology parameters, was underpowered to discriminately evaluate clinical outcomes and complications. Nonetheless, our clinical findings, albeit only observational, are encouraging and warrant further investigations. A subsequent, much larger international study (SUPERIOR-SVG, clinicaltrials.gov identifier: NCT01047449) is currently underway, comparing NT and the standard open technique with the primary outcome being graft patency according to multidetector computed tomographic angiography at 1 year.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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