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Human saphenous vein and coronary bypass surgery: ultrastructural aspects of conventional and "no-touch" vein graft preparations

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Summary. Coronary artery bypass graft surgery (CABG) is routinely used to restore blood flow to diseased cardiac muscle due to coronary artery disease. The patency of conventional grafts decreases with time, which is due to thrombosis and formation of neointima. A primary cause of graft failure is the mechanical damage inflicted to the graft during harvesting, including removal of surrounding tissue accompanied by high pressure saline distension to overcome vasospasm (both causing considerable mechanical trauma). The aim of this study was to compare the ultrastructural features of human saphenous vein (SV) grafts harvested conventionally and grafts prepared using an atraumatic 'no-touch' harvesting technique introduced by Souza (1996). The results of this study showed a better preservation of the lumenal endothelium and medial vascular smooth muscle (SM) in 'no-touch' versus conventional grafts. A 'fast' (within 30 min) response of SM cells to conventional harvesting was noted where features of both SM cell division and apoptosis were observed. It is concluded that the 'preserved' nature of the 'no-touch' aortocoronary SV grafts renders them less susceptible to thrombotic and atherosclerotic factors than grafts harvested conventionally. These features are suggested to contribute to the improved early patency rate described using the no-touch technique of SV harvesting.

Key words: Saphenous vein grafts, Ultrastructure, CABG, Human

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Introduction

Coronary artery disease and atherosclerosis are intrinsically linked with inflammatory disease, where formation of inflamed plaques in the vessel wall reduces blood flow (Ikeda, 2003; Libby et al., 2002). A number of factors are implicated in this process, including circulating low-density lipoprotein particles. These stimulate endothelial and smooth muscle (SM) cells to produce chemokines, which contribute to progression of the pathological processes within the vessel wall (Cortellaro et al., 1993; Nilsson, 1993; Dimmeler et al., 1998; Pocock and Richards, 1999; Rekhter, 2002).

In order to restore blood flow to the cardiac muscle, CABG surgery is performed; the most commonly used conduits are the saphenous vein (SV) and/or internal mammary artery (see Souza, 2002). In conventional procedures, the SV is stripped of the pedicle of surrounding connective tissue, including the adventitia. This surgical trauma causes the vein to go into spasm (Mann et al., 1987), which is overcome by high-pressure distension that is required to re-open the lumen of the vein (see Souza, 2002). With this conventional technique, the occlusion rate of the graft is high, with graft failure occurring in 10% to 20% of patients within the first year (Mannien et al., 1998). This occlusion is thought to be a result of mechanical damage (trauma) to the adventitia and the endothelium during conventional harvesting procedures (Malone et al., 1981; Souza, 1996). The removal of the adventitia during surgery may have drastic implications, as numerous cellular and extracellular components are present in this outermost vessel layer. As for human SV, the adventitia is usually extensive, particularly at the proximal (fat) vein region. It is rich in collagen bundles and networks of elastin, 'islands' of SM, fibroblasts, and autonomic nerve fibres. Also present are the vasa vasorum (Marin et al., 1994), a particularly important component of the adventitia that provides oxygen and nutrients to the SV and which may

penetrate deep into the vein wall (Michel, 1996; Taccoen et al., 1996; Kachlík et al., 2002).

In an attempt to reduce mechanical trauma to the SV grafts, a 'no-touch' harvesting technique has been described (Souza, 1996) where the cushion of surrounding tissue is retained during harvesting. This prevents venospasm, thereby making distension unnecessary. This technique also protects the vein from direct handling by surgical instruments during performing of anastomosis. The aim of this study was to carry out an ultrastructural-transmission electron microscopic (TEM) examination of human SV harvested for CABG using both conventional and no-touch harvesting techniques (Souza, 1996) in an attempt to reveal any structural differences occurring between the two types of graft preparations, and any changes within the grafts.

Materials and methods

Patients: clinical data

Samples of SV were examined from three patients: 2 men (50 and 69 years of age), and one woman (54 years of age), who underwent bypass surgery at the Örebro University Hospital, Örebro, Sweden. Ethical approval and patients' informed consent were obtained for the study. The patients were deliberately selected to represent as closely as possible similar medical history and conditions. All patients suffered from angina pectoris NYHA function class III; were smokers and were receiving aspirin and beta-blockers before surgery. The patients were free of diabetes or other diseases including hepatitis B and HIV. During surgery, a segment of SV was harvested by the conventional technique with an adjacent segment being harvested by the no-touch technique (see below).

SV samples

During the operation the proximal segment of the SV was harvested (duration about 1 h) with its surrounding tissue using the no-touch technique (Souza, 1996), whereas the distal part was stripped of its surrounding tissue and adventitia, and distended with saline at a pressure of 300 mmHg for 1 minute using a syringe connected to a manometer – a conventional technique (duration about 1h 10 min, the extra time being that taken for tissue stripping and distension). Both specimens were stored for about 30 min at room temperature in heparinized patient's blood before implantation.

TEM procedures

Samples of excess SV grafts were then taken from the two vein segments on completion of the proximal anastamosis and fixed by immersion in fixative for 5h at 4 °C. The fixative consisted of 4% paraformaldehyde and 2% glutaraldehyde in 0.1M-phosphate buffer (pH 7.4). After fixation, the specimens were washed in phosphate buffer and then transferred to phosphate buffer containing 0.1% sodium azide and transported on ice (at ~ 4 °C) to the UK for further processing. The specimens were then post-fixed in 1% osmium tetroxide (in 0.1M sodium cacodylate buffer) for 1h at 4°C, washed in sodium cacodylate buffer, stained en block for 30 min at 4 °C in 2% uranyl acetate (in 0.1 M sodium acetate buffer), washed in sodium acetate buffer and then dehydrated in ethanol and propylene oxide, embedded in Araldite and polymerised at 60 ºC for 48 hours. Ultrathin sections (80-85 nm) were cut from 3-5 levels of Araldite-embedded specimens (levels were approximately 30 μm apart). Sections (a group of 2-3 sections from each specimen level) were stained with uranyl acetate and lead citrate and subsequently examined with a JEM-1010 TEM. Araldite semithin sections (1.5-2 μm) were stained with toluidine-blue and viewed on an Olympus BX 500® light microscope and photographed (digitally) where appropriate.

Results

Light microscopy (Fig. 1)

In SV harvested by the no-touch technique, where its cushion of surrounding tissue, including the adventitia, remained intact and the vein was not distended, the intima was thrown into characteristic folds (Fig. 1a). In the preserved adventitia, islands of SM and vessels of the vasa vasorum were present (Fig. 1b). In contrast, in SV harvested conventionally, where the cushion of surrounding tissue was substantially removed and the vein was distended, the characteristic intimal folds were no longer present, and the lumen appeared 'collapsed' (Fig. 1c).

TEM (Figs. 2-6)

The results below focus on the ultrastructural features, which were common either for the no-touch or conventionally, harvested SV grafts.

Intima: endothelium (Figs. 2 and 3)

The ultrastructural comparison of the intima of the no-touch and conventional preparations of the SV are shown in Figs. 2 and 3, respectively. In no-touch preparations, where the handling of the vein was minimal, the intima consisted of a relatively 'healthy' endothelium with preserved contacts (junctions) between individual cells (Fig. 2a). The main body of the endothelial cells, where the nucleus was located, usually protruded to the vein lumen (Fig. 2a). Distal regions of these cells frequently exhibited a 'wavy' or fold-like appearance (Fig. 2b). In addition, small cytoplasmic processes were present on the apical (lumenal) aspect of the cells. The main body of endothelial cells contained a

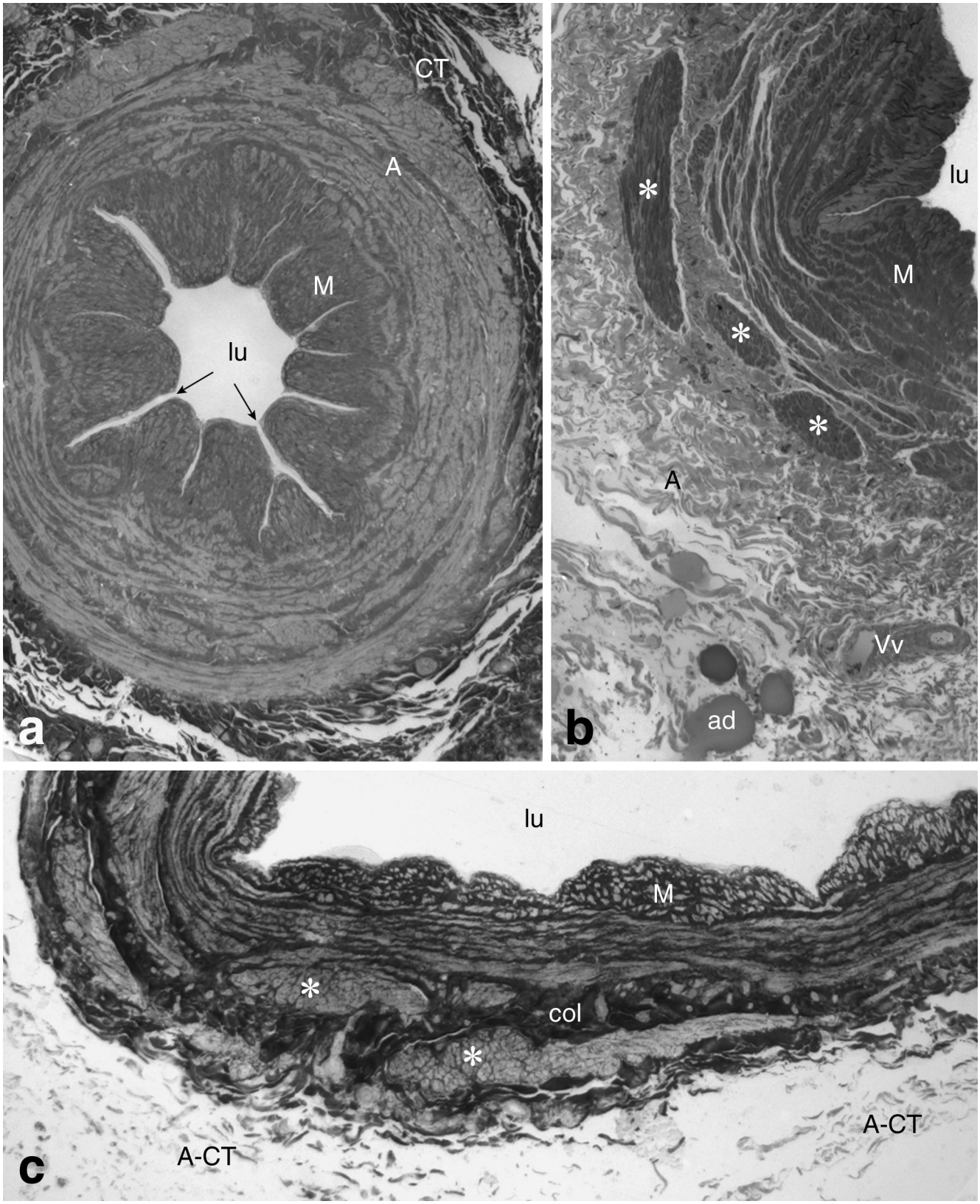

Fig. 1. Light microscopic comparison of no-touch **(a, b**) and conventional **(c)** SV preparations. **a.** Transverse section of SV displays lumen (lu), intimal folds (arrows), media (M), adventitia (A) and peri/paravascular connective tissue (CT). x 92. **b.** A fragment of no-touch SV preparation showing islands of SM (asterisks) located in the adventitia; adventitial vasa vasorum (Vv) and lipids of adipose tissue (ad) are also seen. x 92. **c**. A fragment of a transverse-sectioned SV harvested conventionally showing lack of characteristic intimal folds which together with the media have been distended. Note that only remnants of adventitia and peri/paravascular connective tissue (A-CT) are present. Asterisks indicate islands of SM. col: bundles of collagen intermingled with elastin. x 92

number of intracellular organelles and structures. These included short strands of endoplasmic reticulum, Golgi apparatus, cytoplasmic vesicles, mitochondria and Weibel-Palade bodies (Fig. 2c). The basal aspect of the endothelium contacted subendothelial connective tissue consisting of prominent extracellular matrix with fibrillar collagen and patches of elastin (Fig. 2a).

The intima of the conventional preparations of SV displayed a variety of morphological features (Fig. 3a-d), which were generally absent in the no-touch preparations. Thus the endothelial cells of various shapes and sizes were present (cell polymorphism), often in close proximity to each other (Fig. 3a). Some endothelial cells were 'flattened', lying in a 'horizontal plane' and

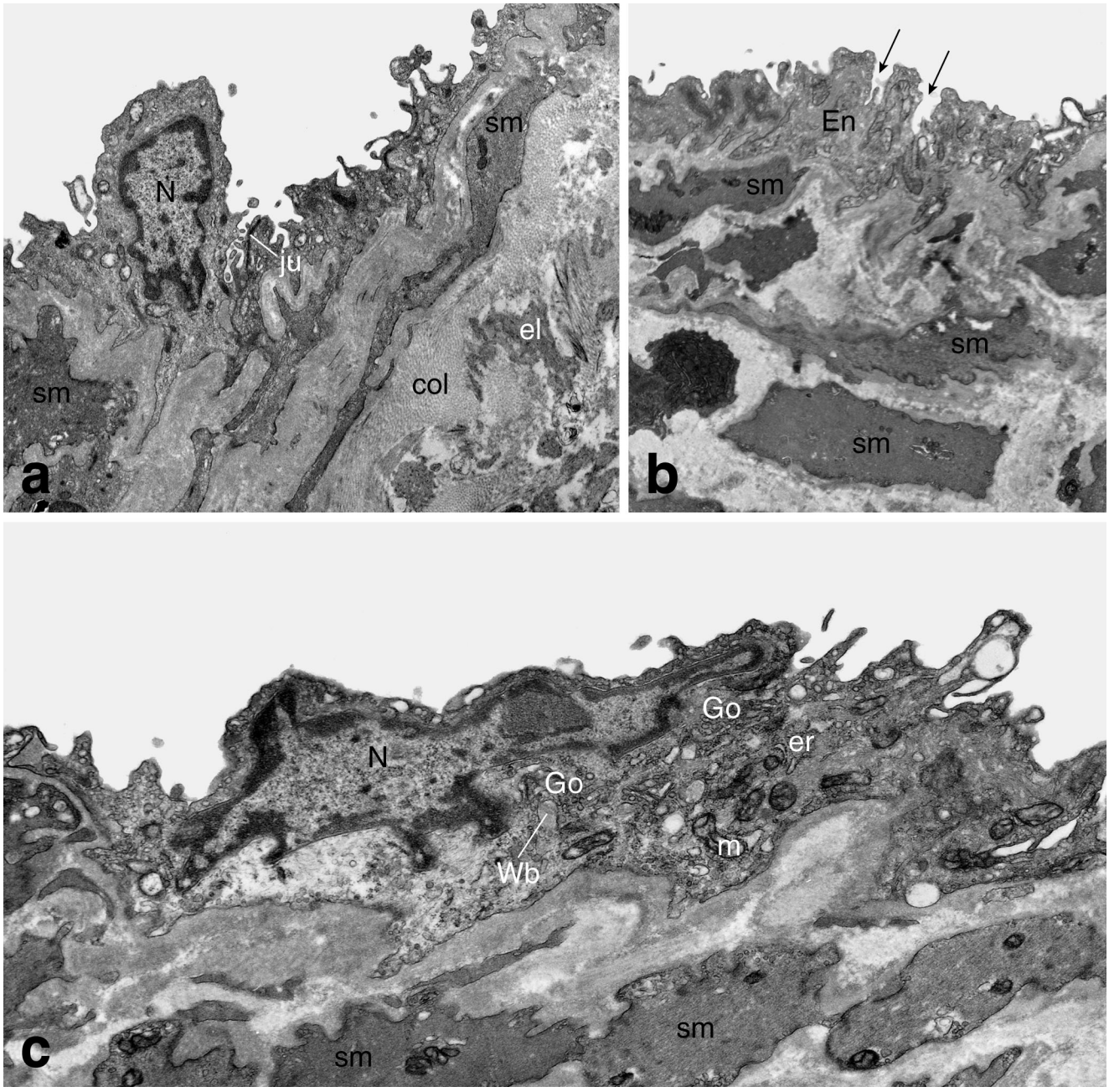

Fig. 2. Endothelium of SV harvested by the no-touch technique. **a.** Note an endothelial cell with the nucleus (N) protruding to the lumen; junctions (ju) between the cells are present. SM cells (sm), elastin (el), collagen fibers (col) are also seen. x 6,000. **b.** Note small folds (arrows) in the endothelial cells (En). x 8,000. **c.** Higher magnified an endothelial cell shows nucleus, endoplasmic reticulum (er), Golgi apparatus (Go), mitochondria (m) and the Weibel-Palade bodies (Wb). SM cells are also seen. x 10,300

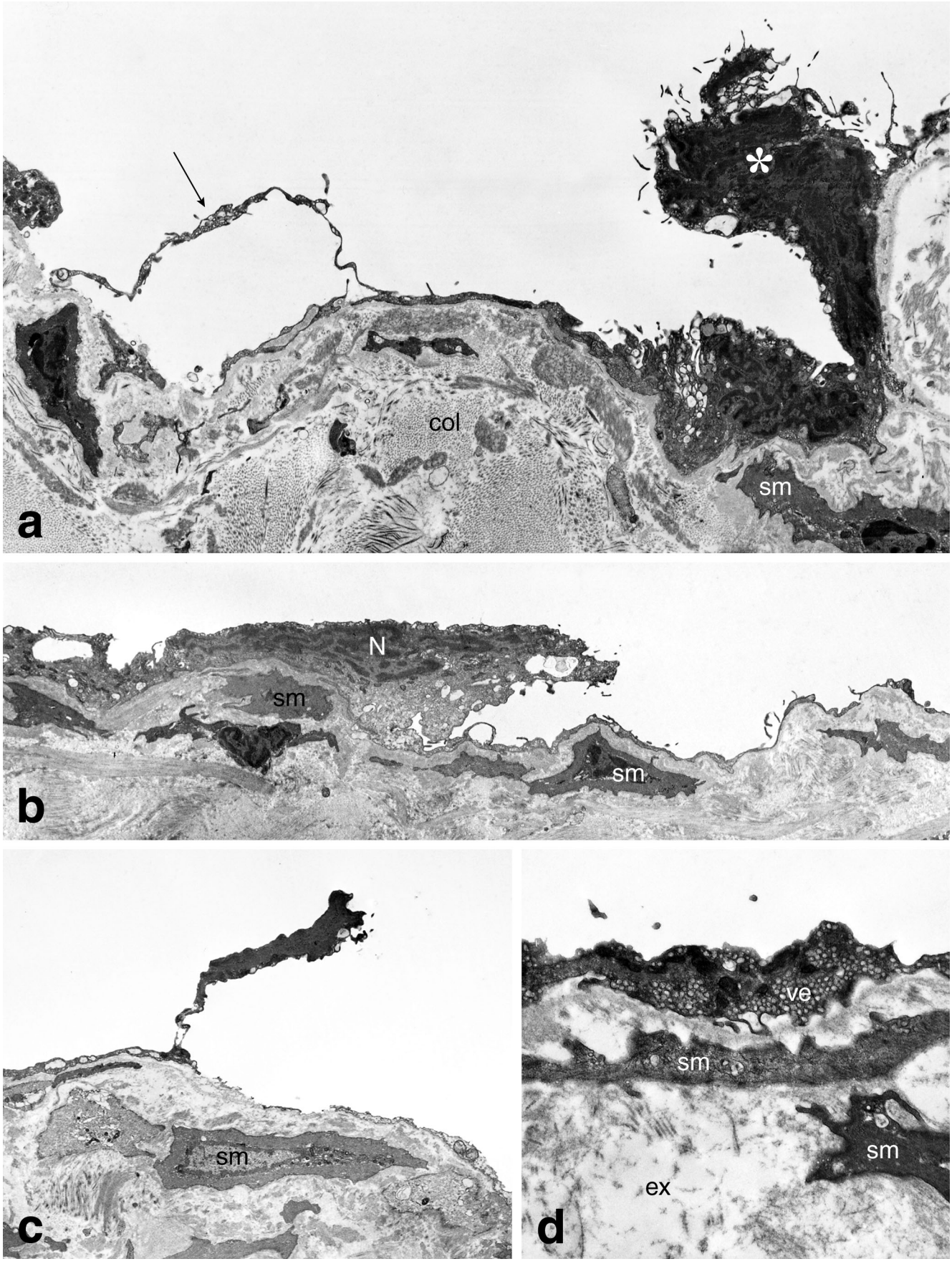

Fig. 3. Endothelium of SV harvested by the conventional technique. **a.** Note polymorphism of the endothelium; cells with 'dark' cytoplasm (asterisk), or very thin cell processes protruding to the vein lumen (arrow) are present. sm: SM cells; col: bundles of subendothelial collagen. x 5,300. **b.** Main body of an endothelial cell with visible nucleus (N) is 'squashed' from the lumenal side. x 5,000. **c.** A thinned endothelial cell containing mostly nucleus or 'dense' cytoplasm is protruding into the vein lumen. x 5,000. **d.** A fragment of a squamous endothelial cell is abundant in electron-transparent cytoplasmic vesicles (ve). ex: extracellular matrix. x 13,100

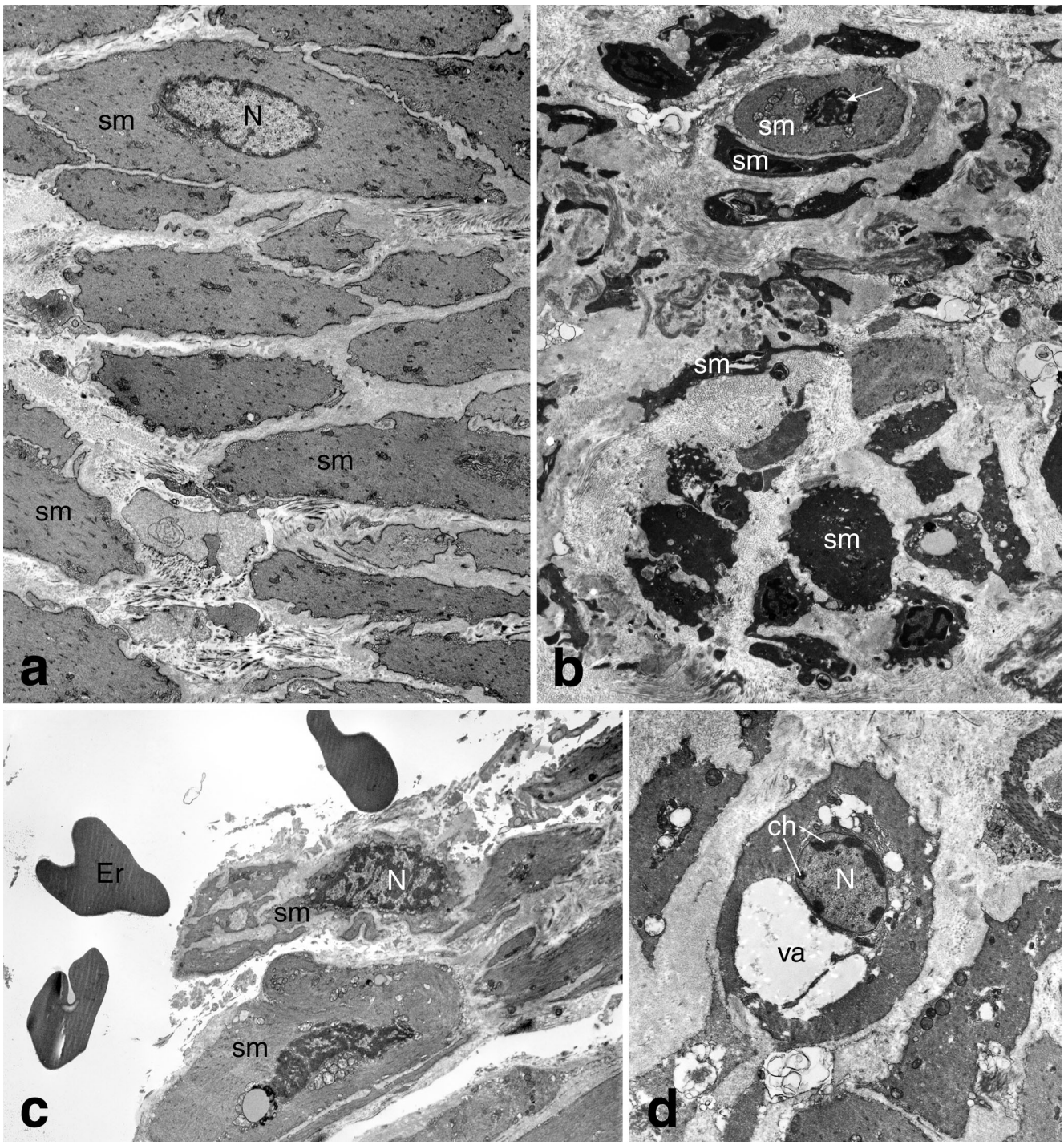

Fig. 4. Media of SV harvested by the no-touch **a** and conventional **(b-d)** techniques. **a.** A 'regular' arrangement of SM cells (sm), which display 'normal' morphological features. N: Nucleus. x 4,600. **b.** Note a polymorphism of SM cells; ovoid, elongated or multi-shaped cells are present. The sarcoplasm of some cells is dark. In one cell, a condensed nucleus is clearly visible (arrow). x 4,500. **c.** A group of SM cells at the site of the removed adventitia is exposed to its surrounding environment; 3 erythrocytes (Er) are also seen in this region. x 4,100. **d.** Note large vacuole (va) in a SM cell; the cell was located near the damaged adventitia. Also, note that the nucleus is undergoing the mitotic activity as indicated by the peripheral distribution of chromatin (ch). x 6,250

Saphenous vein for coronary bypass surgery

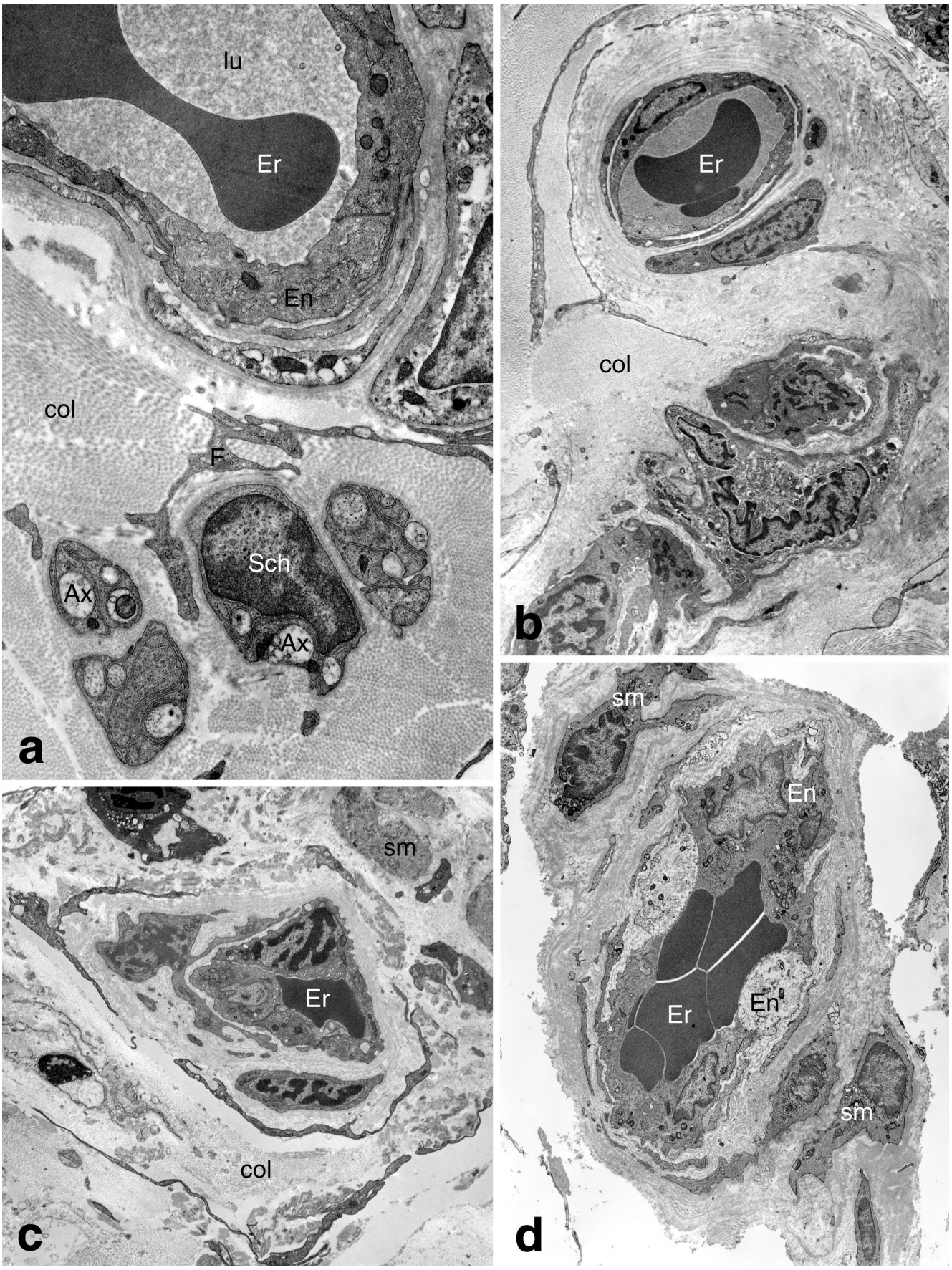

Fig. 5. Vasa vasorum of SV harvested by the no-touch **(a, b)** and conventional **(c, d)** techniques. **a.** A magnified fragment of a vasa vasorum vessel (venule) and surrounding cells. In the vessel note endothelium (En), open lumen (lu) and an erythrocyte (Er). In the vasa proximity note a bundle of autonomic nerves consisting of Schwann cells (Sch) and enclosed axon profiles (Ax). F: fibroblast; col: collagen. x 11,500. **b.** A plexus of the vasa vasorum vessels. Note erythrocytes in the lumen of the vessel. x 4,500. **c.** An example of a vasa vasorum vessel near the medial SM cells (sm). Note an erythrocyte filling the vessel lumen. x 4,500. **d.** An example of the vasa vasorum vessel (a venule) from the periphery of the SV wall. The thin layer of the connective tissue covers the vessel; note the disruptions to the connective tissue (white spaces around the vessel) caused by the harvesting procedures. The lumen of the venule is filled with erythrocytes. Also, note the polymorphism (various cytoplasmic densities) of the venule endothelial cells. x 3,600

displaying normal contact with the subendothelial tissue (Fig. 3b). Other cells were also 'flattened' or 'thinned' but protruding into the vein lumen (Fig. 3c). Intracellular organelles and structures were also observed in endothelial cells. Some of the flat profiles of endothelial cells contained an abundance of cytoplasmic vesicles (Fig. 3d).

Media: SM (Fig. 4)

In the SV harvested with the no-touch technique, the SM cells displayed typical features for this type of cell (Fig. 4a). They were elongated or ovoid, depending on the plane of section, with the nucleus located centrally. Such characteristic SM cells were also seen outside the media, within the adventitia.

In conventional SV preparations, the SM cells frequently had an irregular shape/outline (Fig. 4b). They also displayed polymorphic nuclei (Fig. 4b). The density of the cell sarcoplasm varied but cells displaying 'dark' sarcoplasm were often observed. These cells were present both in the main medial regions as well as the islets of SM cells located in the adventitia. Some of the SM cells located close to the regions of adventitia removed during harvesting were exposed to the external environment (Fig. 4c). In these, now 'peripheral' regions of the SV, SM cells containing large vacuoles were seen and the nuclei of these cells displayed characteristics of mitotic activity (Fig. 4d).

Adventitia: vasa vasorum (Fig. 5)

In no-touch preparations of SV, the connective tissue surrounding the vein muscular wall contained a number of blood vessels of various diameters, including arterioles, venules or microvessels (Fig. 5a,b). These vessels were the integral part of the vasa vasorum system. They were located close to the medial SM cells as well as in the peri/paravascular regions of the adventitia, some distance from the medial SM cells. The lumen of these vessels was usually clearly visible/open (Fig. 5a). Vessels of smaller diameter dominated in the regions closer to the medial SM coat. In the no-touch preparations of SV, fibroblasts were present near the vasa vessels, as were the profiles of the autonomic nerves (Fig. 5a).

In conventional SV preparations, the bulk of the adventitial connective tissue was absent from the vein wall, substantially reducing the number of vasa vasorum. In fact, the occurrence of the vasa vasorum was mostly restricted to the remaining adventitia, adjacent to medial SM cells (Fig. 5c). At times vessels of larger diameter were also present at the peripheral edges of the SV preparations. As with some SM cells, these peripheral vessels were also exposed (or partially exposed) to the external environment (Fig. 5d). In conventional preparations, blood cells were commonly seen filling the lumen of the vasa vasorum vessels (Fig. 5c,d).

Autonomic perivascular nerves (Figs. 5a and 6)

Autonomic nerves consisting of axons and Schwann cells were present in the adventitia of no-touch preparations of SV. These were relatively easy to identify near the vasa vasorum vessels (Fig. 5a). In the other regions of the vast adventitia these nerve profiles were scarce. The axon intervaricosities (regions of axons between varicosities) were usually associated with the Schwann cells, as at times also were varicosities. These structures contained granular and agranular vesicles and mitochondria, displaying 'normal' features (Fig. 6a). However, some axon varicosities were changed and displayed swollen mitochondria and dense multilamellar bodies (Fig. 6b), or were scarce in intraaxonal structures (Fig. 6c).

In the conventional SV preparations, axon varicosities displayed similar features to those observed in no-touch preparations. However, some axon varicosities had prominent polymorphic vesicles and multilamellar bodies. Such axon profiles were located at the peripheral edges of SV preparations, where the remaining connective tissue of the vein wall could be seen (Fig. 6d).

Discussion

This ultrastructural study examined segments of SV harvested for CABG using conventional and no-touch techniques. The results showed better preservation of the ultrastructure of no-touch SV grafts than that those harvested conventionally. This included better preservation of the lumenal endothelium, medial SM and adventitial vasa vasorum. These findings suggest that grafts harvested by the no-touch technique retain a better structural base and that this may contribute to the improved patency rate compared with conventional grafts.

Follow up angiographic studies of patients 18

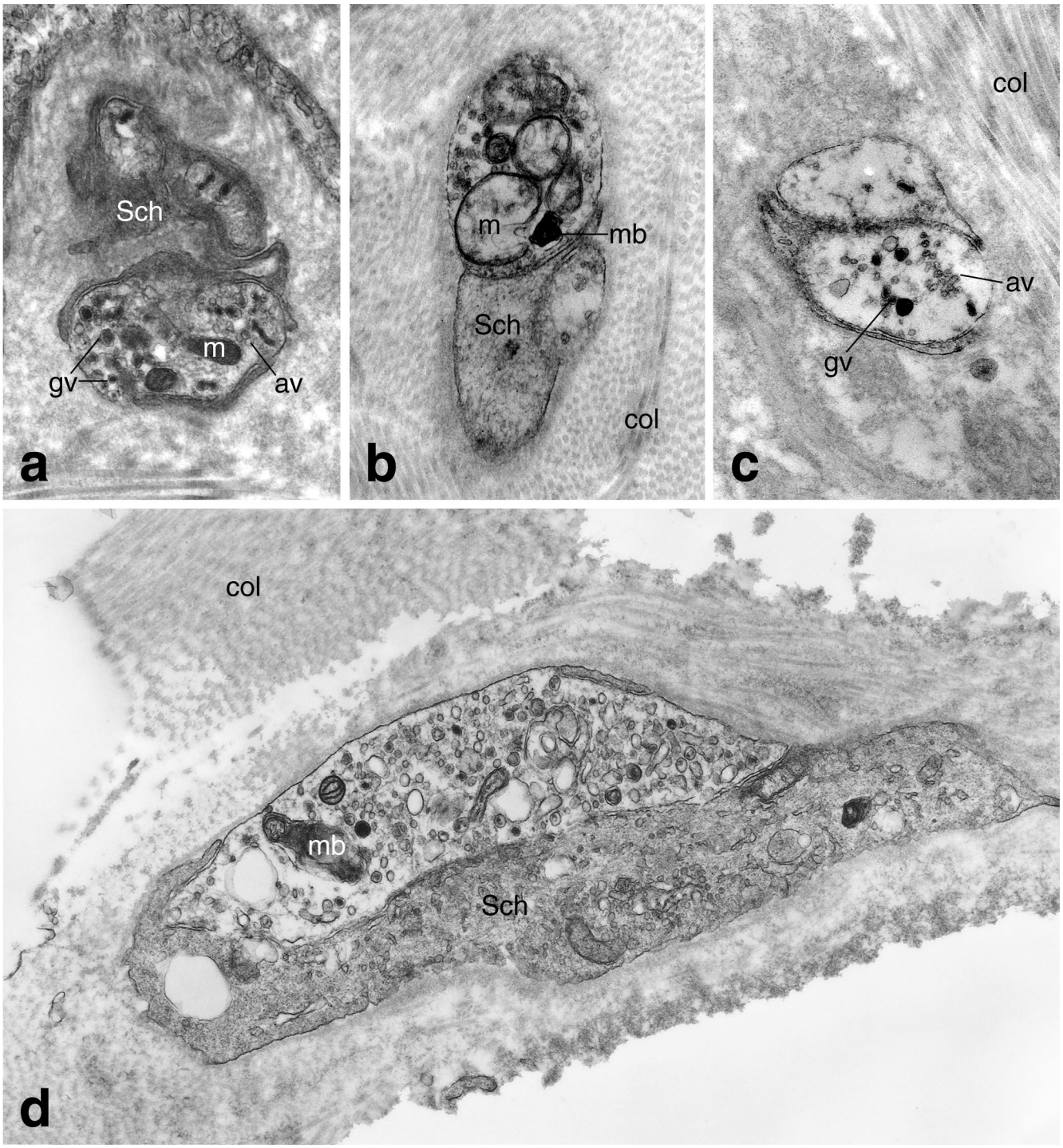

Fig. 6. Polymorphism of the perivascular nerves of SV harvested by the no-touch **(a-c)** and conventional **(d)** techniques. **a.** A varicosity contains several granular (gv) and agranular (av) vesicles and mitochondria (m). Sch: Schwann cell. x 24,000. **b.** A varicosity with prominent swollen mitochondria (m); some vesicles and one multilamellar dense body (mb) can also be seen. col: collagen fibres. x 30,000. **c.** Two varicosities; one is scarce in vesicles, the other has a few polymorphic granular (gv) and agranular (av) vesicles. x 24,000. **d.** An example of an axon varicosity from the adventitial region damaged by the harvesting procedures (the empty spaces are the sites of removed connective tissue). The varicosity contains a number of vesicles of various shapes and densities; a multilamellar body is also seen. x 20,000

months after no-touch CABG surgery showed a much improved early patency rate (<5% occlusion) compared to those with conventional grafts where occlusion rate is 15-25% in the first year (Souza et al., 2001, 2002). According to Mehta et al. (1997), in about 18% of patients who underwent conventional CABG, failure (occlusion) of grafts may occur within the first month, and is increased to approximately 30%, within the first year after surgery where failure progresses at the rate of 2-3% each subsequent year. This drastic difference in graft patency observed between the two harvesting techniques is likely to result from the preservation of potential sources of vasoactive substances, e.g. vasorelaxant nitric oxide (NO), which may prove vital for the physiology of the graft (Tsui et al., 2001, 2002). It is commonly accepted that the potential, and most important, source of NO is the vascular endothelium (see Knowles and Moncada, 1994). Therefore, NO preservation in the lumenal endothelium of the SV graft (Buttery et al., 1996) as well as in the vasa vasorum of the graft may be critical to the maintenance of graft patency.

Endothelium

Thrombotic occlusion is one of the major factors that reduces graft patency in the first year following surgery. It has also been suggested that the combination of endothelial damage together with the haemodynamic changes in the arterialised SV contribute to the occlusion process (Cox et al., 1991). The damage to the endothelium of conventional SV grafts may be implicated in various aspects of graft failure, including an effect on intimal/neointimal development, endothelial content of vasoactive agents and platelet activity (Grabowski, 1990; Allaire and Cloves, 1997).

In addition to NO, the vasoconstrictor endothelin-1 (ET-1) is implicated in graft patency (Dashwood et al., 1998a,b; Tsui et al., 2001, 2002). Both ET-1 and the enzyme responsible for the production of NO – nitric oxide synthase (NOS) are present at the subcellular level in a variety of blood vessels and are associated with both the endothelium and nerves (Loesch and Burnstock, 1998; Loesch, 2002). However, to date, the ultrastructural expression of NO/NOS and ET-1 in SV grafts has not been examined and little is known. A light microscopic study by Tsui et al. (2001) showed that the endothelium of no-touch SV grafts displayed approximately 35% higher levels of NOS (eNOS) than conventional grafts.

The vasoactive substances including NO and ET-1 produced by the endothelium play a major role in both vascular physiology and pathology (Vanhoutte, 2000). The damage caused to the endothelium by distending the SV may therefore have an effect on NO production and subsequent graft patency. Following endothelial injury (e.g. balloon angioplasty), inducible NOS (iNOS) is expressed in the SM cells. Once induced, iNOS may synthesise NO at high rates for some periods without further stimulation (Knowles and Moncada, 1994). This inhibits platelet adhesion and helps to restore blood flow (Yan et al., 1996). The immediate effects of endothelium removal may also lead to peroxynitrite generation, with resultant tissue damage and loss of contractile function (Binko et al., 1999).

Endothelial damage may trigger broad remodelling of the injury site, consequently leading to vessel occlusion (Farb et al., 1997). After implantation of the graft into the coronary circulation, the graft endothelium is immediately exposed to arterial pressure, increased wall tension and shear stress, conditions usually promoting the release of NO (Vanhoutte, 2000). Studies of SV from CABG patients showed, however, that conventional harvesting almost abolishes NO release by the SV graft (Liu et al., 2001). Harvesting may also produce circumferential deformations of SV grafts (after distension), which promote the endothelial expression of adhesion molecules leading to atherosclerosis and thrombosis (Golledge et al., 1997).

Media: SM

Medial SM cells of conventionally prepared SV (stripped of surrounding tissue and saline distended and stored in patients' heparinized blood for approximately 30 minutes) displayed mitotic activity. This seems to result from mechanical damage to the vein, since no dividing cells were observed in SV specimens obtained directly after dissection (unpublished observations). It is therefore likely that the altered mitotic activity of SM cells was time-dependent, stimulated by the surgical trauma. On the other hand, features such as nuclear condensations, blebbing of SM cell membranes or cytoplasmic vacuolisation in conventional SV might reflect apoptotic processes (Keer et al., 1972; Majno and Joris, 1995; Malik et al., 1998), that were triggered by mechanical trauma. It is also possible that both SM proliferation and apoptosis occur early in the conventionally harvested SV. Galea et al. (1999) showed that conventional harvesting increases expression of c-fos mRNA and apoptosis in human SV and reduces cell proliferation when compared with non-distended vein. An imbalance between DNA fragmentation and DNA synthesis has been suggested to contribute to graft instability and failure (Wang et al., 2001). The present study suggests that SM cells in conventional SV grafts responded rapidly to 'harvesting trauma'. Our preliminary study of the distribution of PCNA (proliferating cell nuclear antigen) immunostaining suggests that vein distension quickly brings about (within 30 min) an increase in SM proliferation (Dashwood and colleagues: data unpublished). This strongly indicates that physiological processes that might be crucial to graft patency are initiated during SV harvesting. In the light of the presence of (i) dividing and (ii) probably apoptotic SM in conventional SV grafts, it is worth mentioning that cytokines released e.g. from platelets, cells of the graft itself or from

surrounding tissues, may influence SM proliferation as well as apoptosis (Golden et al., 1991; Scott et al., 1996).

Adventitia: vasa vasorum

The preservation of 'intact' vasa vasorum is one of the necessary conditions ensuring the long-term viability of the graft (Collier, 1992). The vasa vasorum have the capacity to regulate its own tone, independently of the host vessel (Scotland et al., 1999a,b). Decreased blood flow in the vasa microvessels might cause SM and endothelial cell dysfunction in the host vessel (Stefanadis et al., 1993, 1995). Both NO and ET-1 associated with the vasa vasorum are likely to play a role (Dashwood et al., 1993; Scotland et al., 1999a,b; Tsui et al. 2001). For example, ET-1-induced contraction of isolated vasa (from porcine thoracic aorta) is mediated by ETA and ETB receptors (Scotland et al. 1999b). The vasa vasorum is also sensitive to several vasodilators including histamine, purines and NO (Ohhira and Ohhashi, 1992; Scotland et al., 1999a). Functional impairment of vasa vasorum perfusion might contribute to local hypoxia and atherosclerotic changes (Barker et al., 1994). According to Nakata and Shionoya (1996), an occlusion of the vasa vasorum results in intimal thickening and SM proliferation. In addition, disruption of the vasa vasorum results in ischaemic necrosis (Stefanadis et al., 1993) and pronounced SM loss (Kockx et al., 1992). In effect, damage to the vasa vasorum might be associated with long-term development of neointimal hyperplasia and atherosclerosis (McGeachie et al., 1989; Martin et al., 1991; Barker et al., 1993). These are clear disadvantages as to the patency of the graft. Furthermore, the re-endothelialisation process may depend on the extent of adventitial vasa vasorum (Shi et al., 1990).

Since in physiological conditions the vasa vasorum delivers vital nutrients and oxygen to the vessel wall (Brook, 1977; Stefanadis et al., 1993; Lefebvre and Lescalie, 1996; Michel, 1996), the grafts maintaining these structures are likely to be 'healthier' grafts than those devoid of the vasa vessels. In conventional SV grafts only the 'remnants' of the vasa vessels were present. These were mostly near the medial SM cells, were collapsed and filled with blood. It is therefore likely that such vessels had restricted functional significance. Nonetheless, the question arises as to the possibility that an intact vasa vasorum is more likely to establish interconnections with the vasa vasorum of the coronary vessels. In fact, there is little information regarding such connections. Studies of patients with a clinical diagnosis of thromboangiitis obliterans (TAO, Buerger's disease) suggest that the injury and regeneration of minute vessels such as re-canalising vessels and vasa vasorum play a part in the pathogenesis of TAO (Kurata et al., 2000). In normal circumstances SV is supplied by arterial and venous vasa vasorum (Lefebvre and Lescalie, 1996; Kachlík et al., 2002) whereas, in thrombosis, the vasa vessels to the vein are markedly altered suggesting a role of the vasa vasorum in the pathogenesis of thrombotic disease (Lefebvre and Lescalie, 1996).

Adventitia: autonomic nerves

Due to the abundant connective tissue in the no-touch preparations of SV, finding nerve profiles was a difficult task. Nerves were mostly located near the vasa vasorum. There was no great difference between the conventional and no-touch techniques as regards the ultrastructural features of the autonomic perivascular nerves (quantitative differences have not been investigated here).

In general, perivascular nerves may not survive grafting (at least at early stages), since they were severed from their sources, mostly from sympathetic neurones of pelvic ganglia (Herbst et al., 1992). The stripping of the SV connective tissue also removes the nerves from the graft and those remaining are usually damaged, as has been observed in this study. Usually nerve injury and/or regeneration involves many interactions between cellular elements and the extracellular matrix (Beuchew and Friede, 1984; Son and Thompson, 1995; Hall, 1997; Terenghi et al., 1998).

An obvious consequence of graft harvesting is thus the loss of the integrity of perivascular innervation. This may be followed by an adaptive alteration of this innervation (Dashwood et al., 1998a), and an increase in the amount of neural tissue after implantation (McGeachie et al., 1981, 1989). It has been suggested that graft innervation influences neointima formation, possibly through the increased release of catecholamines (Waris et al., 1984). According to Dashwood et al. (1998a) ET-1 plays a role in paravascular neurogenesis. In the regenerating adventitia of vein grafts, there is a marked increase in sites of small profile paravascular nerves, which seem to represent some adaptive phenomenon relevant to arterial conditions (Dashwood et al., 2000). Preservation of vascular nerves as well as the vascular endothelium is important since the plethora of vasoactive agents produced by these components influence vascular cell proliferation, vasomotor tone and blood flow (see Ralevic and Burnstock, 1993; Vanhoutte, 2000).

Conclusions

This study revealed a better ultrastructural integrity of SV harvested by the 'no-touch' technique than those prepared using conventional methods. These observations suggest that the preserved morphology of 'no-touch grafts' may render them less prone to pathological factors. Future studies will focus on the expression of vasoactive factors produced by the endothelium, SM and perivascular nerves and will help to assess the effect of damage to the SV during harvesting for coronary artery bypass

surgery.

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