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The Influence of Tissue Expanders on Grafted Vessels

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Interpositionally grafted arteries and veins were expanded with a 20cc tissue expanders in 50 Sprague-Dawley rats. The grafts were done on both hind legs, one side was expanded and the remaining side was used as control. The average gain in length of expanded grafted arteries and veins was over 4 and 6 times that of the controls respectively. The differences in the patency rates between expanded and control grafts were not statistically significant. Histologic examination revealed that there were no changes in the areas of the media and lengths of the inner elastic laminae of the expanded arterial grafts. In both expanded and control vein grafts, marked intimal thickening was noticed, although these changes were not statistically significant. Expansion of grafted vessels can be safely carried out without loss of vessel patency.

Key Words: Tissue expander, grafted vessels

The physiologic consequences of pressure/tension elongation of blood vessels have not been investigated in detail. These phenomena are observed on the abdominal wall during pregnancy or in tissue adjacent to slowly growing tumors. Increases in capillary blood flow and skin viability as in delayed skin flaps have been reported in expanded tissues (Cherry *et al.* 1983; Sasaki *et al.* 1984). Recently, tissue expanders have been used in our laboratory to expand intact axial blood vessels while maintaining long-term patency. The expanded vessels showed normal histologic appearances and these vessels were found to be functionally normal when severed and reanastomosed (Stark *et al.* 1987; Hong *et al.* 1987). This finding led us to investigate whether grafted vessels could be similarly expanded.

The present study was designed to evaluate the gain in length, patency, and changes in histologic features of the expanded vessels grafted in rats.

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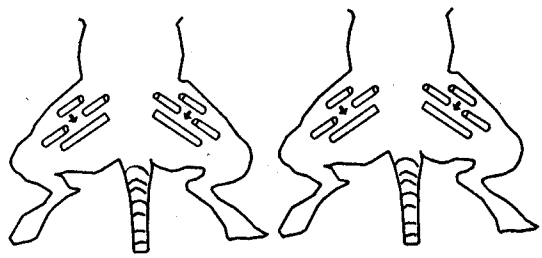
MATERIALS AND METHODS

Grafting Procedure

Fifty male Sprague-Dawley rats, weighing 250 to 350 grams, were used. They were housed in individual cages and fed standard rat chow ad libitum. Sodium pentobarbital (35mg/kg) was used for the anesthesia.

Five mm segments of femoral arteries proximal to the origin of the superficial inferior epigastric artery were removed bilaterally. The same segments were reinserted into 25 rats as interposition arterial grafts. For the remaining 25 rats, the resultant arterial defects were repaired using 7mm segments of the superficial inferior epigastric vein (Fig. 1). The procedure was the



Arterial graft (n=25) Vein graft (n=25)

Fig. 1. *5mm segments of femoral arteries or 7mm segments of superficial epigastric veins are inserted into femoral artery defects.*

Fig. 2. After the anastomoses were completed, the lengths of the grafted vessels were checked again and these measurements were used as base line lengths.

Fig. 4. Tissue expanders were inserted by a lateral approach into a deep submuscular pocket against the femur. The injection port was placed beneath the dorsal skin.

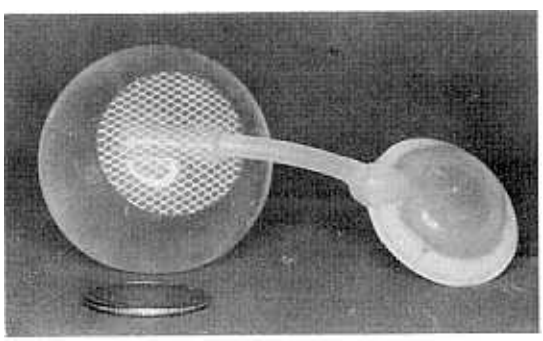


Fig. 3. Twenty cc Spherical Silicone tissue expanders (Cox-Uphoff®) were used.

same as that reported previously (Acland 1980). After the anastomoses, the lengths of the grafted vessels were remeasured and used as the baseline values (Fig. 2).

Expansion Procedure

Three weeks after the initial grafting, six rats (Group I) were sacrificed for histologic examination. The animals were anesthetized with ketamine hydrochloride (40mg/kg i.m.) which is known to have a short active duration time. Of the remaining 44 rats, one side of the grafted vessel was expanded and the other side was used as control.

Twenty cc spherical silicone tissue expanders (Cox-Uphoff®) (Fig. 3) were inserted by a lateral approach into the deep submuscular pockets against the femur. The pockets were created by detaching muscles from the femur. The injection port was placed beneath the dorsal skin (Fig. 4).

The expansion was carried out over 14 days using 4 to 6cc of saline at 3 to 4 day intervals, until a final volume of 20 to 30cc was obtained. Foot circulation was monitored and in five animals, intraluminal pressure was measured by a Hewlett-Packard pressure monitor during injection (Dilley *et al.* 1986; Startk *et al.* 1987)

Three days after completion of expansion, 20 rats (Group II) were examined for changes in vessel length, patency and histology. The remaining 24 rats (Group III) were similarly examined two weeks after completion of expansion.

Histologic Examination

All grafted specimens were harvested and fixed in 10% buffered formalin. Cross sections at the mid-portions and longitudinal sections at the distal anastomotic sites were made and stained with toluidine blue. The thickness of the media in the arterial grafts and that of the intima in the vein grafts were measured. The vessel wall thickness was measured at four different sites (3, 6, 9, 12 o'clock positions) of each cross section. Wall thickness was also measured at two different sites (at 150μm intervals from the distal anastomotic site) of each longitudinal section. All measurements were taken at × 310 magnification with an eye-piece micrometer.

As the specimens were fixed in formalin without perfusion, the cross-sectional areas of the arterial and vein grafts, and the lengths of arterial inner elastic laminae of all cross sections were checked again with a Polar Planimeter and Hipad TM Digitizer with IBM PCXT computer.

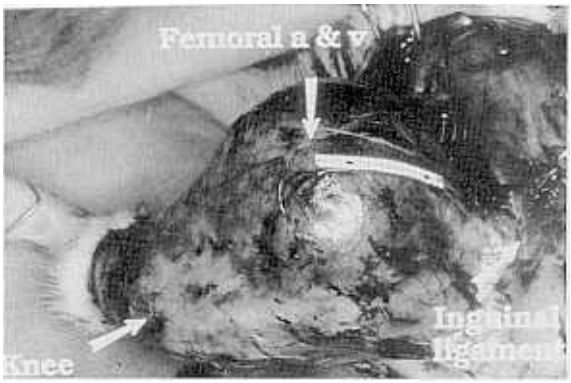


Fig. 5. This picture showing measurement of the elongated grafted vessel after complete expansion.

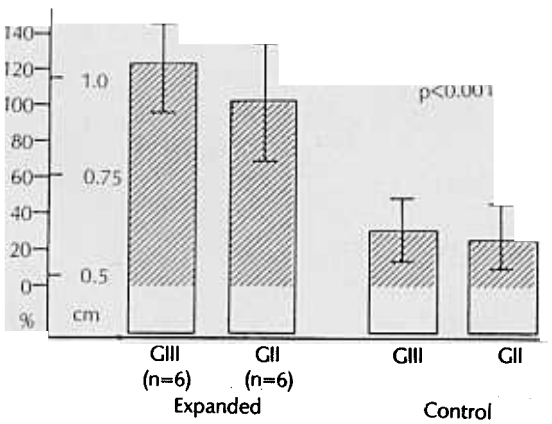


Fig. 6. Gain in arterial grafts length. Both groups of the expanded arterial grafts showed an increase in length over four times that of the controls (p<0.001). (The unshaded area represents the original vessel length, and shaded area the percentage increase.)

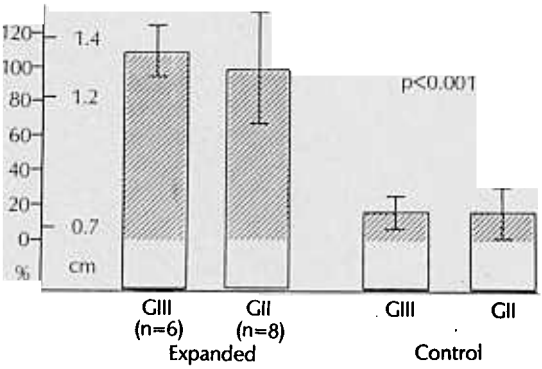


Fig. 7. Gain in vein grafts length. Both groups of the expanded vein grafts showed an increase in length over six times that of the control grafts (p<0.001). (The unshaded area represents the original vessel length, and shaded area the percentage increase.)

Patency Rates of Grafts and Nongrafted Veins.

	Arterial graft (n=17)	Vein graft (n=18)	Normal Vein (n=35)
Expanded	94%	89%	94%
Nonexpanded	100%	94%	100%

Fig. 8. The patency rates of the expanded grafts were as good as in controls and non grafted veins without statistical significances.

RESULTS

Gain in length of grafted vessels

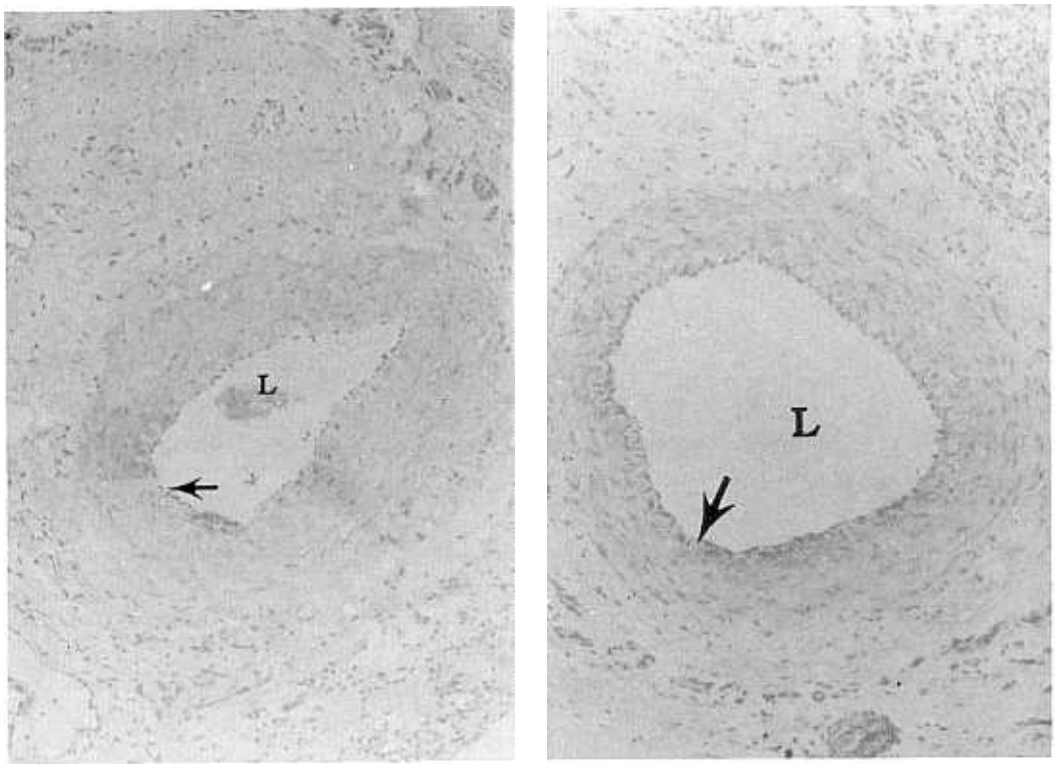
The grafted vessel of the expanded thigh was visualized. A paper scale was placed alongside the expanded vessel with the expander in situ and the length of the expanded grafted vessel was measured (Fig. 5). The control grafted vessels were similarly measured.

In arterial grafts, the average gain in length of the expanded grafts was 100±35% for Group II (evaluated three days after complete expansion) and 120±25% for Group III (evaluated two weeks after complete expansion). In the controls, the increases in length were 24±19% (Group II) and 28±16% (Group III). Overall, the length in both groups with expanded arterial grafts was over four times that of the controls. This was a statistically significant increase in the length of both groups with expanded arterial grafts ($p<0.001$) (Fig. 6). The maximum gain in length of the expanded arterial graft was 167%.

In vein grafts, Group II showed a gain of 97±32%, whereas in Group III it was 107±16% in the expanded grafts. In contrast, the controls showed an increase of 14±14% (Group II) and 14±10% (Group III). Both groups of the expanded vein grafts showed an increase in length over six times that of the control grafts ($p<0.001$) (Fig. 7). The maximum gain in length of expanded vein graft was 150%.

Patency

When expanders were implanted, the patency of



Expanded (G III) Control (G III)

Fig. 9. There was no statistically significant difference in the areas of the media and the lengths of the inner elastic laminae between the expanded and control arterial grafts in both Groups (II, III) ($p>0.05$)
(L: lumen, Arrow indicates inner elastic lamina, Toludine blue stain 40 ×).

the grafts was checked by looking for pulsations distal to the anastomotic site. When the specimens were obtained, the first cut was made distal to the anastomotic site and the flow of blood through the grafted segment was observed.

In arterial grafts, the patency rate of the expanded grafts was 94% and it was 100% in controls (n=17). In vein grafts, the patency rate of the expanded grafts was 89%, and in controls 94% (n=18). The patency rate in expanded normal (non-grafted) veins was 94% (n=35) (Fig. 8).

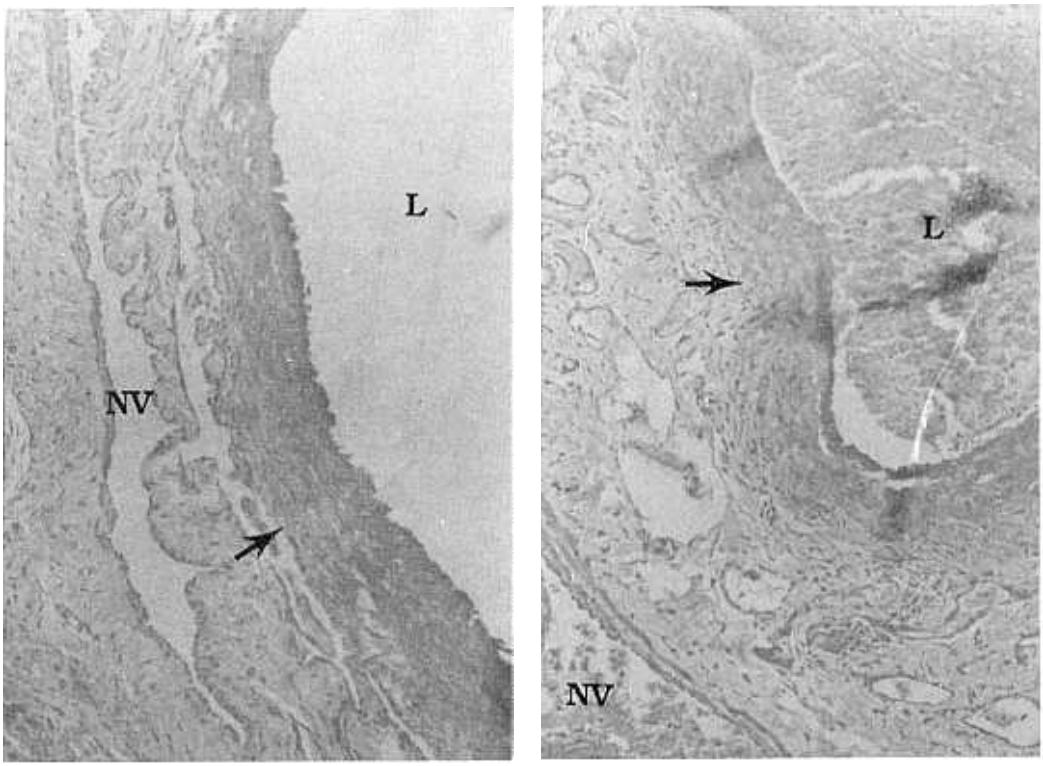
Histologic Examination

The histology of expanded vessel grafts did not show any significant morphologic changes except fibrosis around the expanded vessels, probably due to the expansion procedure. There was no statistically significant difference in the areas of media and the lengths of the inner elastic laminae between the expanded and control arterial grafts in both Groups (II, III) ($p>0.05$) (Fig. 9). In vein grafts, marked intimal thickening was observed in both expanded and control grafts, but the differences in thickness and cross sectional areas were not statistically significant ($p>0.05$) (Fig. 10).

Three week-old vein grafts showed significant intimal thickening at the distal anastomotic site compared to the midportion of the graft ($p<0.05$). No such statistically significant difference was seen in Groups II and III.

Intraluminal pressure

Implant pressure was recorded on three occasions-before, immediately after, and ten minutes subsequent to injection of saline. Pressure always increased after saline injection and gradually decreased with time, eventually returning to similar preinflation values (Fig. 11).



Expanded (G III) Control (G III)

Fig. 10. Vein grafts showed marked intimal thickening in both expanded and control grafts, but the difference in thickeness and cross sectional area was not statistically significant ($p>0.05$) (Toluidine blue stain 40 ×).

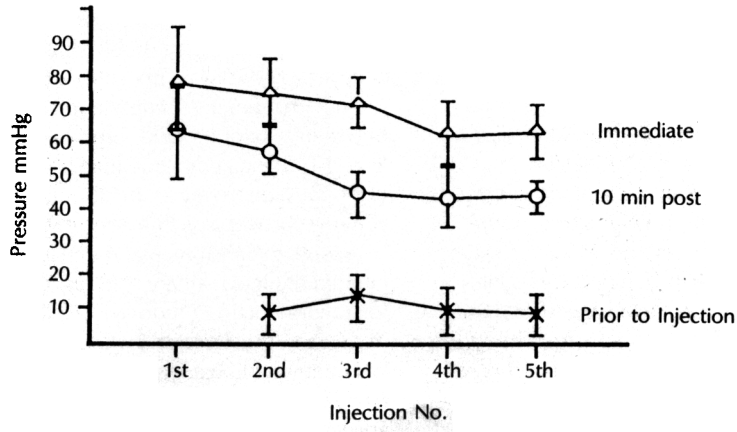


Fig. 11. Intraluminal pressure increased after saline injection and gradually decreased with time, eventually returning to similar preinflation values.

DISCUSSION

In our rat model, we used 7mm segments of vein graft to repair arterial defects. When a 5mm segment of femoral artery was removed, the resultant arterial defect was about 8mm. In a preliminary study, we tried four different sizes (5, 6, 7, 8mm) of vein grafts to bridge the arterial defects and found that the 7mm segments were ideal to obtain tension-free anastomosis, while at the same time avoiding a redundancy of vessel length. In arterial grafts, the same segments were reinserted as interposition arterial grafts without tension at the anastomotic sites.

The extent of intimal hyperplasia in veins grafted into arteries is a major determinant of graft survival (Dilley *et al.* 1986). All vein grafts were noted to be denuded of endothelium two days after grafting and the intimal layer was seen to consist of a thin mural thrombus (Dilley *et al.* 1983). Endothelial cell and smooth muscle cell replication increases markedly during the first week and produces an intact endothelial surface by two weeks (Zwolak *et al.* 1987). The intima in the grafted segment was seen to develop at the anastomotic site as early as 10 days. It was most prominent in the 15 to 28 day-old graft and subsequently became more evenly distributed throughout the graft without further thickening (McGeachie *et al.* 1981; Dilley *et al.* 1986). Our three week-old specimen showed progressive thickening at the mid-portion of vein grafts (Fig. 12). Cellular proliferation almost stops by the fourth week, and a further increase in cross sectional wall area between 4 and 12 weeks was accounted for by accumulation of connective tissue (Zwolak *et al.* 1987). We therefore thought that the critical period of arterialization of the vein graft may be between two to four weeks, and decided to start vessel expansion three weeks after grafting.

This development of the intima termed "arterialization" is regarded as an adaptive process (Dilley *et al.* 1986; Zwolak *et al.* 1987). The thickness of the intima of the grafted vein is not significantly greater than the combined thickness of the intima and media of the adjacent artery (Dilley *et al.* 1986). What factors could regulate this? Some investigators have suggested that the regulatory mechanisms involved may be a response to injury, tangential wall stress and shear stress (Zwolak *et al.* 1987). When abnormal conditions are established in the graft, so as to disrupt the growth control mechanisms, further thickening of intima may occur (Dilley *et al.* 1986). Our histological studies of

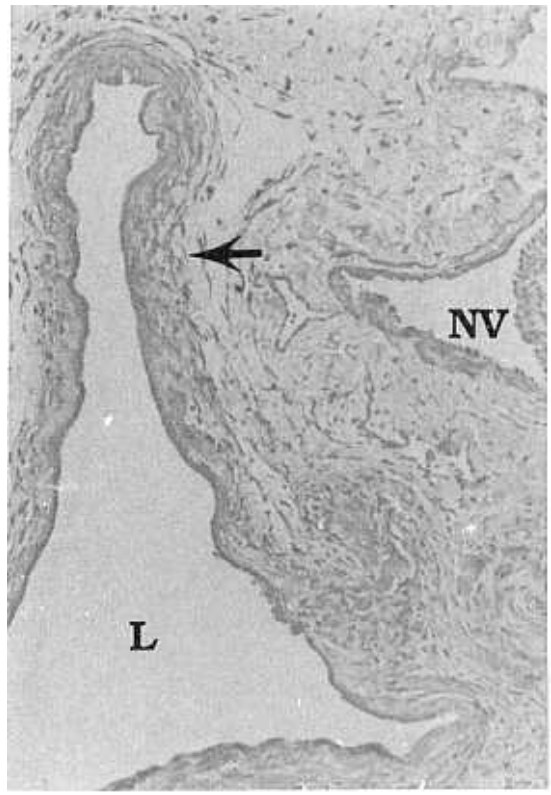


Fig. 12. Three week-old vein graft showed progressive thickening at the midportion of graft.
(L: lumen, NV: normal vein, Arrow indicates inner elastic lamina. Toluidine blue stain 40 ×).

expanded vessel grafts did not show any significant morphological changes, except fibrosis around the expanded vessels and in the media in a small number of cases which was probably due to expansion procedures. There was no statistically significant difference in intimal thickness and cross-sectional area between expanded and control vein grafts ($p>0.05$) (Fig. 10). These findings suggest that tissue expansion does not seem to act as an abnormal condition in the adaptive process of the vein graft.

Tissue expansion is a mechanical stretching of needed tissue to enhance its size to provide a donor for reconstruction (Radovan 1984). The importance of mechanical stress on a variety of cellular activities has been recognized in many diverse systems. Cyclic stretching of smooth muscle cells in culture causes an increase in protein and collagen synthesis (Sottiurai *et al.* 1983). Expansion procedures can also decrease the tissue oxygen tension to extremely low levels

(Argenta *et al.* 1985) and vascular smooth muscle cells can multiply in the hypoxic environment of the vessel wall (Gresham 1983). Therefore, the authors question the role, if any, of these cellular activities in the growth control mechanism of grafted vessels. One can only conclude at this time that tissue expansion does not act as a factor which contributes to further intimal thickening of vein grafts. Although we thought that expansion might have thinned out the grafted vessels, because some expanded arterial graft specimens showed a more tortuous pattern of the inner elastic laminae, this was not found to be statistically different in areas of the media and lengths of the inner elastic laminae between the expanded grafts and controls.

The results showed that the gain in length of the expanded arterial and vein grafts was over four times ($p<0.001$) (Fig. 6) and six times ($p<0.001$) (Fig. 7) that of the controls respectively. The insertion of tissue expanders into the Group III rats was done after the Group II expansions were started, and thus technical difficulties, such as placement of the expander underneath the grafted vessel so that the vessel could pass over the center of the expander as compared to displacement of the vessel were avoided in Group III. Therefore, the statistically significant increase in length of the grafted vessel of Group II might have been even greater. These findings suggest a net gain in grafted vessel length, reiterating the results of previous studies in our laboratory (Stark *et al.* 1987; Hong *et al.* 1987). Others have also proposed that tissue expansion yields a true dividend (Austad *et al.* 1986).

The patency rates between expanded grafts and controls were not statistically different. Moreover, there was no difference in patency rates between expanded grafts and the accompanying normal veins (Fig. 8). Maintenance of vascular patency is the main limiting factor to expansion (Stark *et al.* 1987). In general, no precise timetable for tissue expansion is set. Clinically, fear of extrusion and compromise of overlying tissue have encouraged slow inflation of the prosthesis (Argenta *et al.* 1985). Our results confirm the suggestion of previous studies in our laboratory that gradual expansion is the best method for maintaining patency in expanding rat vessels (Stark *et al.* 1987; Hong *et al.* 1987).

It seems reasonable to conclude that expansion of the grafted vessel does not act as a factor which contributes to further intimal thickening, but rather suggests a net gain in grafted vessel length. It is definite that grafted arteries and veins could be safely expanded and their patency maintained.

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