An Experimental Study of the Sleeve Technique in Graft Anastomosis

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The effects of a sleeve anastomotic technique (SL), which was designed to avoid intimal injury, on the healing characteristics of a polytetrafluoro-ethylene (PTFE) graft were compared with the effects of the conventional end-to-end anastomotic technique (ETE). A segment of canine abdominal aorta was replaced with a PTFE graft. The grafts were explanted after 4 months and the anastomotic portion was examined macroscopically and by light microscopy. The animals were divided into 4 groups (A to D) according to the combination of the anastomotic methods (ETE or SL) used for the proximal and distal anastomosis. No substantial difference was found between the proximal and distal anastomosis for either ETE or SL. The pulling tests between the PTFE graft and the excised segment of the abdominal aorta revealed no significant difference in the breaking loads between ETE and SL. Macroscopic and microscopic examination revealed that SL resulted in better and more regular neointimal extension on the luminal surface of the PTFE graft. The results suggest that SL is less traumatic to the endothelium of the host artery and may be effective in preventing postoperative intimal hyperplasia.

Key words: graft anastomosis, intimal hyperplasia, suture technique

Introduction

Pathologic intimal hyperplasia after vascular procedures has been implicated as a major cause of late obstruction in small to mid-sized arterial reconstructive surgery. Results from experimental and clinical studies have suggested that intimal hyperplasia occurs as a

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wound-healing response following injury to the endothelium.[1-4] Therefore, postoperative intimal hyper-plasia may be prevented by avoiding endothelial injury during graft anastomosis. The purpose of the present study was to ascertain whether a new anastomotic technique designed to avoid injuring the host vessel endothelium is effective for preventing postoperative intimal hyper-plasia. For this purpose, a segment of canine abdominal aorta was replaced with an expanded polytetra-fluoroethylene (PTFE) graft using either routine end-to-end anastomoses or a new sleeve anastomotic technique. The latter is actually a modification of sleeve microarterial anastomosis[5-9] (telescoped or end-in-end anastomosis) for larger arteries.

Materials and Methods

Twenty-eight adult mongrel dogs of both sexes weighing between 9 and 20 kg were used. The study was approved by the Animal Care and Use Committee of Nagasaki University, and all animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Surgical procedure:

Anesthesia was induced by intravenous injection of sodium pentobarbital and maintained by the inhalation of halothane. The animals were intubated and ventilated mechanically with room air using a mechanical ventilator. The surgery was performed under sterile conditions. With the animal in the supine position, a full-length median abdominal skin incision was made and the infrarenal abdominal aorta was exposed. After 2,000 units of heparin was given intravenously, a 1.0-cm segment of the abdominal aorta was excised on the caudal side of the renal arteries. A PTFE graft (8 mm in internal diameter and 2 cm in length) was

implanted to replace the excised segment, using one of the following methods.

(1) End-to-end anastomosis (ETE, control method).

The prosthesis was anastomosed to the host vessel by the routine end-to-end anastomosis with the usual continuous over-and-over suture using 6-0 monofilament polypropylene. In order to facilitate approximation of the intima of the host vessel to the luminal surface of the prosthesis, the anastomosis was made slightly everted relative to the suture line.

(2) Sleeve anastomosis (SL, Fig. 1).

The stump of the host vessel was inserted into the lumen of the prosthesis, and a continuous over-and-over suture with 6-0 polypropylene was placed between the prosthesis and the host vessel. The suture was passed through the wall of the prosthesis close to its free edge and then out through the open end of the prosthesis, picking up the wall of the host vessel; only the adventitia and part of the media were included in the needle pass through the vessel wall. The sutures were tied so that approximately 5 mm of the host vessel was drawn inside the prosthesis; thus, the overlap produced was less than the internal diameter of the prosthesis. The proper construction of anastomosis using this technique kept the luminal surface free of foreign materials.

The animals were divided into 4 groups according to the combination of the methods used for the proxi-

mal and distal anastomoses. In group A (10 dogs), the proximal and distal anastomoses were both ETE. In group B (11 dogs), the anastomoses were made by SL and ETE, respectively. In group C (2 dogs), they were made by ETE and SL respectively, and in group D (5 dogs), both were made by SL. No anticoagulant agent was used postoperatively.

Morphological evaluation

The animals were fed on standard mixed feed and then examined 4 months after surgery. At explantation, the animals were reanesthetized, and the grafts, together with 20-mm lengths of adjacent aortic segments at the proximal and distal anastomoses, were excised en bloc and sectioned longitudinally. The graft surface was examined macroscopically and photographed for later measurement. The grafts were then fixed with 10% formalin and stained with hematoxylin-eosin, Masson and elastica van Gieson stains for light microscopy.

Pulling test:

For pulling tests, 6 anastomoses (3 anastomoses each with the ETE and SL methods) were made between the PTFE graft and the excised segment of the abdominal aorta, which was obtained from 2 dogs not in any of the above-mentioned 4 groups. The breaking loads were measured immediately after making anastomoses using a computerized multipurpose autograph (DDS 500, Shimadzu Co. Ltd., Tokyo, Japan) in 3 anastomotic lines made by each of the 2 suture methods.

Results

There were 5 early deaths (within 2 weeks after surgery); 2 each in groups A and B, and 1 in group C. The causes of death were peritonitis in 2 dogs, and unknown in 3 dogs. The remaining 23 dogs survived to 4 months.

Patency Rate (Table 1):

The patency rates in the 23 surviving dogs at the end of the 4th month were 100% in groups A, C and D, and 78% in group B.
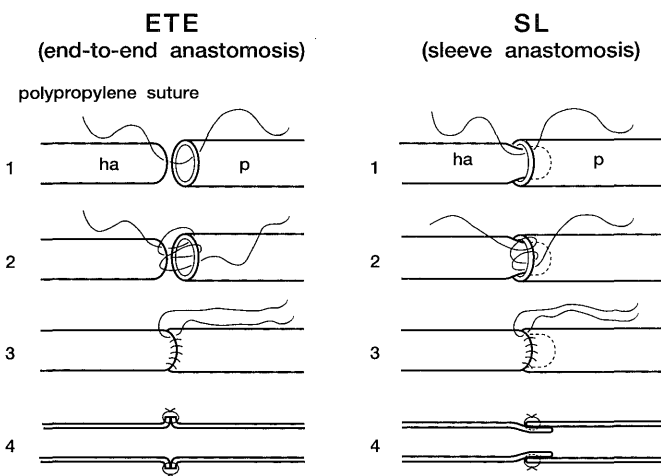

Fig. 1 Suture techniques of conventional end-to-end anastomosis (ETE) and those of sleeve anastomosis (SL): In SL, the stump of the host vessel is inserted into the prosthetic graft lumen. Continuous over-and-over suture with 6-0 polypropylene suture was placed between the prosthetic wall and the host vessel wall; only adventitia and part of the media were included in the needle-pass. ha: host artery; p: prosthesis (PTFE graft).

Table 1 Number of experiments, survival rate, graft patency and patency rates of groups A to D are shown. pro: suture technique of proximal anastomosis; dis: suture technique of distal anastomosis; ETE: end-to-end anastomosis; SL: sleeve anastomosis.

Group (pro//dis)	No. of dogs	Survived	Graft Patency	
			Patent	Patency Rate (%)
A (ETE//SL)	10	8	8	100
B (SL//ETE)	11	9	7	78
C (ETE//SL)	2	1	1	100
D (SL//SL)	5	5	5	100
Total	28	23	21	91

Pulling Test:

There was no significant difference in the breaking load between the 2 methods, with an average value of 4.5-kg weight in the ETE anastomoses and 4.3-kg weight in the SL anastomoses.

Morphological Findings:

No substantial difference of morphological findings (macroscopic and microscopic) was found between the proximal and distal anastomotic sites with either anastomotic method.

Macroscopic Findings:

The ETE anastomotic portion showed an irregular and incomplete neo-intimal cell coverage of the graft at both the proximal (group A and C) and distal (group A and B) anastomotic sites. Suture materials could be seen at the luminal surface of the anastomotic site, with or without neointimal cell coverage over them (Fig. 2). In the SL anastomosis group, no suture material was seen at the anastomotic sites, and nearly the same width of regular neointimal extension from the suture line was observed (Fig. 3).

Histological evaluation:

In the ETE anastomoses, Masson staining revealed suture materials beneath the epithelial cells and medial degeneration (Fig. 4). Localized thickening of the luminal surface of the native artery close to the proximal and distal anastomoses was observed. There was a layer of collagen fibers on the luminal surface of the graft, which was covered with a single layer of intima-like cells. Elastica van Gieson staining revealed that elastic fibers in the media near the anastomotic site disappeared, and no elastic fibers could be seen in the newly developed tissue covering the graft surface (Fig. 5). In the SL anastomotic sites, in contrast, the Masson staining revealed that collagen fibers and smooth muscle cells had smoothly progressed on the luminal surface of the graft. This newly developed tissue was covered with a layer of intima-like cells (Fig. 6). Elastica van Gieson staining revealed interrupted elastic fibers, and there were no elastic fibers at the neo-intimal portion (Fig. 7).
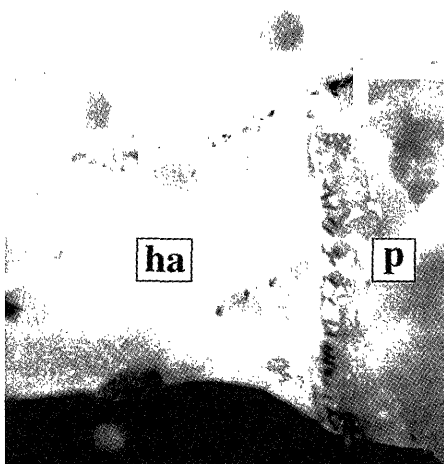

Fig. 2 Macroscopic findings of the end-to-end anastomosis. Suture materials could be seen at the anastomotic site.
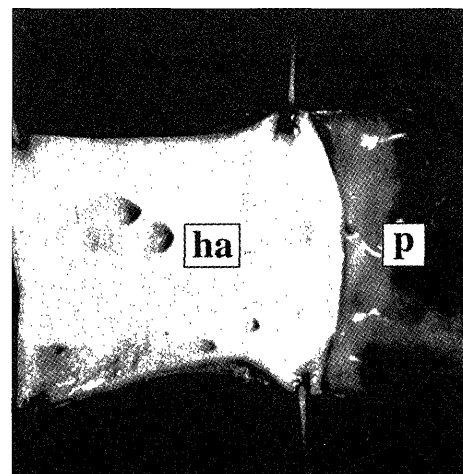

Fig. 3 Macroscopic findings of the sleeve anastomosis. There were no suture materials at the anastomotic site, and regular neointimal extension from the anastomotic line was seen.
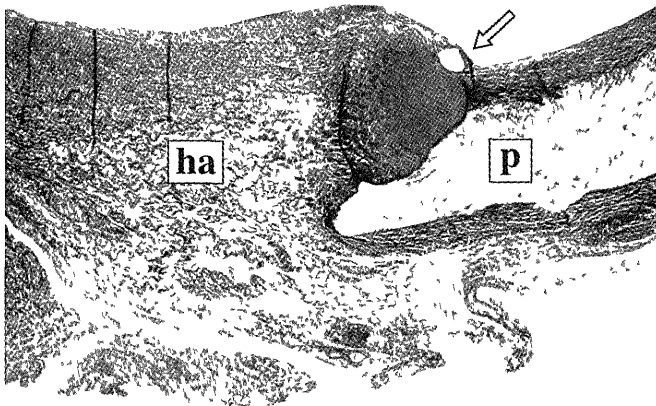
Fig 4 Microscopic findings of the end to end anastomosis (Masson stain x 50) Suture material was seen beneath the epithelial cells (arrow) Localized thickening of the host artery close to the anastomotic site was observed
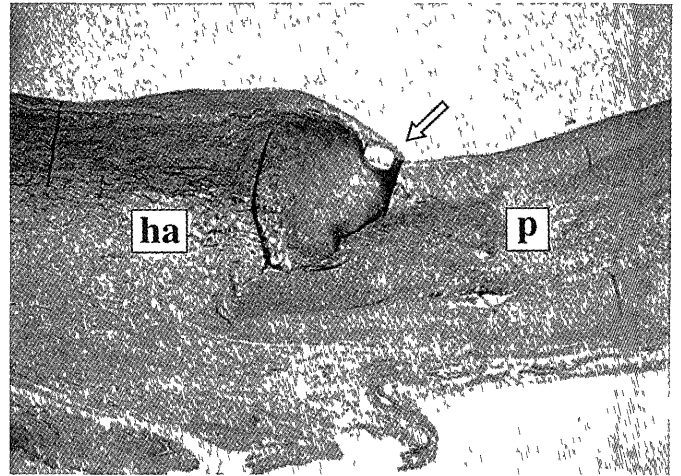

Fig. 5 Microscopic findings of the end to end anastomosis (elastica van Gieson stain x 50) No elastic fibers could be seen in the newly developed tissue covering the graft surface
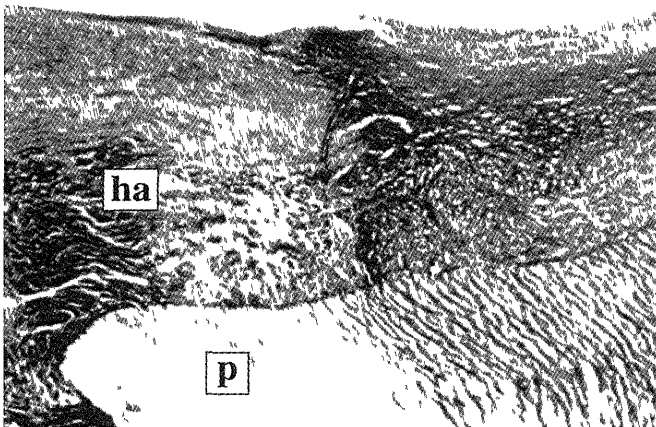

Fig. 6 Microscopic findings of the sleeve anastomosis (masson stain x 120) No suture material was seen at the anastomotic site
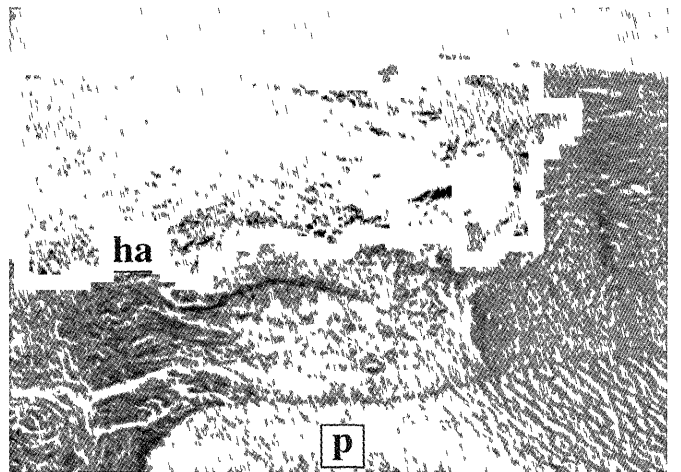

Fig. 7 Microscopic findings of the sleeve anastomosis (elastica van Gieson stain x 120) No elastic fibers could be seen in the neo intimal portion

Discussion

SL anastomosis was introduced by Lauritzen[5] in 1978 as a method of microarterial anastomosis for arteries in the 1 mm external diameter range This anastomosis is technically simpler and faster than the conventional end to end anastomosis In the SL method, the vessels to be anastomosed are joined with a male to female connection using only 2 extraluminal sutures, and a high patency rate has been reported[6,9]

The SL method used in this study was a modification of the above technique for the anastomosis of a larger artery (about 8-mm internal diameter) The purpose of this technique is to prevent the mechanical injury of the intima at the stump of the host artery by avoiding passing sutures through it In relation to the direction of the blood flow, the microvascular sleeve anastomosis has been used only for proximal anastomosis, because when used in distal anastomosis, the free edge of the host artery may be turned over into the lumen by the force of the blood stream and may cause subsequent graft occlusion Therefore, for evaluating the influence of anastomotic sites (proximal or distal), the animals in the present study were divided into 4 groups according to the suture techniques used at the proximal and distal anastomoses One hundred percent patency was obtained even in the distal SL anastomosis (groups C and D), thus the problems mentioned above were avoided However, further testing

is necessary, including the examination of the inser-
tion of the host vessel further than 5 mm.

In the present series, occlusion of the graft occurred
in only 2 cases in group B, among the 23 dogs that
survived for 4 months. The whole length of the graft
lumen was totally occluded with granulomatous tis-
sues in both cases, and thus it was not clear which
side was responsible for the graft occlusion. There was
no significant difference in the patency rate between
the ETE and SL anastomoses; they were both satisfac-
tory.

Compared with the anastomoses by the SL method,
the ETE anastomoses showed partially exposed suture
materials in the luminal surface of the graft which
were covered or uncovered with a neo-intimal layer.
Neo-intimal cellular progression into the graft was also
uneven. Histologically, degeneration of the tissue near
the sutures and intimal thickening were observed. In
the SL anastomoses, in contrast, newly developed inti-
mal cells together with underlying collagen fibers and
smooth muscle cells smoothly progressed on the lumi-
nal surface of the graft. Based on these findings, the
SL technique is considered to be superior to the ETE
technique in preventing intimal thickening of the ana-
stomotic site.

We know from studies of wound healing in animals
and humans that luminal narrowing of the lumen of a
graft is due largely to smooth muscle cell (SMC) prolif-
eration and connective tissue deposition in the inti-
ma.[1-4,10,11] During the response to injury, SMCs undergo
a change in phenotype, losing the capacity to contract,
gaining the capacity to divide, and increasing the syn-
thesis of extracellular matrix molecules.[12] The prolif-
erative activity of SMCs is physiologically regulated
by both promoters and inhibitors.[12-14] Possible candi-
dates for promoters include platelet-derived growth
factor, interleukin-1 and endothelin. Inhibitors include
heparin sulfates, endothelial-derived relaxing factor, and
prostaglandins E_1 and I_2. It is likely that vascular in-
jury allows SMC growth by disrupting the physiologic
balance between SMC growth inhibition and growth
stimulation. SMCs respond to the injury stimulus by
first proliferating in the media, then migrating from
the media, and finally proliferating in the intima to
form an intimal thickening. Intimal SMCs may become
nonproliferative when the overlying endothelial layer
is reestablished.

Chamley et al.[15] observed in SMC cell cultures that
most single cells lost their contractile properties and
gained dividing properties during the first few days in
culture. After 7 days of culture, these dedifferentiated
SMCs underwent extensive proliferation. If sufficient
cells were present in the culture inoculate, a continuous

monolayer formed at about 9 days of culture, and
redifferentiation of the SMCs began. After 8-10 days of
culture, the muscle bundles reformed and foci of syn-
chronous contractions developed. However, in cultures
where a continuous monolayer was not formed at 9
days, isolated cells did not redifferentiate.

In the present SL anastomoses, less intimal injury
by the suture material and less distortion of the host
vessels were observed at the anastomotic sites, com-
pared to the ETE anastomoses. This beneficial condi-
tion of the anastomotic site led to more regular and
smooth neointimal extension, and may have promoted
an early end of the proliferative activity of the intimal
SMCs. These results suggest that the SL anastomosis
would be effective in preventing postoperative patho-
logic intimal thickening of the anastomotic site.

Conclusion

The SL anastomotic technique, designed to avoid
intimal injury, was experimentally compared with the
conventional ETE anastomosis. Although there was no
significant difference in patency rates between the two
anastomotic methods, the SL anastomosis showed bet-
ter and more regular neointimal extension on the lumi-
nal surface of the prosthesis, as assessed both macro-
scopically and microscopically. From these results, SL
anastomosis is considered to be effective in preventing
postoperative intimal thickening.

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