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<th>Title</th>
<th>An Experimental Study of the Sleeve Technique in Graft Anastomosis</th>
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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 anastomatic 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 wound-healing response following injury to the endothelium. Therefore, postoperative intimal hyperplasia 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 hyperplasia. For this purpose, a segment of canine abdominal aorta was replaced with an expanded polytetrafluoroethylene (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 (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 proximal 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.
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.

<table>
<thead>
<tr>
<th>Group (pro/dis)</th>
<th>No. of dogs</th>
<th>Survived</th>
<th>Graft Patency</th>
<th>Patency Rate (%)</th>
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<tbody>
<tr>
<td>A (ETE/SL)</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>B (SL/ETE)</td>
<td>11</td>
<td>9</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>C (ETE/SL)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>D (SL/SL)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>23</td>
<td>21</td>
<td>91</td>
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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 neo-intimal 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 neo-intimal 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 neo-intimal 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 theneo 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 insertion 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 tissues 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 satisfactory.

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 intimal cells together with underlying collagen fibers and smooth muscle cells smoothly progressed on the luminal 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 anastomotic 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) proliferation and connective tissue deposition in the intima. During the response to injury, SMCs undergo a change in phenotype, losing the capacity to contract, gaining the capacity to divide, and increasing the synthesis of extracellular matrix molecules. The proliferative activity of SMCs is physiologically regulated by both promoters and inhibitors. Possible candidates for promoters include platelet-derived growth factor, interleukin-1 and endothelin. Inhibitors include heparin sulfates, endothelial-derived relaxing factor, and prostaglandins E1 and I2. It is likely that vascular injury 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. 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 synchronous 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, compared to the ETE anastomoses. This beneficial condition 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 pathologic 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 better and more regular neointimal extension on the luminal surface of the prosthesis, as assessed both macroscopically and microscopically. From these results, SL anastomosis is considered to be effective in preventing postoperative intimal thickening.

**References**


