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**EXPERIMENTAL STUDIES ON MODIFIED HUMAN URETER AS
AN ARTERIAL SUBSTITUTION FOR RECONSTRUCTION OF
SMALL CALIBER VESSELS**

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INDEXING WORDS

small caliber vessel; bioprosthesis; human ureter; polyepoxy compound; glutaraldehyde;
expanded polytetrafluoroethylene (e-PTFE)

SYNOPSIS

Human ureters were experimentally employed as vascular biografts for small-caliber vessels. They were produced by tanning with polyepoxy compound or glutaraldehyde, and also were compared with saphenous veins tanned by polyepoxy compound and/or ready-made vascular graft of polytetrafluoroethylene (e-PTFE). These produced grafts, which had enough elasticity and excellent durability were successfully implanted to the carotid arteries in Japanese white rabbits. The grafts were sacrificed after 1 month observation. In this model there were no evidence of rejection, aneurysmal formation and/or infection. Polyepoxy compound fixed human ureter (n=6), polyepoxy compound fixed saphenous vein (n=6) and e-PTFE (n=3) showed good patency, but all glutaraldehyde fixed human ureters (n=3) were completely occluded with severe intimal hyperplasia. Especially, polyepoxy compound fixed human ureter showed excellent patency as well as function, and the histological findings revealed monolayer endothelial cells covering the surface of the graft. No intimal hyperplasia was demonstrated at the anastomotic site. This study suggested that the polyepoxy compound fixed human ureter could serve as a satisfactory blood conduit of reconstruction for the small caliber vessels.

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Our preliminary data also suggests that longer observation up to 6 months in polyepoxy compound fixed human ureter graft and 18 months in polyepoxy compound fixed saphenous vein graft after surgery, arteriovenous shunt (A-V shunt), long bypass with a graft length of 2 to 5cm), Y-graft model, sequential model and reinforcement graft by dacron cloth give good results.

INTRODUCTION

The need for an ideal bioprosthesis for reconstruction of small caliber vascular vessel has been recent because the number of patients with severe coronary artery disease or peripheral occlusive arterial disease has been increasing. At present, autogenous saphenous vein has been currently employed as first choice for arterial reconstructions at the sites of below knee or in the coronary arteries. However, there are a few patients for whom saphenous vein can not be used because of too small, absent, varicosities or inadequate length of the vein. On the other hand, ready-made prosthetic vascular grafts have many problems such as durability of the material, immunity and patency rate on the long-term period.^{1,3,4,9,10,12,14,16,18} This experimental study documents the efficacy of the original modified human ureter for small-caliber arterial prosthesis.

MATERIALS

(1) Human cadaver ureters were collected within 12 hours after death in autopsy . After informed consent was taken from the all family members of the cadavers according to the regulation of human research of Kobe University School of Medicine, 12 ureters were taken out from 6 cadavers. After cleansing of retained blood with cold isotonic saline solution containing 200 units /dl of heparin and removing the fat and collagen tissue from the ureters, ureters were tanned for 48 hours either with 1% glutaraldehyde (pH 4.3, 20°C) -group I, or 5% polyepoxy compound (pH 7.4, 20°C) -group II with a 2 to 4 mm (in diameter) polyester mandrel placed inside the ureters, respectively. All tanned ureters were preserved in 40% ethanol solution. The diameters of the grafts ranged from 2 to 4 mm with smooth inner surface (Fig.1 A and B). Glutaraldehyde fixed human ureters were brownish and slight firm as compared to polyepoxy compound fixed human ureters. Histologically, the polyepoxy compound fixed human ureter and the glutaraldehyde fixed human ureter revealed that the epithelial cells of the ureter were almost vanished and the ducts produced from ureter were uniformly formed by basement membrane and muscle layer (Fig.2 A).

(2) Human saphenous veins were harvested at the time of coronary artery bypass grafting (CABG) or stripping operation for varicose veins, as well as in autopsies (6 bodies,

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12 saphenous veins). After cleansing blood with cold isotonic saline solution containing 200 units /dl of heparin, these veins were tanned for 48 hours with 5% polyepoxy compound (pH 6.0, 20 °C) -group III with a polyester mandrel of the diameter 2 to 4mm inside. The tanned saphenous veins were preserved in 40% ethanol solution. The diameters of the vein grafts also ranged from 2 to 4 mm and had a smooth inner surface (Fig.1 C). Histologically, endothelial cell layer and the other structures of the vessel were well preserved (Fig.2 B and C) in each of the veins.

METHODS

Methods I (Physical characteristics of the each grafts in vitro)

The prepared grafts (Group I, glutaraldehyde fixed human ureter (n=6); Group II, polyepoxy compound fixed human ureter (n=6); Group III, polyepoxy compound fixed reserved saphenous vein (n=6)) were studied in vitro.

(1) Tensile strength test

Tensile strength test was carried out for each graft. We used a 2 cm long segment with a 5 mm diameter and 5-0 monofilament polypropylene suture was anchored at the one side of each graft wall, and tensile strength 1.8 gram per 2 second from one side was added continuously. Subsequently, tensile strength at the time of graft tearing was measured. Strength tolerances in 10% glutaraldehyde fixed human ureter, in 5% polyepoxy compound fixed human ureter and 5% polyepoxy compound fixed human saphenous vein were measured. Furthermore, expanded polytetrafluoroethylene (e-PTFE) vascular graft (n=6) and modified human umbilical cord vein graft (Dardik biograft®)(n=6) were used for comparison.

(2) Pressure tolerance test (Response to the increased intraluminal pressure)

In all grafts (glutaraldehyde fixed human ureter, polyepoxy compound fixed human ureter and polyepoxy compound fixed human saphenous vein), sphygmomanometer was connected to the one end and another end of the graft was clamped. The internal pressure of the graft was increased continuously to test the pressure tolerance. The diameter of the each graft was measured by ultrasonography at each pressure level of 50mmHg, 100mmHg, 150mmHg and 200mmHg. Furthermore, the internal pressure of the graft was added up to the pressure level of 300 mmHg, at which point the tolerance limits were investigated.

Methods II (Graft interposition to animal arteries)

All grafts (Glutaraldehyde fixed human ureter (n=3), Polyepoxy compound fixed

human ureter (n=6), polyepoxy compound fixed saphenous vein (n=6) and e-PTFE (n=3)) were implanted in female Japanese white rabbits (n=18) weighing between 3.0 to 3.5 kilograms. Anesthesia was induced with ketamine hydrochloride 15 mg/kg intramuscularly and pentobarbital sodium 15 mg/kg intravenously according to the "Guidelines for Animals Experiment in Kobe University School of Medicine". The animals were heparinized systemically with an intravenous bolus injection of 500 units. Interposition graftings were performed by using a 2 cm long segment of a graft in diameter for the internal carotid arteries (1.5-2 mm in diameter). The artery was resected and each graft was interposed with proximal and distal end-to-end anastomoses by using 7-0 or 8-0 monofilament polypropylene as a suture material. Doppler ultrasonography was performed at the completion of the reconstruction (Fig.3). Follow-up observations were carried out 1 month after surgery and then the animals were sacrificed. All grafts were evaluated with Doppler ultrasonography, angiography and histological examination with hematoxylin and eosin and with van Gieson elastic staining.

Methods III (Other types of graft implantation in animals)

Arterio-venous shunt (A-V shunt), long bypass (graft length, 2-5cm) used for curving model or looped model, Y-graft model and sequential model were carried out and evaluated experimentally. Reinforcement of graft with Dacron graft was also evaluated.

In A-V shunt, polyepoxy compound fixed human ureter of 2 cm length was implanted between the left carotid artery and the left jugular vein. For this procedure, end-to-end arterial anastomosis and end-to-side venous anastomosis were used. In long bypasses more than 3cm of graft length, S-curve shaped graft model or looped model were made as well. Polyepoxy compound fixed human ureters in 2 cm, 3 cm, 4cm and 5cm in length were implanted to the carotid arteries. End-to-end anastomosis was used on both proximal and distal sites. Y-graft was made by creating an end-to side anastomosis into a polyepoxy compound fixed human ureter with sutures with 6-0 monofilament polypropylene. The proximal portion of the graft was anastomosed to the left carotid artery in end-to-end fashion and the two distal portions were anastomosed to the bilateral carotid arteries in end-to-side fashion. Then the right proximal carotid artery was ligated. In sequential graft implantation, a 4 cm polyepoxy compound human ureter was interposed to the left carotid artery, then side-to-side anastomosis in a graft loop was made to the right carotid artery which was ligated proximal to the anastomosis. For the reinforcement of the graft, Dacron cloth manufactured by Dacron graft was placed and fixed around it. In all experiments, Doppler ultrasonography was performed to confirm the good flow of the each graft at the time of completion of the procedure and at 1 month after surgery.

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Arterio-venous shunt (A-V shunt), long bypass grafts (graft length, 2-5 cm) using s-curve shaped model or looped model, Y graft and sequential graft were evaluated by the same methods as well. In a case of polyepoxy compound fixed human ureter the maximal follow-up span was 6 months after implantation, and in a case of polyepoxy compound fixed human saphenous vein it was 18 months.

RESULTS

Result I (In vitro : physical characteristics of the graft)

1) Analysis of tensile strength test

The results of the mean tensile strength tests are presented in Fig.4. In this experiment using 5-0 monofilament polypropylene, the sutures were easily placed in all grafts except the e-PTFE graft.

2) Pressure tolerance test and elasticity of the grafts

In the polyepoxy compound fixed human ureter and polyepoxy compound fixed human saphenous vein, the diameters of the grafts for each level of internal pressure were measured. The diameter of the polyepoxy compound fixed human ureter showed the following values in each internal pressure: internal diameter of 1.7mm in 50mmHg, 2.0mm in 100mmHg, 2.2mm in 150mmHg and 2.3mm in 200mmHg (Fig.5 shows the actual images). The diameters of the 5% polyepoxy compound fixed human saphenous veins revealed the following values in each pressure: 1.9mm in 50mmHg, 2.2mm in 100mmHg, 1.9mm in 150mmHg, 2.2mm in 200mmHg. In this experiment, all grafts (glutaraldehyde fixed human ureter (n=6), polyepoxy compound fixed human ureter (n=6), polyepoxy compound fixed human saphenous vein (n=6)) had enough elasticity and flexibility to tolerate internal pressure of up to 200 mmHg.

Furthermore, tolerance limits were up to 300 mmHg in each graft without transformation.

Result II -I (Patency and macroscopic findings of the implanted graft)

Implantations of all grafts (Group I, glutaraldehyde fixed human ureter (n=3); Group II, polyepoxy compound fixed human ureter (n=6); Group III, polyepoxy compound fixed saphenous vein (n=6); Group IV, e-PTFE (n=3)) were successfully carried out in 18 carotid arteries of Japanese white rabbits and good blood flow was recognized by Doppler ultrasonography at the each of implantation period in all grafts. Histological findings were evaluated in all grafts. Subsequently, aneurysmal formation and/or infection was not seen

in any of the grafts. These data could be summarized as follows.

Group I, glutaraldehyde fixed human ureter

Macroscopic observation of the glutaraldehyde fixed human ureter grafts (graft length, 2cm) showed severe intimal hyperplasia at both anastomotic sites in all explanted segments (Fig.6 A). All grafts (n=3) were completely occluded at 1month.

Group II, 5% polyepoxycompound fixed human ureter

All grafts (n=6) were patent at 1 month. The luminal surface of the graft was smooth and lustrous. Thrombus or intimal hyperplasia were not seen (Fig.6 B). Good blood flow was recognized by Doppler ultrasonography and excellent patency was also observed on the arteriogram (Fig.7 A).

Group III, 5% polyepoxy compound fixed saphenous vein

Polyepoxy compound fixed prereserved saphenous vein grafts (graft length, 2cm) showed good blood flow on the Doppler ultrasonography, but arteriogram showed moderate (~severe) stenosis in the anastomotic sites (Fig.7 B). All grafts (n=6) were patent at 1 month. The luminal surface of the graft was lustrous but irregular. Thrombus was not observed, but moderate intimal hyperplasia was seen (Fig.6 C).

Group IV, expanded polytetrafluoroethylene (e-PTFE) vascular graft

Good blood flow on the Doppler ultrasonography was observed in e-PTFE vascular grafts and excellent patency was found on the arteriogram in all grafts (Fig.7 C). All grafts (n=6) were patent at 1 month after implantation. The luminal surface of the graft was mottled with red color but lustrous and smooth (Fig.6 D). Thrombus was not observed but minimal intimal hyperplasia was seen and an intramural bleeding in the pores of e-PTFE was observed.

Result II -2 (Histological findings)

Group I, 1% glutaraldehyde fixed human ureter

After 1 month of implantation, thick pannus (severe intimal hyperplasia) was observed, and all grafts (n=3) were completely occluded at 1month after implantation. Inflammatory cells Infiltration around the ureter and the both anastomotic sites was observed. Nevertheless, the structure of the ureter was preserved completely.

Group II, 5% polyepoxycompound fixed human ureter

The luminal surface of all grafts was relatively smooth and lustrous. Subintimal fibrosis was not seen, and peripheral inflammatory reaction around the anastomotic site was found but it was graded as minimal. Endothelial cells extended from the both anastomotic sites and covered the inner layer of the graft (Fig.8 A and B). Infiltration of the inflammatory cells around the ureter and the anastomotic site was not observed. Hence the

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structure of the ureter was preserved completely as it was before.

Group III, 5% polyepoxy compound fixed preserved saphenous vein

All grafts at 1 month after implantation were lustrous. However, moderate inflammatory reactions around anastomotic sites were observed (Fig.9 A). Moderate intimal hyperplasia was observed as well. Moderate infiltration with many inflammatory cells was observed around the graft. The structure of the vein was not destroyed.

Group IV, expanded polytetrafluoroethylene (e-PTFE) vascular graft

The luminal surface of the graft was lustrously mottled with faintly red color but it was smooth. No subintimal fibrosis around the both anastomotic sites and in the native artery were seen, but minimal inflammatory reaction around both anastomotic sites was observed. Endothelial cells covered the inner layer of the both anastomotic sites and the pores of the e-PTFE (Fig.9 B and C). Moderate intimal hyperplasia and moderate infiltration of the inflammatory cells around the graft were observed.

Result III (Fate of other grafts after implantation)

The number of grafts in each of these groups was one. A-V shunt model and long bypass model of polyepoxycompound fixed human ureter graft is shown in Fig.10. Doppler ultrasonography revealed unobstructed blood flow in all grafts. The Y-graft and the sequential models are shown in Fig 11 A and B. In these cases, good blood flow was seen by Doppler ultrasonography as well. Polyepoxy compound fixed human ureter graft reinforced by Dacron cloth is shown in Fig.11 C. Until the follow-up period of surgery, polyepoxy compound fixed human ureter showed good pulsation and unobstructed blood flow by Doppler ultrasonography and the maximal follow-up period was 6 months. The maximal follow up period of polyepoxycompound fixed human saphenous vein graft was 18 months as well. However, during the follow-up period of 6 months, another case of polyepoxycompound fixed human saphenous vein graft showed obstructive and calcified changes.

DISCUSSION

Much attention has been paid to an ideal small-caliber vascular graft for coronary artery bypass or peripheral artery bypass. However no satisfactory grafts for long-term patency have yet been developed.^{1,3,4,9,10,12,14,16,18} At present, the first choice of vascular graft for reconstruction of small caliber vessel is autologous saphenous vein graft, because the prosthetic vascular graft have some problems such as price, unsuitability and surgical morbidity. So far numerous pilot studies have been performed for the purpose of vascular

reconstruction with the prosthetic grafts especially for small-caliber vessels. They should be freely available, pliable, easy to use and most importantly should have excellent long-term patency. Hence in this study, human ureter grafts were harvested from cadavers at autopsy, treated with glutaraldehyde or polyepoxy compound, and sterilized with ethanol. Human saphenous vein grafts were harvested at the time of aortocoronary bypass surgery or venous stripping operation, treated with polyepoxy compound, and sterilized with ethanol. They are therefore inert and more easily available. Their mechanical and biological compatibility with the host blood vessels should be taken into consideration when they are used as vascular prostheses. Previous reports demonstrate vascular xenograft cross-linked with glutaraldehyde or dialdehyde starch in the reconstruction of small caliber biological vessels.^{7,8,17)} However, side-effects including degeneration, aneurysmal formation, and cytotoxicity related to use of glutaraldehyde were still clinical problems. Recently, polyepoxy compound have been introduced as a new cross-linking agent to overcome the above-mentioned disadvantages of glutaraldehyde or dialdehyde starch.^{5,11,13)} Judging from easy operability and durability the modified human ureter graft or modified human saphenous vein could be available as a proper arterial conduit.

The *in vitro* study showed that the human ureter grafts fixed with polyepoxy compound grafts had adequate pliability and elasticity. Furthermore, they had enough strength and durability because they could tolerate internal pressure of up to 300 mmHg.

In our study all human ureter grafts tanned by 1% glutaraldehyde were completely occluded at 1 month because of severe intimal hyperplasia. However, human ureter grafts tanned by 5% polyepoxy compound and human saphenous vein graft tanned by polyepoxy compound showed good patency. Especially, human ureter grafts tanned by 5% polyepoxy compound revealed excellent results in the patency as well as the function, and the histological findings showed that endothelial cells covered the surface of the graft without intimal hyperplasia. Human saphenous vein grafts tanned by polyepoxy compound sometimes had moderate (~severe) intimal hyperplasia. In e-PTFE graft, endothelial cells covered the inner surface of the graft and it was mottled with red color which was probably an intramural bleeding or thrombus as has been mentioned in previous reports, too.^{2,6,15)}

In polyepoxy compound fixed ureter, good pulsation was observed at 6 months, while in polyepoxy compound fixed human saphenous vein graft, one case showed obstruction and severe calcification. Based on these results, polyepoxy compound fixed human ureter grafts could be used as a proper vascular conduit for reconstruction of small-caliber artery.

Polyepoxy compound fixed human ureter graft has many advantages, such as lack of branches and valves, controllability of length and optimal luminal diameter (2-4 mm), host tolerance, tissue compatibility, easy operability, flexible, elasticity and resistance to

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biodegradation. Human ureter might be clinically useful for the reconstruction of small-caliber arteries, and we are continuing this study with long time observation and more animal experiments are needed for confirming these promising results.

CONCLUSION

Vascular bioprostheses for small-caliber vessel could be produced from human ureter and preserved human saphenous vein. It could be concluded that polyepoxy compound fixed human ureter graft had an excellent potential for vascular reconstructions of small-caliber vessel.

Some points of these works were presented at the Japanese Society for Artificial Organs (candidacy of originated prize) held in Osaka, Japan, November 7-9, 1995, and Japan Surgical Society held in Chiba, Japan, April 10-12, 1996.

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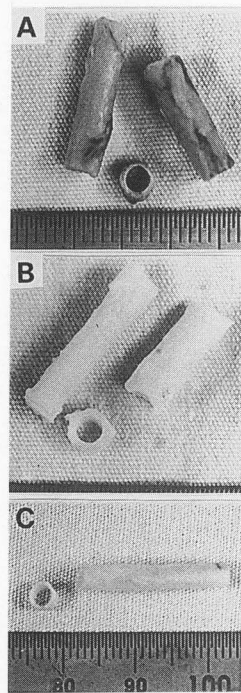


Fig. 1.

Models of each small caliber vascular graft .

A: Glutaraldehyde fixed human ureter.

B: Polyepoxy compound fixed human ureter .

C: Polyepoxy compound fixed human saphenous vein .

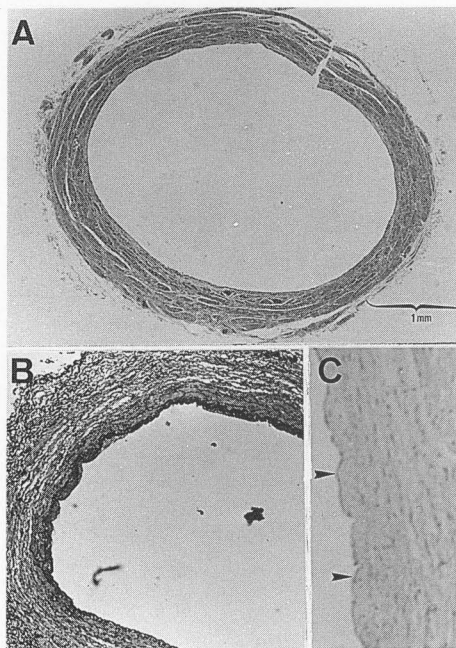


Fig. 2.

Microscopic findings of the grafts.

A: At polyepoxy compound fixed human ureter, epithelial cells of the duct were almost vanished and the duct was uniformly formed by basement membrane and musclelayer.(H.E. x20)

B: At polyepoxy compound fixed human saphenous vein, endothelial cells were preserved together with the other structures of the vessel. (van Gieson x30)

C: Factor VIII related antigen stain of the polyepoxy compound fixed human saphenous vein.

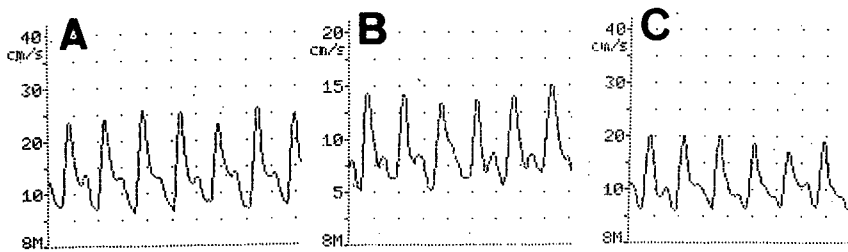


Fig. 3.

Doppler ultrasonography was performed at the completion of implantation and prior to explantation of the graft.

A: the center portion of the carotid artery.

B: the graft.

C: the peripheral portion of the carotid artery.

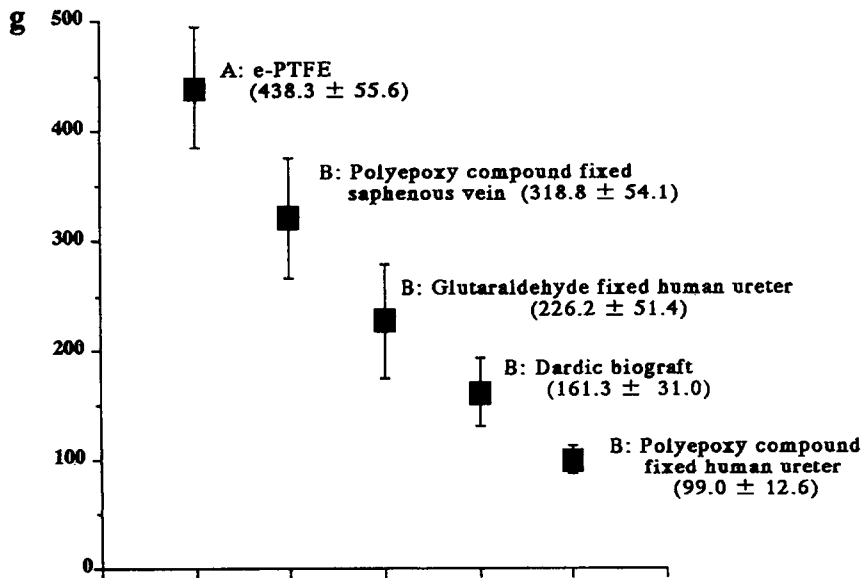


Fig. 4.

Tensile strength test. The spots indicate the means of the weight needed for the tear off. Values are mean $\pm$ standard deviation (SD) of the mean.

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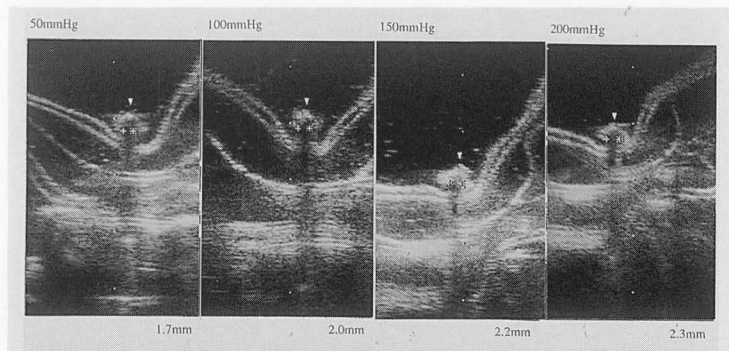


Fig. 5.

The elasticity of the polyepoxy compound fixed human ureter; the transverse sections of the polyepoxy compound fixed human ureter at different levels of intraluminal pressure were shown.

▽:Polyepoxy compound fixed human ureter.

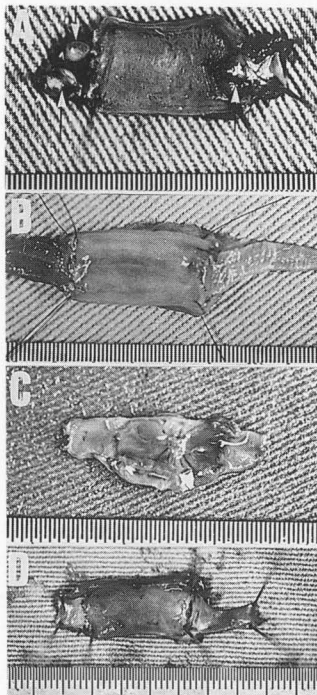


Fig. 6.

Macroscopic findings of the grafts at 1 month after reconstruction.

A: Glutaraldehyde fixed human ureter.

Severe intimal hyperplasia was shown (↑).

B: Polyepoxy compound fixed human ureter graft at 1 month.

The luminal surface of the graft remained smooth and lustrous.

C: Polyepoxy compound fixed human saphenous vein graft at 1 month.

The luminal surface of the graft showed lustrous but irregular.

D: e-PTFE graft at 1 month.

The luminal surface of the graft was mottled with faintly red but lustrous and smooth.

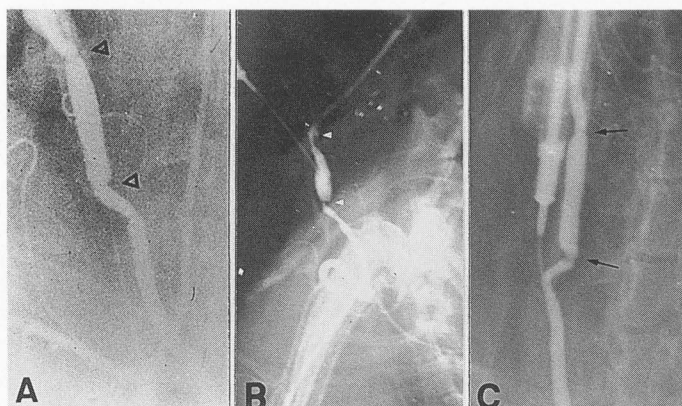


Fig. 7.

- A: Angiogram of the implanted polyepoxy compound fixed human ureter graft showed good patency without stenosis at 1 month.
- B: Angiogram of the implanted the polyepoxy compound fixed human saphenous vein graft showed patency with moderate (~severe) stenosis of the anastomotic sites at 1 month.
- C: Angiogram of the implanted e-PTFE graft showed good patency without stenosis at 1 month.

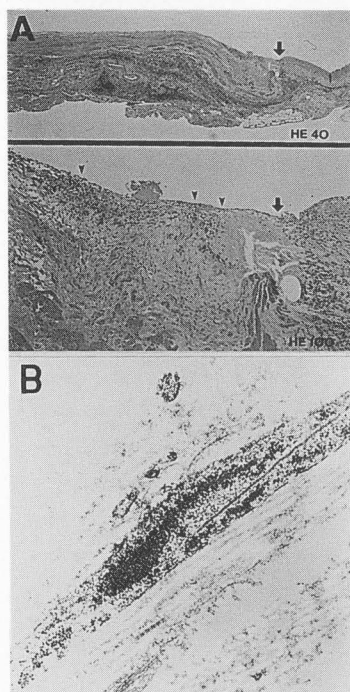


Fig. 8.

- A: Histological findings of the polyepoxy compound fixed human ureter graft at 1 month. Monolayered endothelial cells (↓) covered the luminal surface of the graft starting from the anastomotic site and showed no intimal hyperplasia.
- B: Electron microscopic findings detect the cells covering the luminal surface of the same graft.

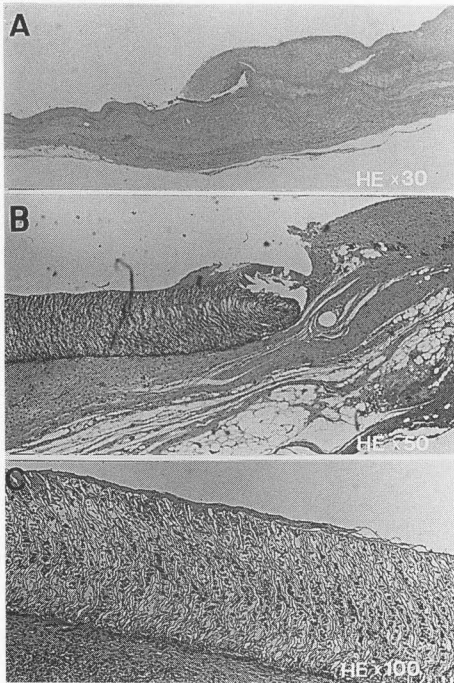


Fig. 9.

A: Histological findings of the polyepoxy compound fixed human saphenous vein graft at 1 month. Moderate intimal hyperplasia was seen at the anastomotic site.

B, C :Histological findings of the e-PTFE graft at 1 month. The luminal surface of the graft was mottled but lustrous and smooth. Minimal intimal hyperplasia and intrawall bleeding were observed.

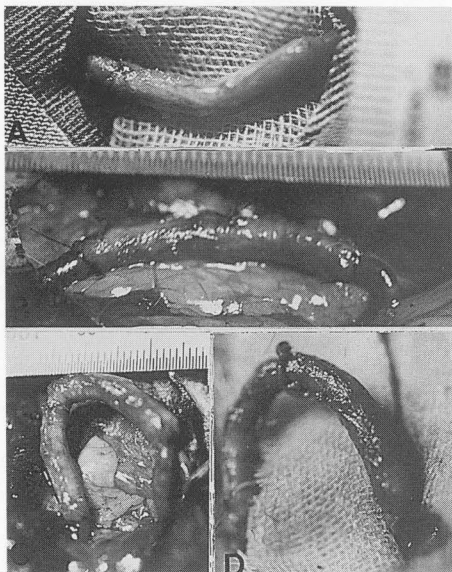


Fig.10.

Long bypass model. The graft lengths were 2cm (A), 3cm (B), 4cm (C), and 5cm (D).

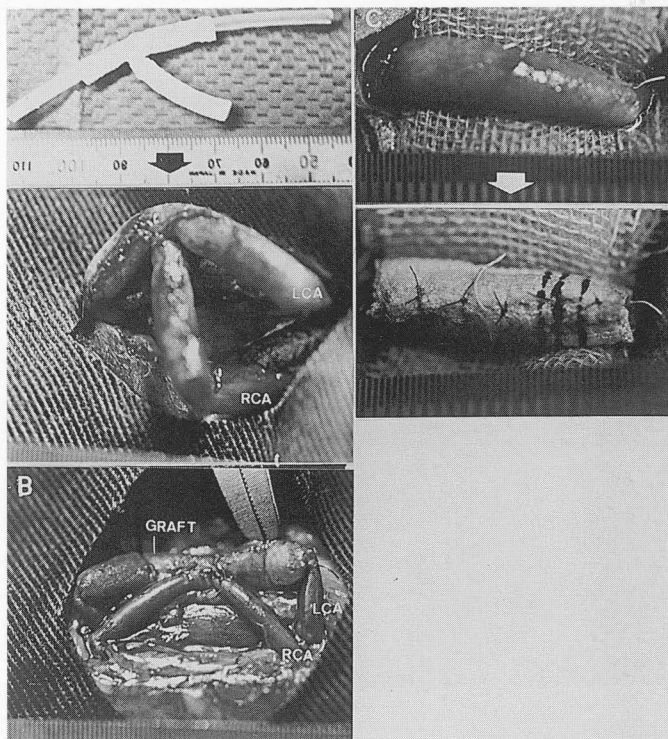


Fig. 11.

- A: Y-graft model of the polyepoxy compound fixed human ureter graft.
B: Sequential model of the polyepoxy compound fixed human ureter graft.
C: Polyepoxy compound fixed human ureter (C, top) reinforced with a Dacron cloth manufactured by Dacron graft in place (C, bottom).