The long-term clinical results of vascular prostheses sealed with autologous adipose tissue fragments

Norihisa Karube, Tamitaro Soma, Yasuharu Noishiki*, Ichiya Yamazaki*, Takayuki Kosuge*, Yukio Ichikawa*, and Yoshinori Takanashi*

ABSTRACT

Purpose: Tissue engineering can improve the former limitations of artificial organs. This article reports the long-term clinical results of grafts constructed with fragmented adipose tissue (FAT grafts). Method: We did a retrospective analysis of a series of 53 patients with lower leg ischemia that received 69 fragmented adipose tissue grafts (FAT grafts) implantation in our institution. The mean follow-up period was 36.0 months. Results: After 1, 2, 3, and 5 years, the primary patency rates were 85.3%, 83.3%, 73.8%, and 67.7%, respectively. The lumen of occluded areas not only at anastomotic sites but also in areas far from the anastomotic sites was occupied by a thickened neointima, which had a great number of capillary blood vessels, elastic laminae, smooth muscle cells, fibroblasts, and collagen fibers. This type of intimal hyperplasia was a characteristic finding in FAT grafts. Conclusion: From the results of the present clinical trial, we conclude that the FAT grafts are acceptable as vascular prostheses for ischemic low extremities. The intimal hyperplasia at sites far from the anastomotic lines suggested the possibility of neointima formation throughout the luminal surface of the grafts.

INTRODUCTION

Tissue engineering can improve former limitations of artificial organs. Two major problems of vascular prosthesis for reconstruction are the resultant delay in tissue healing with endothelialization over the graft luminal surface and development of anastomotic intimal hyperplasia. In an attempt to overcome these problems, we devised an original method of tissue engineering to accelerate the neointima formation of fabric prostheses made with autologous tissue embedded in the pores of the grafts, and we succeeded in producing a graft that may accelerate tissue healing and prevent late thrombosis occlusion. The aim of this study is to demonstrate the short-term and long-term results of our newly concepted graft.

MATERIALS AND METHODS

Patient data

Between July, 1992, and July, 1995, a total of 69 autologous tissue fragmented graft (FAT grafts)

prostheses were implanted in 53 patients with lower leg ischemia in our institution. There were 44 males and 9 females. The mean age was 68.9 years. All the patients had arteriosclerosis obliterans in a lower peripheral artery, and according to the Fontaine classification 39 were grade 2, 7 were grade 3, 6 were grade 4 (mean grade 2.4±0.7).

Sealing of fabric vascular prosthesis

Approximately 30 g of subcutaneous adipose connective tissue was resected from the lower abdominal subcutaneous layer of the patient and chopped up with scissors in small peaces and suspended into saline solution according to our original method (1). Micron (Intervascular, Clearwater, FL, USA), Bard Sauvage EXS (C.R.Bard, Inc., Billerica, MA, USA), Micronit (Golaski Laboratories Inc., Philadelphia, Pa, USA), and Intervascular Low Porosity (Intervascular, Inc., Clearwater, FL, USA) were used for the framework of the graft. To obtain a smooth-tissue fragmented luminal surface, the fabric Dacron vascular prosthesis was turned inside out, except for the Sauvage EXS, and the adipose tissue fragments suspension was injected several times with pressure to force the fragments into the interstices of the fabric. Injections were repeated until it was no longer possible to pass any liquid through the graft wall by manual injection through a 20ml syringe. The sealed graft was then turned right side out to yield a fregmented adipose connective tissue graft with smooth luminal and rough outer surfaces.

Because Sauvage EXS, which was externally supported, cannot be turned inside out, the graft preparation was performed without this maneuver. A Teflon rod (Sanplatec corp., Osaka, Japan) was inserted in the prosthesis to preserve the smooth surface during further manipulation. A heparin slow release system was added, in order to mask the positively charged collagen fibers of the tissue fragments, to increase the antithombogenicity of the graft (2). The size of straight grafts ranged from 5 to 10 mm, and the size of Y-grafts was 16-8 mm.

Operative techniques

The procedure was performed under general inhalation anesthesia combined with epidural anesthesia. A piece of adipose connective tissue was obtained from the subcutaneous layer of the lower abdomen, and the vascular prosthesis was prepared intraoperatively as described above. All patients had reconstruction with the FAT grafts. All the anastomoses were performed using the continuous polypropylene suturing technique. During the operation, systemic heparinization was performed (100IU Heparin/Kg) intravenously. The reconstructive procedures employed for the graft were 34 above-knee femoropopliteal bypasses, 10 aorto-unilateral femoral bypass, 9 aorto-bilateral femoral bypasses (Y grafts), 4 below-knee femoropopliteal bypasses, 4 aorto-iliac and femoral bypasses (Y grafts), 3 aorto-iliac bypasses, and 5 others. The patients were routinely treated after the graft implantation with antiplatelat

medication.

Follow-up

The patency of the reconstructed vessels was assessed every 2 months during the first year and every 6 or 12 months thereafter. Follow-up information was obtained from recent clinic visits. Graft patency was determined by clinical pulse examination, ankle/brachial pressure index measurements, and angiographies whenever symptoms recurred or there was a suspicion of threatening graft failure. In cases where recent follow-up was not possible, graft patency was determined at the time of the most recent examination. The mean follow-up period was 36.0 months (range, 0 to 97).

Statistics

All results were analyzed by actuarial methods and presented in the form of life tables, according to procedures established by the Society for Vascular Surgery/International Society for Cardiovascular Surgery committee on guidelines for reporting lower extremity ischemia (3).

RESULTS

Perioperative complications

In the 30-day postoperative period three patients died, one of acute myocardial infarction, one of acute mesenteric artery occlusion, and one of pneumonia. In two patients early graft thrombosis occurred (within 2 weeks) as a result of a technically flawed anastomosis. Immediate thrombectomy and reconstruction of the anastomosis was performed leading to long-term patency in both cases. Eight patients (15.4%) had delayed wound healing because of resection of subcutaneous adipose connective tissue for sealing the fabric vascular prosthesis.

Long-term results

During follow up, a total of 8 patients died, one each of acute myocardial infarction, cerebrovascular accident, pulmonary thromboembolism, lung cancer, ruptured esophageal varix, peritonitis, pneumonia, and hemolytic anemia. No patients underwent amputation during the period of follow-up. In one patient a fulminating infection around an FAT grafts developed, necessitating removal of the graft after 2 months.

The life table analysis for cumulative patency is summarized in Tables 1 and 2, and life tables for primary and secondary graft patency are displayed in Figs.1 and 2. At 1-year, 2-year, 3-year, and 5-year follow-up, the primary patency rates were 85.3%, 83.3%, 73.8%, and 67.7%, respectively (Table 1). Secondary patency (achieved by additional intervention) rates were 90.0%, 86.1%, 76.8%, and 70.8% at the same intervals.

Occluded or infected FAT grafts at the late follow-up times were removed and evaluated histologically. Graft occlusion did not occur at the distal and proximal sites, but in areas far from the proximal anastomotic sites. The lumen of the occluded areas was occupied with a granulomatously thickened neointima, and other areas throughout the graft had a thick neointima and thrombus in the remaining narrow cavity. Under the microscope, the neointima had a great number of capillary blood vessels, elastic laminae, smooth muscle cells, fibroblasts, and collagen fibers (4).

DISSCUSSION

Long term patency

From the results of the present clinical trial, it is concluded that the FAT grafts is capable of long-term patency: at 1-year, 3-year, and 5-year follow-ups, the primary patency rates were 87.1%, 83.2%, and70.7%, respectively. These results must be compared with the use of other grafts for low extremity ischemia. Budd *et al* reported that the 5 year patency rates were 41% for in situ veins, 62% for reversed saphenous veins, 31% for porytetrafluoroethylene(PTFE) grafts, and 29% for human umbilical vein grafts (HUV) at above and below the knee femoropopliteal bypasses(5). In randomized clinical trials between PTFE and HUV by Aalders *et al,* at 5 years the patency rate was 38.7% for PTFE and 75.1% in HUV(6). Veith *et al* compared autologous saphenous vein (ASV) and PTFE grafts, and primary patency rates at 4 years for above knee grafts were 61% for ASV and 38% for PTFE(7). El-Massry *et al* reported good results with externally supported knitted Dacron grafts for femoropopliteal bypasses, and 5-year primary patency rates for the entire series, for above knee grafts, and for below knee grafts were 70%, 71%, 57%, respectively(8). Since in this report, we counted all patients who received an FAT graft, we could not compare our results with other grafts directly. But we confirmed that our FAT grafts is equal to other kinds of grafts in long-term patency. From the long-term results, we conclude that the FAT graft is acceptable as a vascular prosthesis for ischemic low extremities.

Tissue engineering procedures and hybrid vascular prostheses

Recently, organ construction and creating tissues by tissue engineering techniques have been attempted in various fields of medical sciences. Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biologic substitutes that restore, maintain, or improve tissue function (9).

In the field of vascular prostheses, up to this time, an endothelial cell lining on a fabric graft has never been observed except at the anastomotic sites(10). For a vascular prosthesis to

maintain long-term, stable patency it is necessary for the luminal surface of a vascular prosthesis to become covered with antithrombogenic endothelial cells rapidly and completely, not only near the anastomotic sites but also over the total length of the graft. The problem has been how to accelerate the endothelialization so that the entire luminal surface of vascular grafts becomes covered with endothelial cells.

Therefore, the combination of various types of hybrid vascular prostheses with autologous, heterologous, and homologous tissue has been studied as a tissue engineering approach to this problem. Herring et al showed that if the graft was pre-clotted with fresh blood, endothelial cells could be increased. Vascular prostheses with his method were the first hybrid vascular prostheses. They expected that antithrombogenic and antistenotic features could be gained by reconstruction of blood vessels with cell components and matrix level (11). However, the surface of the graft could not become covered with endothelial cells simply by directly seeding a prosthesis with endothelial cells.

In an experimental study, what we did to accelerate endothelialization was to transplant autologous venous tissue fragments onto the fabric vascular wall, so that neointima formation was clearly accelerated and become complete (12). The tissue fragments contain endothelial cells, smooth muscle cells, and fibroblasts (13). Tissues and organs are always composed of various kinds of cells. They create a cell community that works together synergistically, performing different roles and aiding survival. We have learned that a mixed cell community is important for the design of new hybrid artificial organs. We recognized the efficacy of venous tissue transplantation, but we could not obtain enough venous tissue for clinical use. Therefore, we tried adipose tissue instead of venous tissue for clinical applications after basic animal experiments (14,15).

Neointima formation

In an experimental study, we demonstrated the accelerated endothelialization and neointima formation of the FAT grafts. In clinical works, occluded and infected FAT grafts in the late follow-up period were also removed and inspected histologically. In general, vascular prostheses implanted in humans do not heal (16). Only the areas near anastomotic sites have been able to form a neointima (10,17-19). Other areas are usually covered with a fresh thrombus layer (20-22). In comparison, occlusion in FAT grafts did not occur at the distal and proximal sites, but in areas far from the proximal anastomotic sites. The lumen of the occluded areas was occupied with a granulomatously thickened neointima, which contained a great number of capillary blood vessels, elastic laminae, smooth muscle cells, fibroblasts, and collagen fibers. These findings indicate that the FAT grafts has a possibility of healing its

own luminal surface.

A limitation of the FAT grafts is the existence of excessive neointima healing in some cases. It is possible that too rapid and excessive neointima healing lead to the graft occlusion. We could not control the neointima formation, so that if cells on the tissue fragments are too active, and growth factors are synthesized in too great amounts, the graft will be quickly occluded. We need to learn how to control the remodeling process using still more in vivo tissue engineering techniques. But we wish to emphasize that FAT grafts appear to have a dynamically metabolized neointima, which has not been observed in any other kind of vascular prosthesis implanted clinically.

FAT grafts are only a first step in the tissue engineering approach. The concept of FAT grafts and other tissue engineering techniques will give us better vascular prostheses with more biocompatibility and long-term patency

CONCLUSION

From the long-term results of the present clinical trials, we concluded that FAT grafts are highly acceptable as vascular prostheses for ischemic low extremities. The FAT grafts appear to have a dynamically metabolized neointima, which has not been observed in any other kind of vascular prosthesis implanted clinically.

FABRIC VASCULAR GRAFTS MADE OF ULTRAFINE FIBERS

Yasuharu Noishiki, M.D., Ph.D.

1. Toray Graft History

Background

In 1952, A.B. Voorhees reported for the first time that a porous tube made of a synthetic fabric material could be applied as a blood vessel substitute. The tube became the model for the fabric vascular grafts used at the present time. Before that, waterproof polyethylene or polyvinyl chloride, or human and animal natural blood vessels had been used as blood vessel substitutes. Voorhees showed in his animal experiments that a tube made of synthetic polymer fabric could be used as a vascular graft even though it was not waterproof. Later, Edwards and Tapp reported that a fabric vascular graft having a crimped structure was easy to handle for implantation because of its flexibility which prevented the kinking.

In 1957, the Committee of American Society of Vascular Surgery of which O. Creech was the Chairman reported that, the synthetic polymer materials which many chemical companies including DuPont were actively developing at that time, Dacron and Teflon had less deterioration in the living body, and were superior as materials for vascular grafts because they showed less foreign body reaction, cytotoxicity and carcinogenesis in the human body. Accordingly, the committee recommended them as raw materials for vascular prostheses. As a result, production of Dacron and Teflon vascular grafts began. Dacron, which was superior in handling as a fiber, was finally used for fabric vascular prostheses. Teflon was not frequently used for fabric grafts, but later it was used for expanded PTFE grafts. A Dacron vascular graft was used clinically in 1958 for the first time by Dr. M.E. DeBakey. He successfully used a Dacron fabric prosthesis for replacement of an aortic aneurysm.

In 1962, S.A. Wesolowski showed in animal experiments that in tightly-knitted vascular grafts (less porous fabric vascular grafts) from which blood did not leak, there was difficulty in forming neointima to cover the luminal surface of the vascular grafts after implantation, but that in loosely-knitted vascular grafts (highly porous vascular grafts) from which the blood could leak, neointima was formed more quickly and was maintained without degeneration such as calcification or necrosis due to metabolic problems. So he recommended the use of highly porous vascular grafts. In order to prevent blood leakage, a preclotting technique was developed, i.e, fresh blood was poured on highly porous fabric grafts, so that fresh thrombi were

formed on the outer and inner surfaces of the prosthesis and also occupied the interstices of the Dacron fibers. L.R. Sauvage and his colleagues reported their own method of preclotting for highly porous vascular grafts. After these successes, highly porous vascular grafts were generally used in clinics.

However, bleeding problems occurred with highly porous fabric vascular prostheses after implantation. Thrombi formed on the vascular prosthesis surface and in the interstices of the textile fibers were dissolved by fibrinolysis. Unexpected bleeding occurred in some cases after implantation of the highly porous fabric prostheses. Especially in case of the thoracic aortic surgery, the bleeding was sometimes uncontrollable. Moreover, systemic heparinization was recognized as a useful supporting method for prevention of unexpected thrombus formation during cardiac and vascular surgery, and heparin was frequently used during vascular prosthesis implantation. So even though preclotting was used, it became evident that the danger of bleeding from highly porous fabric prostheses could be avoided. Accordingly, since the safety of the patient at the time of the implantation is more important than healing of the neointima in aortic surgery. Fabric vascular grafts of low porosity were recommended for the replacement of large arteries, especially for thoracic aortic surgery.

In 1972, K. Burger reported that fabric vascular prostheses implanted in humans did not heal. Even though the prostheses maintained their patency, the luminal surfaces were not healed and neointima did not form after a long time implantation. Thus, if neointima healing cannot be expected, it is better that there be no danger of bleeding and the surgical procedure be safe, and vascular grafts of low porosity were favored particularly for surgery of the thoracic aorta.

However, there was still strong need for neointima healing, and since low porosity vascular grafts were usually tightly knitted like tent cloth, it was difficult to handle. Accordingly, the need for softer vascular grafts was stressed by many surgeons. In order to meet those demands, development of moderate porous vascular grafts temporarily sealed with biologically absorbable materials such as collagen, gelatin, etc., was begun. With these sealed grafts, they were no bleeding problems after implantation without preclotting, and they were soft and easy to handle during implantation. However, in the beginning around 1965, many problems had to be solved, such as how to immobilize and insolublize the coated collagen on the prosthesis surface, how to sterilize the coated grafts, how to maintain flexibility and humidity of the collagen and gelatin, how to preserve the products after the manufacture, how to maintain a clean environment during the process of the coating, etc. Therefore, the development of the vascular graft coated with biologically absorptive materials was temporarily

suspended until the middle of 1980.

However, Meadox Co. Ltd. began again to develop collagen-coated vascular grafts with the cooperation of M. Chvapil, who was a vascular surgeon and biomedical chemist and worked for the University of Arizona, and developed a coated product named "Hemashield" which could be clinically used after 10 years of testing.

Teflon was also recommended as raw material for blood vessel prostheses by the Creech Committee, but Teflon fabric vascular grafts were slippery due to the low friction ratio in the fiber state. The grafts made of Teflon fibers easily lost their original shape, and therefore were not used very much. But Teflon again appeared in a new shape in the vascular graft field. These were the e-PTFE grafts. Testing of e-PTFE vascular grafts started in 1972 and drew much attention because they could be used for veins and small arteries field of about 4 - 8 mm in internal diameter.

In e-PTFE vascular grafts, even though preclotting was not done at the time of implantation, bleeding from the vessel wall did not occur. Because of its non-adhesiveness, which is a characteristic of Teflon, thrombi did not easily adhere to the e-PTFE surface. Expansion of the PTFE caused numerous cracks, resulting in flexibility, elasticity, and high porosity, so that there was superiority in bending and handling the product at implantation. Therefore, the use of the products in large veins and small diameter arteries with an inner lumen of 4 – 8mm has been increasing worldwide.

Problems of Clinically- Used Vascular Grafts

Vascular grafts which were clinically used for the thoracic aorta around 1985 were tightly-knitted low porosity ones so that bleeding did not occur at the time of implantation. As mentioned above, these were stiff and were unsatisfactory in terms of handling. It is difficult to suture stiff low porosity vascular grafts, particularly to arteriosclerotic blood vessel walls or weak and thin blood vessel walls that are about to rupture. There were many cases that required special techniques for implantation. The operation is getting more difficult, resulting in longer operation times and bent needles. At this time, collagen coated vascular grafts had already begun to be developed, but had not yet been perfected. Also remarkable foreign body reactions appeared when collagen and gelatin coated vascular grafts were used and there were few vascular grafts which could be said to be ideal and safe.

Furthermore, most of these vascular grafts had a plain woven structure, so that they frayed easily at the cut edge. Vascular grafts are cut on a slent and a slightly curved and sharply cut edge is formed depending on the

operation. When such special cutting methods are used the cut edge becomes frayed in grafts with a plain woven structure. As a result, the sutured threads can come loose from the cut edge of the graft, which is dangerous. To preventing this, it is necessary to insert the sutures far from the cut edge. This causes a lump in the vascular graft at the anastomotic part, resulting in slight stenosis. Therefore, a vascular graft which can be easily sutured, is soft, does not fray, and does not bleed even without preclotting is required.

Development of the Toray Graft (Ultrafine Polyester Fiber Vascular Graft)

(1) To solve the problems

Y. Noishiki undertook the development of new vascular grafts together with Mori Y. Their purpose was to solve the problems concerning vascular grafts for the thoracic and abdominal aortae. Namely, they wanted a graft that would not bleed even though it was not coated with tissue absorptive materials such as collagen, etc., did not need preclotting procedures, was soft, and did not fray no matter how it was cut. e-PTFE produced favorable results in relatively small vascular grafts with an internal diameter of about 6mm, and so vascular grafts in this range were not targeted.

(2) Deterioration of Ultrafine Fiber

To accomplish those purposes, we obtained the ultrafine polyester fibers which Toray produces. We obtained cloth fabricated from ultrafine polyester fibers, and sutured it into the form of a tube. The advantage in using ultrafine fibers is that even though the weave is tight, the cloth does not become stiff. So it seemed that one problem could be solved. However, when a graft is manufactured only from ultrafine fiber, there could be a problem if deterioration of the fiber progressed year by year. In other words, the fiber would corrode from the outer surface, so that it would suddenly weaken at a certain period of time after implantation, resulting in the possibility of a rupture. Accordingly, the quantity of ultrafine fibers was limited such that the form of the vascular graft could maintained even when all the ultrafine fibers deteriorated; normal sized fibers were used to fill out the weave. That is, the idea of a hybrid polyester fabric of normal and ultrafine fibers was established.

However, we thought that for vascular prostheses, highly crystallized polyester fibers should be adopted in order to prevent deterioration during implantation. Therefore, we evaluated our ultrafine polyester fibers. The ultrafine polyester fibers for our vascular prosthesis were implanted into the subcutaneous layers of

experimental dogs for three years. We found that the deterioration of our ultrafine polyester fibers was negligible during the observation period. There was no change in molecular weight of the polyester polymer in the ultrafine polyester fibers when compared with unimplanted controls. From this in vivo experiment, we concluded that highly crystallized ultrafine polyester fibers were safe as far as deterioration was concerned.

Just at that time, M. King, a polymer chemist in Quebec, Canada, reported that the deterioration of Dacron fibers of a vascular graft which had been implanted in a human body for 19 years was less than 10%. The Dacron fibers were polyester fibers made by DuPont Co. Ltd., and their crystallization was not changed by their use as a vascular graft. It was the same Dacron as is clothes. The deterioration of these fibers was extraordinarily small, and the ultrafine polyester fibers which we used also had such small deterioration that it could not be measured even after three years. Accordingly, it was concluded that a vascular graft fabricated from ultrafine fibers only would be safe from rupture.

(3) Countermeasures against fraying of the cut edges

Regarding the countermeasures against the fraying problem, which was the next target, it was proved from a sample vascular graft which we manufactured, that fraying could be effectively prevented by making the ultrafine fibers entangled with each other. Any fibers, including polyester can get entangled, but since the ultrafine polyester is extraordinarily soft, it is especially easy to entangle the fibers. We used a water jet method, i.e., high pressure water is jetted from fine nozzles so that the ultrafine fibers become completely entangled. To accelerate the entanglement automatic fluffing of the ultrafine fibers inside the wall of the vascular graft was done in advance. We used this method for making a complicated structure of ultrafine polyester fibers inside the graft wall. Details such as the size of the nozzles, water volume, water pressure, speed of the nozzle movement, repetition of the procedure, and other appropriate conditions were determined by trial and error. A method of weaving which facilitated fluffing was also adpoted. These measures were successful in preventing fraying.

(4) Countermeasures against bleeding problems

The next problem was preventing bleeding without the use of preclotting or collagen and gelatin coating. Even if serious fibrinolysis occurs, the bleeding need to be controlled by the prosthesis.

The problem was successfully solved by using the ultrafine polyester fibers. The water jet procedure

effectively produced tight matting of the ultrafine fibers. The porosity of the prostheses, i.e., water permeability, was then measured. Water at a pressure of 120mmHg was forced through the graft cloth and the volume passed per cm2 per minute was measured. It was 1500ml to 4000 ml per minute for high porosity grafts, 500ml to 1500ml for medium porosity grafts, and less than 300ml for low porosity grafts. Less than 100ml is desirable for the grafts when heparin is used systemically. The water permeability of the ultrafine polyester vascular grafts was about 90ml. As a result of those efforts, vascular grafts having less bleeding could be manufactured.

(5) Low porosity vascular graft

However, when our low porosity grafts were actually implanted inside the human body, it was found that the blood leakage was even less than anticipated. This is because innumerable ultrafine fibers were twisted and entangled in the graft wall, so the overall surface area of the polyester fiber is extraordinarily large, and when this large area touches fresh blood, blood platelets adhere easily to the surface of the fibers. Furthermore, since a fibrin net easily becomes entangled, small thrombi occur everywhere in the blood vessel walls, producing favorable hemostatic. Thus, even though the water leak is 100ml, blood leak does not occur at all. Therefore, even when the vascular graft is used in an operation where heparin is used, bleeding does not occur and a very safe vascular graft can be implanted.

(6) Discovery of cellular affinity of ultrafine fiber

While the vascular grafts were being implanted in animals during development, an interesting phenomenon become evident at the cellular level. That is, neointima healing inside the vascular graft was more favorable than expected. Initially, it was unclear why this phenomenon occurred. However, it was found that host cells such as fibroblasts, which play the most important role in healing of a wound, adhered along each ultrafine fiber. Also, such cells easily gather at the site where the ultrafine fibers exist, and migration of the cells was accelerated at the sites where there were ultrafine polyester fibers. Cell migration to the areas without ultrafine fibers was always delayed.

Why does this phenomenon occur? Because cells characteristically adhere along a very thin fiber, sharp edges, etc. For example, when cells are cultivated in a petri dish, if there is a crack at the bottom of the dish, the cells form a line along the edge of this crack at first. This phenomenon is called "contact guidance", which

is the instinctive nature of many kinds of cells.

When we were developing the vascular grafts, there was a hypothesis that blood platelets might acknowledge the size of minute microspheres. In other words, microspheres of about 1 micron are not taken up by blood platelets, but microspheres of less than 0.5 micron are. Also, there was a study which examined whether or not white blood cells acknowledge the thickness of fibers. It was found that blood cells could be selectively removed from the blood if the blood was passed through a filter whose fibers were less than 3 microns. Accordingly, we tested various sizes of ultrafine polyester fibers to see whether or not contact guidance could be observed. We finally found that cells adhered to fibers of less than 3 microns in diameter. As a result, it was decided to use fibers of less than 3 microns in all vascular grafts to be manufactured. For reference, the weight of the polyester fiber used for normal vascular grafts is from 1.2 to 2.0 deniers, and the cross section diameter of the fiber is 16 to 20 microns. In contrast, the ultrafine fibers for vascular prostheses are less than 3 microns in thickness and less than 0.5 denier in weight.

(7) Introduction of Endothelial Cells into Vascular Graft Walls

When this kind of vascular graft is used, the host cells migrate into the vascular graft walls along the thin fibers at early stage after implantation, actually in one to two weeks after implantation. After migration of these host cells such as fibroblasts, numerous capillary blood vessels follow for their nutrition supply. It is assumed that at first, many blood vessel growth factors are produced by the cells which migrate, but later, innumerable cells and capillary vessels infiltrate the vascular graft walls, so that a new blood vessel network starts to formed. Capillary blood vessels are made up of numerous endothelial cells. They make a tube and infiltrate into a new tissue. Therefore, infiltration of capillary vessels into the vascular graft walls causes introduction of innumerable endothelial cells into the vascular graft walls. Therefore, the graft wall can create a neointima covered with endothelial cells quickly.

M. B. Herring and his colleagues reported in 1979 that seeding of endothelial cells into the vascular graft walls accelerated neointima formation. After the report, there was many endothelial cell seeding experiments in the 1980s, but it was finally found that there were problems with the stability of these cells. Most of the endothelial cells seeded on vascular prostheses did not survive after implantation. They were washed away by bloodstream. But in the graft which we made, endothelial cells are introduced naturally into the vascular graft walls without artificial seeding. As a result, contrary to our expectations, good neointima could be formed

after implantation of the grafts.

(8) Birth of Toray Graft

As development progressed, Toray applied for patents for the various technologies developed by Y. Noishiki and Toray engineers including Dr. Y. Mori, and the patients have also been obtained in the U.S. Vascular grafts for clinical use started to be manufactured with this technology and Toray called them "Toray Grafts".

Since the results of the animal experiments were very favorable, they were reported at the Annual Meetings of the American Society for Artificial Internal Organs. They were also reported in Japan and drew the attention of many researchers and clinical doctors. U.S. evaluation was made by Dr. M. E. DeBakey at Baylor College of Medicine and he was very satisfied with the results. With these results, Toray Co., Ltd. obtained the U.S. FDA approval (510K). Then the company applied to MHW for the manufacturing approval in Japan and obtained it without problem.

Clinical use started at University Hospitals with which Noishiki was associated as a part-time lecturer at that time: Kyoto Prefectural University of Medicine, Tokyo Women's Medical College, and Yokohama City University School of Medicine. As a result, Toray grafts were used for more than 400 clinical cases. There have been no clinical problems in the results in terms of the clinical use for about 10 years, although the vascular graft field is generally prove to problems. This is proof that the product is safe and easy to handle.

2. Why Toray decided to halt sales because of DuPont's announcement

DuPont made the following announcement on January 15, 1993.

As of January. 15, 1993, DuPont will begin to phase out sales of materials to customers using our materials in medical articles intended for permanent implantation in the human body or in permanent contact with internal body fluids or tissues. The firm intends to complete the phase-out as soon as possible, but not later than January. 31, 1994.

This announcement was made in light of the fact that Dow Corning was subjected to many lawsuits due to problems with tissue-implantable silicone for breast implants, and lost many of them. If there is a problem with medical materials, there is a possibility of a lawsuit even against a manufacturer of the raw materials. If a large company supplies some particular raw materials, a suit may be filed against it in the hopes of financial

gain. Accordingly, DuPont was against implanting Teflon or Dacron in the body for more than 30 days, or using them as medical devices which would have contact with body fluids.

With this announcement, materials manufacturers all over the world followed DuPont's policy. Therefore, Toray Co. Ltd., immediately halted the manufacture and sales of the vascular grafts. They had just started to manufacture Toray grafts at that time, and were about to sell them because they had obtained MHW approval. However, Toray Co., Ltd. suddenly canceled everything as orders from the president.

3. How Dr. Noishiki, one of the joint developers, received all patents related to the manufacture of vascular grafts from Toray.

The decision to halt the manufacture and sales of the vascular grafts by Toray severely upset Dr. Noishiki, one of the developers, and cardiovascular surgeons of Tokyo Women's Medical College, Kyoto Prefectual University of Medicine, and Yokohama City University, which had used Toray grafts. They could not imagine that a superior vascular graft like should be taken out of use. After the report of O. Creech, Chairman of the Committee of American Society for Vascular Surgery in 1957, more than one million Dacron vascular prostheses were safely implanted into the human body. There was no problem in implanting Dacron fibers inside the human body. Despite the fact that there has never occurred even one case of complications caused by Dacron fibers, their use is prohibited. Suspension of the delivery of Dacron fibers created a big stir regarding other implantable artificial organs as well.

Therefore, Profs. H. Koyanagi, Y. Imai, and M. Hashimoto of Tokyo Women's Medical College, Prof. A. Matsumoto of Yokohama City University, and Prof. T. Oka of Kyoto Prefectual University of Medicine petitioned Mr. Maeda, President of Toray Co., Ltd. To resume manufacture and sales. They also requested MHW to speak to Toray on their behalf. As a result, a responsible person in MHW repeatedly called the person in charge at Toray, and asked him to cooperate in providing safe and superior medical devices to the Japanese people.

This movement was well understood by any cardiac surgeon who had ever used Toray grafts. However, Toray refused all requests, commenting that it was company policy. However, the request was so strong that Toray proposed to supply of Toray grafts free of charge for one year to the three Universities. The professors accepted this proposal in order to use the products for the time being, and the products were supplied as promised. (It seems that they were actually supplied for nearly two years.)

Apart from the above movement, the professors mainly from the cardiac surgery field in Japan negotiated with the Japanese Society of Thoracic Surgery, the Japanese Society of Cardiovascular Surgery, the Japanese Society of Vascular Surgery, the Japanese Society for Artificial Organs, and others, and asked MHW for aid and Mr. Maeda, President of Toray, for resumption of the manufacture and sales of Toray grafts. This request was officially made in writing, so that Toray was unable to ignore the matter. A strong movement arose inside Toray in favor of resuming the manufacture and sales of vascular grafts. However, Mr. Maeda remained opposed in order to protect the company. Under the social situation In light of the Dow Corning trials and DuPont's decision, the decision to resume the manufacture and sales was not made and Mr. Maeda conveyed his intention that the vascular grafts would never be handled in the future to his successor upon retirement from the company. Accordingly, nothing has not yet been done, and the manufacturing equipment were disposed of.

MHW, the Japanese Society of Thoracic Surgery, the Japanese Society of Cardiovascular Surgery, the Japanese Society of Vascular Surgery and many doctors negotiated with Toray for more than three years, but in vain. However, Toray Co., Ltd. decided that Toray and Dr. Noishiki would jointly apply for all patents regarding development of Toray grafts, and they agreed that Toray would do all the paperwork and pay all expenses incurred.

Furthermore, if vascular grafts using ultrafine fibers are manufactured by Noishiki or developed by Noishiki together with a third party, even though breach of patent may occur, it was agreed that Toray will not file an objection. However, Toray stated that they would not ship the ultrafine polyester fiber they are manufacturing.

As a result of these events, Noishiki obtained the rights to manufacture vascular grafts using ultrafine fiber at his own disposal. (1998)

4. Future Competitiveness of the Ultrafine Fiber Vascular Graft

The ultrefine finer vascular graft has the advantage that a similar product cannot be manufactured with normal fibers. As for vascular grafts coated with collagen, gelatin, etc., it is possible that grafts with similar flexibility and cellular inductivity may be produced. However, a vascular graft with the flexibility and touch of the ultrafine polyester fiber cannot be manufactured from the usual size of polyester fibers. Any person who has ever touched an ultrafine fiber vascular graft would agree. A soft tube can be manufactured only

with ultrafine polyester fibers. Cloth made from ultrafine fibers has already been introduced into the clothes market under the name of "Ultrasuede", "Ecsene", etc. Since it is used as a high quality synthetic leather for seats of luxury cars, furniture, a high quality suits, etc., there seem to be many people who can remember its softness.

It is generally known that collagen provides a good matrix for cell migration in collagen-coated vascular grafts. Accordingly, some people may think that collagen-coated vascular grafts can become clinically useful. However, glutaraldehyde and formaldehyde, which are cytotoxic chemicals, are used on collagen-coated vascular grafts to prevent the collagen from being dissolved. In addition, glycerol, etc. is included so that the collagen will not dry out and to help retain flexibility. Therefore, the cellular affinity of collagen does not actually work due to the cellular toxicity of these chemical substances, and this prevents collagen-coated vascular grafts from having good cellular affinity.

The sterilization of the ultrafine fiber vascular graft can be done with gas sterilization or by autoclave in the same manner as other vascular grafts. The sterilization of the vascular grafts coated with collagen and gelatin is restricted, and heating up after implantation and foreign body reaction are also problems. Those problems are never found with the ultra fine fiber vascular graft. Handling during the operation is very simple and easy, and bleeding does not occur even during operations using heparin. Since these characteristics are unrivaled, our graft seems to be very strong by competitive in this field for the foreseenable future. Again, for vascular grafts in the range of 4 to 6mm in internal diameter. Collagen coating may be needed. The thinner the graft becomes, the softer it needs to be. Accordingly, the softness of the ultrafine fiber vascular grafts comes in handy in this field, too. A vascular graft which is soft and even after coating with collagen or gelatin cannot be manufactured by other methods. If collagen or gelatin coating is needed, an ultrafine fiber vascular graft is more advantageous than a graft made of the usual size fibers. This is because fibrin and blood platelets get entangled in the ultrafine fibers and strong thrombogenic tissue is formed even when collagen or gelatin coating is used.

Furthermore, considering future prospects, tissue engineering is being introduced into many fields now, and it will be introduced into the vascular graft field as well. For this purpose, the cellular affinity which the ultrafine fibers have will be very useful. There are no other vascular grafts that have the power of contact guidance. For tissue engineering, basic materials which are soft and cell-affinitive will be required, and the superiority of the ultrafine fiber vascular grafts will make itself felt.

5. Manufacturing Coats

a. Manufacturing equipment (units/day)

b. XXX

c. Personnel, etc.

d. Others

I do not know any details of the manufacturing coats.

We entrusted the manufacture of Tory grafts to their usual factories. About 200 m of knitted graft can be easily manufactured with one knitting machine in one day and about 10 m of woven graft can be manufactured in one day. It seems that special machinery is not necessary for the weaving equipment. It is good that that this can be done with simple weaving equipment. Other vascular graft manufacturers had trouble with fraying, so they had to use weaving equipment which costs more than ¥50 Million is used. On the other hand, when the ultrafine fibers are used, even though the weave is simple it finally becomes a compact structure through the water jet procedure, and the edges do not fray. Therefore it do not seem that the manufacturing costs would be prohibitive.

However, the basic ultrafine fiber cloth needs to be subjected to the water jet procedure. Accordingly, the machines generally used in the fiber industry and the water jet equipment need to be improved such as by miniaturization, etc. Some expenses for these improvements will be required. Because the machines are simple, the costs will probably not very high.

6. Time required from the start of manufacture until sales, and other necessary matters (approval, clinical trials, etc.)

One year is required for establishing the conditions of manufacture. If permit application is made with 510K, it seems that the manufacturing permit can be easily obtained. Two additional years seem to be necessary for efficiency evaluation in animal experiments, etc. If collagen or gelatin coating is done, the approval cannot be obtained with 510K. So some clinical trials will be required. The period depends on how the clinical trials are planned, but about three years will be necessary.

7. Future efficiency of ultrafine fibers, Stent Graft, etc.

There is a tendency to think that ultrafine fibers are weaker than fibers of normal thickness. For instance, if the fibers are one-fifth the thickness of normal fibers is thought that the strength is also one-fifth of the normal strength. However, the characteristics of fibers are not so.

By nature, each polymer in a fiber is arranged in the direction it is pulled. The stronger and thinner a fiber is pulled, the more regular the arrangement of molecules becomes. Thus, the strength increases logarithmically according to the regularity of the arrangement of molecules.

A product utilizing this characteristic is a super strong fiber well-known by the name of Kevlar, and it is used for bulletproof vests and airplane wings. Thus, since ultrafine fibers are manufactured by pulling them extremely thin, each polyester molecule contained in them is regularly arranged, so that they become very strong. It is anticipated that the utility value of ultrafine fibers will rise in the future.

Stent grafts have begun to be widely used in clinical practice, but ultrafine fiber vascular grafts can be just as easily used in this field.

Usual size polyester fiber is used for the cloth of vascular grafts in stent grafts in many cases. However, as it is desirable that a stent vascular graft be inserted into as a thin sheath as possible. However, if the cloth made with usual size fibers becomes thin, it also becomes highly porous or it becomes a simple structure, with danger of blood leakage. Also, the fiber weave is easily broken because of the thinning. The cloth also becomes weak, and there is doubt about its durability in the long-run for use in the aorta.

Those problems can be solved by the use of ultrafine fibers. Ultra fine fibers are thin and strong, and can be made into cloth. Moreover, if ultrafine fibers get twisted, even when it is thin, its form is not easily disruptured, and the damage which might occur to the cloth upon inserting it into the sheath and strongly pushing it can be minimized. Also, because the cloth is thin, a thin sheath can be used, and it can be used in any stent type vascular graft.

When a vascular graft made from general size fibers is inserted into a thin sheath by force, the fibers of the vascular prosthesis are frequently damaged. Polyester fibers are also composed of polymer material, and polymer materials are generally strong in regard to pulling force, but weak in regard to shearing force. If a particular part of the fiber is slightly damaged, the pulling force becomes a shearing force at the damaged part, and it can be easily torn. This is why a strong nylon climbing rope can be damaged by a rocky edge and is broken. Accordingly, in a vascular graft, if small injuries inflicted when passing into the sheath, there is a possibility that a fiber break will occur there in the future. In vascular grafts manufactured from normal size

fibers, electron microscopy has shown that innumerable injuries occur in the fiber when it posses through the sheath. However, if ultrafine fibers are used, pressure on the fibers is dispersed, and furthermore the fiber is soft. So injuries to individual fibers are less common, reducing the possibilities of fiber break. Regarding ultrafine fiber vascular grafts actually passed through a sheath, damages to the fibers have not been found so far. This characteristic can also be utilized for other purposes.

8. Problems : Obtaining Ultrafine Fibers

The biggest problem for manufacturing ultrafine fiber vascular graft is obtaining ultrafine polyester fibers. Ultrafine fibers were developed by Mr. M. Okamoto who worked for the Textile Institute of Toray Co. Ltd. about 30 years ago. Since the patent for the manufacturing method has already expired, any fiber maker can manufacture it. Also, there are many makers which manufacture ultrafine fibers with methods different from that of Mr. Okamoto. There are many companies that manufacture ultrafine fibers in Japan. In other words, almost all fiber makers in Japan have the techniques to manufacture ultrafine fibers, and fiber makers in Korea and Taiwan which have ties with those companies also manufacture ultrafine fibers in the same manner. Many of them manufacture fibers which are used in cloth, shoes, wall cloth, etc. In the medical-related field, these fibers are only used for blood filters used temporarily outside the body. These fiber manufacturers have not been agreeable to the use of ultra fine fibers for implantable medical materials since DuPont made its announcement. This means that they are against the use not only of ultrafine fibers, but also of other materials for implantable artificial organs. Accordingly, it seems that there will be difficulty in obtaining ultrafine fibers from these makers at present time.

However, attitudes toward implantable artificial organs are now being changed. This is due to the book titled "Science on Trial", published in 1997. The writer of this book is Marcia Angell, M.D., who is the chief editor of "The New England Journal of Medicine", which is very authoritative in the medical world. In this book, she scientifically describes in detail the FDA position, Dow Corning's position, the actual circumstances of the trial, academic evidence, etc. in regard to the silicone medical trial of Dow Corning, by checking the process against fact, and introducing the facts with logical explanations. This book has great persuasive power. The U.S. trial, particularly the actual circumstances of the trial under the jury system, academical handling of the facts, activities of the lawyers, etc. are described in detail.

The medical trials in general have been affected since this book was published. We also learned that