完成品の動物植え込み実験および臨床治験

ーーーあらゆる現象を詳細に観察するために少なくとも6カ月以上の観察が必要である。

コラーゲンの吸収と細胞侵入との関連は動物実験での最大の関心事である。さらに異物反応の最終確認のためには動物実験は必修である。臨床試験に関してはすべての評価が満足できる結果として明らかになった時点で行えるものである。これに関してはかなり慎重に行う必要があるが、我々はこれまでに培ってきた人脈を活用して、輸入品での副作用を経験した、信頼のおける医師に委託するつもりである。

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

the materials manufacturers attitudes are being gradually changed.

Polyestrer fiber is very stable inside the human body and not prone to deterioration, and side effects like carcinogenicity, etc. have not occur in clinical cases of many polyester fiber products including sutures and heart valve pedestals, as well as in vascular grafts in more than one million cases. In a previous study Noishiki demonstrated that Dacron is a hydrophobic substance, so that when Dacron fibers are implaned, they become covered with a thin membrane, similar to the outer half of cell membrane, which is created by the host. The membrane is formed by a single layer of lecithin molecule in in vitro experiments. Therefore, Dacron fibers that are already insulated by the covering of the special host membrane at the molecular level will not show foreign body reaction if the fibers are not contaminated during fabrication. Accordingly, there does not seem to be a problem of safety, and there is good possibility that the makers will supply ultrafine fibers if we negotiate with them.

9. Others ; FDA, MHW Approval, etc.

Since Toray Graft has already obtained FDA and MHW approval in the U.S. and in Japan, approval for new grafts fabricated from ultrafine polyester fibers does not seem to be difficulty in the application with 510K.

ハイブリッド型人工血管の開発

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今日は人工血管開発の初期の頃のお話と、最近最も注目されておりますハイブリッド型人工血管のお話をいたします。

人工血管は動脈が膨らんで、破れそうになった動脈瘤の治療や、動脈が狭くなって、血液を送ることができなくなった、動脈閉塞の治療に広く用いられておりますが、昭和３０年以前には、それは不可能なことでありました。

古い話をしますと、人工血管の開発には一つの事件がきっかけとなっております。今からちょうど１００年余り前の、１８９４年、フランスの大統領Marie Francois Sadi Carnotが、イタリア人の無政府主義者Santo Caserio、によって刺される事件が起きました。大統領は直ちに開腹手術を受けましたが、お腹の中の門脈という血管が傷ついておりました。しかし当時だれひとり血管を縫うことも、勿論人工血管で繋ぐ事もできませんでしたので、みんなが手をこまねいているうちに、大統領は出血死、してしまいました。

この事件のあと、血管の手術方法の開発と、人工血管の開発が真剣に考えられるようになりましたが、試行錯誤の連続でありました。しかし当時フランスの大学を卒業したばかりの、Alexis Carrelという、若いお医者さんがこの研究を精力的に進め、血管外科の基本的な手技を確立させました。彼はその成果が認められて、1912年にノーベル医学賞を受賞いたしましたが、それと平行して行っていた人工血管の開発では、象牙の管やアルミニューム管、ガラス管など、ありとあらゆる物を人工血管として試し、全て失敗に終わっております。当時は血液が身体の外に出たり、人工物に触れると、なぜ固まってしまうのかといった理由も分かっておりませんでしたので、無理のないことだったかも知れません。その後、第二次世界大戦のあとまで、人工血管の必要性は日増しに高まるものの、ポリエチレンチューブで一時的に繋ぐか、または保存しておいた、動物やヒトの血管を使用していたにすぎませんでした。

したがって当時は、動脈瘤であると診断がついても、治療法がありませんでした。例えば、今世紀最大の科学者の一人とされております、アインシュタイン博士は１９４９年、腹部大動脈に動脈瘤があると診断されました。主治医は博士に、死亡した人の動脈をもらってきて、それを移植できるかも知れない、と説明しましたが、博士は「自分はこれまで十分に生きてきました。今更わざわざその様なことをする必要はありません」といって断り、６年後の１９５５年、つまり昭和３０年に動脈瘤が破裂して、亡くなられております。当時は人工衛星の打ち上げが計画され、宇宙時代の幕開けであり、

医学の世界では腎臓移植がすでに始まっていました。しかし人工血管開発の分野は大変遅れておりました。

この様な情けない状況でありましたが、動物実験では新しい光がさしこんでいました。と言いますのはVoorheesと言う、アメリカの若い外科医者が、布を丸めて管を作り、動物実験で、それが人工血管として使える事を証明したのであります。当時は、水を流しても漏れることのないチューブが、人工血管として考えられていましたので、布切れで人工血管ができたことに、みんなとても驚きました。そしてこの話が伝わると、ありとあらゆる合成繊維の布が人工血管として試みられ、その結果、１９６０年、つまり昭和３５年頃から、ポリエステル繊維で作られた布製人工血管が、臨床で使えるようになりました。現在では、胸部や腹部の大動脈、及び大腿部の、太さ６mmから３０mm程度の、動脈領域に、人工血管は使われております。

人工血管はこのように、生まれてまだ４０年足らずでありますが、この歴史の新しさにも関わらず、世界中で多くのヒトの命を救ってきました。そして今日では心臓の冠動脈が閉塞した時などにも使用できる、冠動脈バイパス用の、３mmより細い人工血管の開発が、求められるようになりました。しかしこれがなかなかの難問で、世界中で研究が続けられていますが、突破口が見いだせずに今日に至っております。

人工血管を植え込みますと、内皮細胞という、天然の血管の内面にあって、血液を固まらせない仕事をしている特殊な細胞が、細胞分裂を繰り返して、次第に人工血管の内面を覆うことで、天然の血管壁と同じように血栓がつかなくなると、期待されておりました。しかし実際には、内皮細胞には分裂の限界があって、期待通りには進みません。

内皮細胞は特殊で、高級な細胞ですので、７０回ほど細胞分裂を繰り返しますと、老化現象によって細胞分裂ができなくなります。そのため人工血管のごく一部しか、内皮細胞は覆うことができず、それ以外の内面には血栓が付着して、それが厚くなったり、剥がれたり、さらに新しい血栓が付着するなど、落ちつかない状態が続くこととなります。細い人工血管では、この血栓によって閉塞してしまうこともありますので、血液が固まらないようにする薬を、一生の間、飲み続けないといけません。しかし長い間そのような薬を使っていますと、その副作用として、歯ぐきから出血しやすくなったり、怪我をすると出血が止まらなくなります。また、脳出血でも起こすと致命的な状態になったりいたします。

この問題を解決するには、人工血管内面に、積極的に内皮細胞を移植するとか、内皮細胞を初めから人工血管に組み込んでおく、という考え方が当然持ち上がって参ります。これが話題のハイブリッド型人工血管であります。

ハイブリッド、というのは、動植物では雑種とか混血を意味しますし、物では、異質の要素からなる合成物を、意味します。最近では環境問題から、電気モーターとガソリンエンジンとを組み合わせたハイブリッドカーが、話題になっておりますように、人工血管におきましても、合成高分子材料と、生きている細胞との組み合わせが、ハイブリッド型人工血管と呼ばれて、話題となっております。

ハイブリッド型人工血管の研究は、１９７９年にアメリカで始まり、細胞培養技術を用いて内皮細胞を身体の外で大量に培養して、それを人工血管の壁に張り付ける、という手段で、精力的に行われました。しかしせっかく大量の内皮細胞を付着させたのに、体の中に植え込みますと、ほとんどの細胞が激しい動脈の血流によって剥がされてしまいますので、この研究は次第に下火となってしまいました。

しかしこれらの研究の中にあって、世界中で三つのグループが良い結果を示しました。ひとつは大阪にあります国立循環器病センターの松田武久先生のグループであります。松田先生は細胞増殖のための足場、これはマトリックスと呼ばれていますが、そのマトリックスに工夫をこらして、３種類の細胞が重なって存在するハイブリッド状態を作りました。つまり、マトリックスの内面に内皮細胞を、そしてその下に平滑筋細胞層を、そしてさらにその下に線維芽細胞層をそれぞれ層状に配置することによって、天然の血管壁に良く似た構造を、体の外で、細胞培養の技術を用いて作りました。しかしながら、これには余りにも高度な技術と、長い時間が必要ですので、臨床には使われておりません。

第二のグループはアメリカの、アリゾナ大学の Williams 先生の研究グループであります。Williams 先生は、皮下脂肪組織から内皮細胞だけを純粋に分離して人工血管に絡ませて、動物に植え込みました。すると人工血管のすべての内面が、内皮細胞によって覆われたと言うのです。この方法は手術中に内皮細胞を分離せねばなりませんので、技術的に難しく、時間もかかりますので、実用化には至りませんでしたが、内皮細胞だけを移植した他のグループはすべて失敗したのに、なぜ Williams 先生だけが成功したのか、私にはとても興味がありました。

ある時私は Williams 先生 の講演を聴く機会があって、先生の示すスライドをじっくり見せてもらいました。すると彼のスライドで示すところの内皮細胞のなかには、無数の平滑筋細胞や線維芽細胞が混ざっていることを私はみつけました。異種類の細胞が混入していたことは、細胞におけるハイブリッド状態となって、期せずして成功を呼び込むことになったと私は考えております。

さて、ハイブリッド型人工血管の開発に関して成功を収めた、第三のグループは、私どもであります。私どもは人工血管の内面が、長期間経過しても内皮細胞に覆われない状態を、血管壁の難治性潰瘍、とみなしました。その様に考えますと、皮膚に於ける難治性潰瘍では、皮膚を切り刻んで、潰瘍部分に播くことで、潰瘍をなおす事実が参考になります。また、いつまでたっても治らない骨折では、骨を細かく砕いて骨折部に詰めますと、骨折がなおる、といった現象があります。そこで血管壁における難知性潰瘍にも、この原理が応用できるのではないかと、考えられます。そのようなことから、私どもは血管を細かく切り刻んで、人工血管に播いてみました。するとそこから内皮細胞、平滑筋細胞、線維芽細胞が、それぞれ活発に出てきて、わずか２週間で新しいハイブリッド状態の血管壁が完成し、それ以降、ずっと安定した状態を保ったのであります。この方法は、単に組織を切り刻んで人工血管に絡める、約１０分間の作業ですから、これまでに５０人余りの患者さんがこの手術をうけ、現在はその長期成績を観察している段階に来ております。

以上説明しました３グループの研究の特徴は、いずれも内皮細胞以外の異なった種類の細胞を、ハ

イブリッド状態で共存させたことでありました。これに似た現象は人工皮膚の開発においても見ることができます。人工皮膚では、皮膚の表面にあります表皮細胞だけを培養するのではなくて、その下にあります線維芽細胞も同時に培養しますと、それが表皮細胞にとってのFeeder cellとしての働き、つまり成長因子を出して、表皮細胞の成長、分裂、増殖を助ける働きをします。また一方の表皮細胞は、外界の刺激から線維芽細胞を守ることで、２種類の細胞は分業が成立し、共存共栄の細胞社会を形成します。

人工血管においても、内皮細胞や線維芽細胞、平滑筋細胞などを混在させますと、内皮細胞は表面に出たがる性質がありますし、線維芽細胞は下に潜って、他の細胞の支えになりたがる性質がありますし、平滑筋細胞は張力のかかる場所に行きたがる性質がありますので、身体の中では細胞固有の棲み分け性によって、ハイブリッド状態での共存共栄の細胞社会が築かれてゆくのであります。

この様に種々の細胞の本能的性質を利用することで、ハイブリッド型人工血管は現実の物となりました。

ではどの様な条件で、これらの細胞を混在されば良いのでしょうか。これが成功の鍵を握ることとなりそうです。

種々の細胞を組み合わせて新しい臓器を作る、これは最近話題となっていますTissue Engineeringの領域であります。皆様はTissue Engineeringという言葉を、お聞きになった事があるでしょうか。膵臓の島細胞や肝臓の肝実質細胞を、立体的にグループで培養して、人工膵臓や人工肝臓を作るといった試みが、その例であります。この様なTissue Engineeringでは、どの様な種類の細胞を組み合わせて使うか、それに細胞同士の言葉といわれています、サイトカインという蛋白質のうち、どのようなサイトカイン、つまりどのような言葉を使うか、そしてさらに、この様な細胞をいかなる足場で、つまりいかなるマトリックス上で活動させるか、といった、細胞、サイトカイン、マトリックス、の三つの要素の設定が工夫のしどころとなってきます。

このTissue Engineeringの考え方に立って、ハイブリッド型人工血管を発展させた例としまして、最近注目を集めております、私どもの開発しました手法を、紹介しましょう。

まず細胞に関してですが、最も効率よく血管壁を完成させるには、細胞をいち早く増殖させねばなりません。しかし、内皮細胞は高級な細胞で、細胞分裂に限りがありますので、内皮細胞にかわって未分化な細胞を用います。そうしますと、細胞分裂が早く、しかも環境に応じて機敏に棲み分けをし、その場に適した特殊な細胞に、自ら分化してくれるのではないか、という期待が沸いて参ります。つぎに細胞同士の言葉でありますサイトカインと、細胞の足場でありますマトリックスですが、それらを初めから含む組織を用いれば良い、という事となりますので、その条件を満足させるために、私どもは骨髄組織に注目しました。骨髄組織は多くの未分化細胞を持っていますし、さらに多くのサイトカインを出し、細胞にとって最適なマトリックスも含んでおりますので、先ほど説明しました、細胞、サイトカイン、マトリックス、の三要素はこれで万全となります。このようにして骨髄組織を絡ませた人工血管で動物実験をしましたところ、期待した通り、人工血管の内面には内皮細胞が、その下層