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Bone marrow transplantation in vascular prostheses

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Abstract

We obtained natural cytokine synthesis by bone marrow transplanted autologously in a synthetic vascular prosthesis. Bone marrow contains young, primitive cells with a strong survival potential which can differentiate into many kind of mesenchymal cells and can produce many kind of cytokines. These cytokines are useful if we can employ them in the right way at the right time. As an example of the use of natural cytokines, bone marrow transplantation into a synthetic vascular prosthesis will be described. Transplanted marrow cells survived in the prosthesis and accelerated neointima formation on its luminal surface. Bone marrow contains numerous undifferentiated cells which can differentiate into various kinds of cells depending on thier environment. We expected the marrow cells to differentiate into cells such as fibroblasts, endothelial cells, and smooth muscle cells. But we could not find any signs of differentiation of the transplanted marrow cells.

Introduction

We have recently achieved natural cytokine synthesis by bone marrow transplanted autologously in a synthetic vascular prosthesis[1]. Bone marrow contains young, primitive cells with a strong survival potential which can differentiate into many kind of mesenchymal cells and can produce many kind of cytokines[2]. How to use cytokines and growth factors efficiently in clinical medicine is one of the most interesting fields of research[3,4]. Gene transfer technologies using viruses, liposomes, DNA-coated micro projectiles, and infected cells are major methods for this purpose[5,6]. Some basic experiments have shown promising results, but there are also undesirable side effects[7,8]. Instead of genetically modified cells, we used autologous bone marrow.

In an ordinal environment, marrow cells works as a physiological tissue synthesizing their usual cytokines[9,10]. In unusual conditions, however, they synthesize various other cytokines for survival. Therefore, if we transplant them ectopically, they will synthesize cytokines actively and continuously. These

cytokines are useful if we can employ them in the right way at the right time. In this communication, as an example of the use of natural cytokines, bone marrow transplantation into a synthetic vascular prosthesis will be described. Transplanted marrow cells survived in the prosthesis and accelerated neointima formation on its luminal surface.

Problems with synthetic vascular prostheses

Vascular prostheses used in clinical applications have a number of problems, such as lack of anti-thrombogenicity, lack of protection from infection, less compliance than natural arteries, and low affinity for host tissues[11,12]. The most undesirable problem is the delay of neointima formation with a natural anti-thrombogenic endothelial cell lining. In fact, most of the prostheses implanted are not endothelialized, and the inner surfaces remain thrombogenic long after implantation[13,14]. Dacron fibers do not cause foreign body reaction[15], but have no anti-thrombogenicity[16,17]. Therefore, when the prosthesis is exposed to the blood, the coagulation system works against the Dacron fibers and red thrombi adhere to its luminal surface. Usually, from the anastomotic sites, approximately 2 cm areas are healed with endothelial cell lining. There was no thrombus deposition in these areas. These areas are covered with endothelial cells which have crept in from the host arterial wall at the anastomotic sites. Endothelial cells prevent thrombus formation and adhesion on the luminal surface of natural blood vessels[12]. On the areas far from the anastomotic sites, however, thick thrombi adhere to the surface because there is no endothelial cell lining. In clinic, vascular prostheses are not endothelialized except at anastomotic sites.

Reasons for the delay and efforts to overcome the problem

There are several reasons for the delay in healing, i.e., foreign body reactions of the prosthesis, inflammation, hypercoagulation, cell aging, and unusual blood flow dynamics. The major reason is the aging of endothelial cells. After more than 70 cell divisions, endothelial cells form another generation due to aging[18,19,20]. The maximum area covered by 70 endothelial cell divisions is less than 2 cm from the suture lines. In clinical practice, we used 30 to 60 cm long vascular prostheses in a patients with peripheral arterial occlusive disease. Therefore, most of the luminal surface except very limited areas of anastomotic sites are not covered with endothelial cells. As a result, the vascular prosthesis cannot acquire natural anti-thrombogenicity of endothelial cells even after a long period of time. This is the one of the most

difficult problems to overcome in a small diameter arterial regions. Details of pathological observations have been described elsewhere[21].

Background of our new trial

As explained, vascular grafts in humans do not endothelialize[13,21]. This is a protracted ulcer in the blood vessel wall. Transplantation of autologous tissue fragments has been used effectively to accelerate the healing process of protracted skin ulcers and prolonged bone fractures[22,23,24]. Tissue fragments contain various kinds of cells. In the skin, fibroblasts act as feeder cells to epidermal cells[25]. In equivalent experiments, smooth muscle cells and fibroblasts were shown to enhance endothelial cell growth[26,27]. We have applied a similar technique to vascular prostheses, since endothelial cell proliferation is greatly improved with an underlying base of feeder cells. Satisfactory results in both animal experiments[28,29] and in clinical practice[30] were obtained with venous and adipose tissue fragments in prostheses. Capillaries for endothelialization originated from the transplanted fragments and complete endothelialization was observed in a canine study[28]. We also showed that tissue fragments transplanted into the fabric prosthesis wall synthesized high amounts of cytokines such as bFGF from the early stage of implantation, resulting in rapid capillary ingrowth into the prosthesis wall[31].

A new trial

From theses evidences, the procurement of autologous tissue containing a satisfactory quantity and quality of proliferate feeder cells become desirable to accelerate endothelialization. In order to regulate the healing process, we designed a vascular prosthesis which would have growth factors during endothelialization. Autologous bone marrow tissue containing various proliferative and differentiative cells with feeder cells and cytokine secretion[32] was chosen for the source of fragments and was transplanted into the vascular prosthesis wall[21]. With this background, we applied bone marrow transplantation technology to the field of vascular prosthesis as shown in the following animal experiment.

Preparation of the graft

As a basic matrix, an expanded polytetrafluoroethylene prosthesis (e-PTFE prosthesis) with fibril length of 60 to 150 μm (average 90 μm) was used. The prosthesis was donated by the Vascular Group, Baxter

CVS Division (Irvine, CA, U.S.A.). Approximately 0.5 ml of bone marrow was obtained and stirred into 20 ml of lactated Ringer solution to create a bone marrow suspension. This suspension was sieved through the prosthesis wall by repeated and pressurized injections into the closed prosthesis with a syringe. The residual suspension that passed through the prosthetic wall was then injected again. During the repetition of this sieving procedure, bone marrow tissues were trapped in the pores of the prosthesis wall. Then the prosthesis was washed several times with lactated Ringer solution to remove the free remnants of tissue from the luminal surface, and it was implanted as a vascular substitute in the same dog from which the bone marrow was taken. As a control, a similar E-PTFE prosthesis without any bone marrow transplantation was used.

Prosthesis implantation

Twenty-four adult dogs of both sexes, weighing 7 to 12 kg, were used for implantation of the vascular prostheses (inside diameter 6 mm; length 6 to 8 cm) in the abdominal aorta. Twelve of them were used for the implantation of the treated prostheses, and 12 for the control prostheses. The abdominal cavity was entered via a midline incision in the abdomen. The abdominal aorta was exposed and mobilized by sacrificing several branches between the renal arteries to the trifurcation. A 5 cm segment of the aorta was resected and replaced by the prosthesis. During the operation, an antibiotics (1 gm Cefazolin sodium, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) was administered into the abdominal cavity, and no anticoagulants were used at any time.

All animal care was in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 80-23, revised 1985).

Prosthesis removal

The prostheses were removed from the animals at 3 days, 3 weeks and 3 months. Before harvesting, heparin sodium (100 IU/kg) was administered intravenously to prevent clotting. All the removed specimens were rinsed with saline solution to remove excess intraluminal blood and inspected macroscopically and microscopically.

All of the animals with prosthesis implantation looked healthy during the observation period. Chronic ileus symptom with a distended abdomen due to intestinal adhesion was noticed in one test animal, but

it survived the observation period. In the treated group, 2, 4, and 6 prostheses were retrieved at 3 days, 3 weeks, and 3 months, respectively. One prosthesis out of 4 at 3 weeks was occluded, but all the other prostheses were patent. In the control group, 2, 4, and 6 prostheses were retrieved at 3 days, 3 weeks, and 3 months, respectively. All of them were patent.

Macroscopic appearance of the removed grafts

The treated prostheses removed at 3 days were covered with fresh, thin, red thrombus, and were colored red. There was no thick thrombus deposition on the surface. At 3 weeks, all the prostheses were dark red in color along the entire luminal surface, and there was no fresh thrombus at all. The prosthesis wall was soft and pliable. There was no unusual adhesion, hematoma, seroma formation, inflammatory reaction or scar tissue around the prosthesis. The one occluded prosthesis was hard along its entire length and dark brown in color. Its lumen was completely occupied by connective tissue. But there was no inflammatory reaction either inside or outside. At 3 months, the luminal surfaces were still red in color, but the degree of redness was less than at 3 weeks. There was also no thrombus in the luminal surface. On the adventitial side, there was no unusual response at all. The prostheses were soft and pliable. Some areas of the luminal surface were red like thrombus deposit, but they had no thrombus deposition at all. The redness had arisen from the inside of the wall. The adventitial side was white in color composed of very loose connective tissue without unusual reactions.

In the control group at 3 days, thin red thrombus covered the luminal surface. No thick thrombus was present. At 3 weeks, the luminal surface looked slightly red in color due to a thin layer of red thrombus . Near the anastomotic sites, however, the lumen was white and glistening. There were no unusual reactions around the prosthesis. There was no hematoma, scar formation, seroma formation, or inflammatory reaction. At 3 months, the prostheses were surrounded with very loose connective tissue without any unusual responses. The luminal surface was red in color with thin red thrombus adhesion except for the anastomotic sites, where the surface was glistening and white for about 10 mm from the suture line. At the adventitia side, the prosthesis was surrounded with loose white connective tissue without unusual reactions.

Light microscopic appearance

Before implantation, the treated prosthesis had numerous bone marrow tissue including megakaryocytes and erythroblasts in the interstices of the Teflon fibrils of the E-PTFE prosthesis. Stem cells were not identified. The cells, including small pieces of bone marrow tissue, were enmeshed and accumulated in the interstices of the Teflon fibrils near the luminal surface. But numerous cells including peripheral red blood cells were present all over the prosthesis wall.

At 3 days after implantation, the prosthesis wall contained numerous cells including bone marrow cells. These marrow cells were immunohistochemically reactive. At 3 weeks, the luminal surface of the treated prosthesis was completely lined with a continuous layer of endothelial-like cells. These cells could be stained with the PAP method, indicating that they had factor VIII, and were identified as endothelial cells. There were many capillary blood vessels near the luminal surface. The thickness of the neointima was about 20 μm. A small pannus about 30 μm thick extended approximately 2 mm from the proximal anastomosis. There were no giant cells around the prosthesis, which was surrounded with connective tissue containing a large number of fibroblasts and collagen fibers. Inside the prosthesis wall, numerous macrophages could be seen. Many capillary blood vessels were also observed inside and outside the wall. Hemopoiesis areas with erythroblasts were present sporadically inside and outside the graft wall. Around these areas there was always some capillary blood vessel ingrowth. Because of the hemopoiesis and the numerous capillary blood vessels, the wall contained a great amount of endothelial cells and blood components.

At 3 months, the luminal surface of all the prostheses was covered with a layer of endothelial cells. The thickness of the neointima was between 10 to 100 μm. There was no thrombus on the luminal surface. At the anastomotic sites, small panni from 1 to 3 mm in width along the suture line and 20 to 100 μm in thickness were seen, but there was no intimal hyperplasia at all. These panni were formed to make the surface smooth between the host aorta and the prosthesis surface. There were some areas with hemopoiesis associated with capillary blood vessels. But the number of these areas was less than at 3 weeks. Inside the neointima, there were small pieces of bone fragments. They were embedded in the neointima without any inflammatory reactions or intimal hyperplasia. Hemopoietic activity was still present at 3 months. We observed some erythroblastic islands which are typical sites of hemopoiesis in the interstices of prosthesis fibers. Around the erythroblastic islands, numerous capillary blood vessels were present.

In the control group at 3 days, the prosthesis was covered with a fibrin layer with numerous erythrocytes. Inside the prostheses wall, a fibrin layer containing erythrocytes and leukocytes occupied all

areas. At 3 weeks, the surface near the proximal anastomotic site had a pannus. It was between 0.5 to 1.5 mm in width and 20 to 50 μm in thickness. The areas about 5 mm from the anastomotic site were endothelialized. The other areas far from the anastomotic sites were not endothelialized, but were covered with a thin layer of fibrin. Inside the prosthesis wall there were numerous macrophages. On the adventitia side, fibrin layers of 50 to 200 μm in thickness were attached sporadically to the outer surface. Connective tissue composed of fibroblasts and collagen fibers was also seen. There were some capillary blood vessels inside the connective tissues on the adventitia side. But there were no capillary blood vessels inside the prosthesis wall, i.e., in the interstices of the PTFE fibrils. In immunohistochemical examination, the control graft showed no bFGF reactive in any areas of the graft wall.

At 3 months, the panni at the anastomotic sites of the control prostheses were about 1 to 3 mm in width and 20 to 50 μm in thickness. A continuous endothelial cell lining from the suture line was noticed beyond the pannus edge, but the other areas far from the anastomotic sites were covered with a thin layer of thrombus without endothelial cell lining. Inside the wall, numerous macrophages were seen. But there was only a very small number of capillary blood vessels inside and outside the graft wall. On the adventitial side, connective tissue layer composed of fibroblasts and capillary blood vessels could be seen. There was a fibrin layer on the adventitia side.

There was no intimal hyperplasia in the pannus layer of the anastomotic sites. These endothelial cells were located on the fibrin layer or on the connective tissue containing fibroblasts. Inside the wall, a small number of macrophages were seen. Some capillary blood vessels were also observed. On the adventitial side, a thin layer of fibrin 1 mm in thickness and 5 mm in width directly adhered to the outer surface. Outside the fibrin layer, connective tissue composed of fibroblasts and collagen fibers was seen. A small number of capillary blood vessels were observed here and there.

Acceleration of the neointima formation observed in the results

As shown in the results, neointima formation was obviously accelerated by the bone marrow transplantation. The treated prostheses showed more rapid endothelialization than the controls. Without exception, all of the retrieved prostheses showed extremely rapid healing. Endothelialization of the center area was quicker than at the anastomotic sites. In the control group, endothelialization always started from the anastomotic sites even at 3 months. There was no endothelial cell lining in the center areas of the control

prostheses. The patterns of endothelialization of the treated prostheses and the controls showed great differences.

In humans, endothelialization always starts from the anastomotic sites[13] as in the control prostheses of the current experiment. If it could start from the entire luminal surface at a very early stage after implantation as in the treated prostheses of the current experiments, the prostheses could quickly obtain the natural anti-thrombogenicity of endothelial cells, which would be of great benefit in small diameter vascular prostheses.

In order to obtain complete endothelialization, endothelial cell seeding techniques have been tested during the past two decades. Some of them produced favorable results[33,34,35,36] , but they remain unavailable for general use because they require special cell culture techniques and facilities. They are also not available for emergency use, because the cell culture requires an extended period of time. In endothelial cell seeding experiments previously reported, the survival rate of the seeded cells were extremely low. Almost no seeded cells are available for endothelialization[37,38]. Some experiments showed that the cells in the newly formed neointima did not originate from the seeded cells but from the surrounding host tissue at the adventitia side. These experiments were not successful because the seeded cells were washed away by the circulation. Enzymatically separated cells have difficulty surviving in vivo.

Survival of the transplanted cells

It was obvious that the bone marrow cells survived and maintained their activities during the sieving procedure and after implantation. They created colonies and acted as bone marrow tissue inside the prosthesis wall. This result is greatly superior to the low survival rate of enzymatically derived single cells. We need clumps of endothelial cells to minimize washoff. In the metastasis of cancer cells, it is assumed that a single cell that has migrated from the original tumor does not survive to cause a metastasis. A certain number of cells is required to start a new colony. In the current experiments, the survival rate of the bone marrow cells *in vivo* was not calculated, but bone marrow tissues formed clumps of cells and it is evident that these bone marrow tissues certainly survived and continued their hemopoiesis.

Our hypothesis regarding the cell survival is as follows. The interaction of different kind of cells is probably of crucial importance. In artificial skin grafts, new skin can not be produced with cultured epidermal cells alone. However, with cultured fibroblasts underneath the epidermal cell layer, a skin-equivalent graft can be produced[25]. Fibroblasts are considered to act as feeder cells for the epidermal cells. Fibroblasts

do not suppress the epidermal cell growth in vivo. The combined use of different cell types is important for organ reconstruction. Wildevuur et al. found that seeding of smooth muscle cells enhanced endothelialization of vascular prostheses[26]. Tissue fragments containing endothelial cells, smooth muscle cells, and fibroblasts also enhanced neointima formation of a fabric vascular prosthesis[27,28]. As shown in our in vitro cell culture results, bone marrow contains various cell types. Therefore, the bone marrow in the vascular prosthesis was in a desirable condition for their survival.

Ectopic hemopoiesis of the transplanted marrow cells

It was surprising that the hemopoiesis took placet in the treated prosthesis wall. Colonies of erythroblasts were always associated with capillary blood vessels. During the hemopoiesis, the marrow cells require nutrition for survival and raw materials for hemopoiesis. Therefore, capillary blood vessels are required quickly. There is natural angiogenesis. As a result of this activity, the prosthesis wall can obtain numerous capillary blood vessels. This is the reason why the luminal surface of the treated prostheses looked red without fresh thrombus deposition.

In general, bone marrow obtained by needle puncture contains some endothelial cells. The amount of endothelial cells is not high. But these cells could survive to make colonies to promote marrow cell survival, producing the entire luminal surface lining within a short period of time. This activy is quite aggressive in the young, primitive cells of the bone marrow. We can obtain an active bone marrow tissue even from elderly patients. Therefore, bone marrow transplantation into the vascular prosthesis wall can induce this activity in the healing process of neointima very efficiently.

Bone marrow transplantation technology

Exogenic hemopoiesis on these vascular prostheses was characteristic. During hemopoiesis, some cytokines might aid capillary ingrowth into the prosthesis wall. Marrow cells need nutrition for their survival, raw materials for producing blood cells during hemopoiesis, and routes for shipping out their products, i.e. "blood cells". As a result, capillary ingrowth was required. These requirements of the marrow cells might be translated by cytokines and growth factors. Remarkable capillary ingrowth shortly after the prosthesis implantation indicated the existence of strong angiogenic properties. Detection of bFGF in the transplanted marrow cells suggests that its production continued throughout the prosthesis wall as long as those

cells existed. bFGF has gained attention due to its strong angiogenic property and ability to accelerate capillary ingrowth to form collateral circulation for the ischemic heart[39,40]. The dark-red color of the treated prostheses observed macroscopically might have come from the newly formed capillaries and numerous immature blood cells at hemopoiesis . The size of the capillaries decreased in parallel with the regression of the hemopoiesis and at that time the prosthesis turned light pink. These four factors, i.e., bFGF, capillary ingrowth, hemopoiesis, and graft color seem to be interrelated.

The dose of cytokine and growth factors sufficient for angiogenesis and neointima formation might be small, but continuous release would be essential for the cells to endothelialize the prosthesis lumen, since these factors' efficacy lasts a short time[40,41,42]. However, there appears to be an autoregulating system in the bone marrow treated prostheses, since endothelialization was complete within 3 weeks and was arrested without endothelial cell hyperplasia or hemangioma formation, whereas hemopoiesis continued for more than 3 months.

Cytokines synthesized from bone marrow

The activity might be stimulated by cytokines such as bFGF synthesized by the transplanted bone marrow. We have shown that in a vascular prosthesis made with fragmented autologous tissue, a high amount of bFGF was synthesized soon after implantation[31]. There was much capillary ingrowth in the prosthesis wall and the luminal surface was endothelialized within 1 month. In the current experiments, we did not identify any synthesized cytokines other than bFGF. During hemopoiesis, however, marrow cells could continuously produce various cytokines that accelerate the neointima formation. In effect, we transplant a system that slowly releases cytokines and supplies young cells. Such systems could be useful not only in vascular prostheses, but also in various hybrid artificial organs and in surgery in general. Thus we were able to produce neointima formation on vascular prostheses without recourse to the use of gene transfer technology.

Differentiation of cells

Bone marrow contains numerous undifferentiated cells which can differentiate into various kinds of cells depending on thier environment. In the current experiments, we expected the marrow cells to differentiate into cells such as fibroblasts, endothelial cells, and smooth muscle cells. But we could not find any signs of differentiation of the transplanted marrow cells. If such differentiation could be effected with the

aide of some kind of cytokines, bone marrow technology would be useful to design future hybrid artificial organs with the ability of cytokine synthesis .

Conclusion

Active angiogenesis in the bone marrow tested prosthesis and accelerated endothelialization throughout the luminal surface seemed to be the result of co-operation of the transplanted cells and cytokines secreted from them. The prosthesis appears to be an autocrine artificial organ producing growth factors for itself under an autologously controlled system. Based on these observations, we could understand how useful the ectopic transplantation of bone marrow was. We expect that this technology will be applied in all medical fields.

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組織工学研究における「走性」の活用

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はじめに

先の研究から、幼弱な細胞、遊走能の活発な細胞、分裂能の高い細胞、等を意図的に集めることが可能であることが明らかとなった。これは一般の体組織内であっても、通常な何ら活発な動きのみられない組織内であっても、何らかの刺激でそのようなことが惹起されることが判明した。この手法を用いると、用意にBlastogenesisの現象を引き出すことができる。そうなると、細胞組込型人工臓器を作成するに置いて、この現象を積極的に取り入れると、成熟した組織においても幼弱な細胞を集めて、その細胞の活力を生かすことが可能となる。

さらにこのような時に置いて、積極的に細胞に働きかけると、意図的に細胞を選別しつつ集めることも可能となるはずである。具体的に言えば、前回行った「細胞ほいほい」の装置を用い、使用したコラーゲンスポンジにVEGF (vascular endothelium Growth Factor) の如き細胞成長誘導因子を用いていれば、その特異性によって特定の細胞を集めることが可能となるはずである。

一般的に言うと、細胞や単細胞生物など、勿論多細胞の生物にも未羅列現象であるが、それらの個体の本能的な動きの特徴の一つに「走性」という性質がある。この性質を活用すると、無理なく多くの細胞を集めることが可能となる。「走性、Taxis」の項目を理化学事典で引くと、以下の様な説明がかかれている。

走性、Taxis. 自由運動の能力を持つ生物が外部からの刺激に反応して運動を起こし、この運動に方向性が認められたときに、これを走性という。走性は刺激の種類によって化学走性、重力走性、電気走性、温度走性、流れ走性、音波走性、などに分けれれ、いずれの場合にも刺激源に向かって進むときには正、刺激源と反対方向に進むときには負、とよばれる。走性は下等度物の行動において、きわめて重要な意義を持っている。(Chemotaxis, Aerotaxis, Phototaxis, Thigmotaxis, Osmotaxis, hygrotaxis, Geotaxis, Electorotaxis, Thermotaxis, Rheotaxis, Phototaxis etc)

このような、細胞の持つ本能的な性質をいかに活用するかが、Tissue Engineeringの重要なキーポイントとなるであろう。しかもそれをin vivoで用いると、さらに顕著に現象が現れ、素直に、無理なく細胞組込型人工血管を作成することができるであろう。このたびの研究はこのようなことを基本に考えて、人工血管作りを目指した試みである。

材料と方法

1，使用した人工血管の基材

ポリエステル繊維で編まれた布製人工血管 (Micron, knitted graft, water permeability,

1,200ml/min/cm2）を枠組みとして用いた。次に、アテロコラーゲン（株式会社高研、東京）を重量比で５％となるように蒸留水内に入れてコラーゲン分子を膨潤させた後に、徐々に加熱してコラーゲン分子を熱変性によってゼラチン化させ、結果的に５％の親ゼラチン液を得た。さらにこれとは別に、0.5%になるように繊維性コラーゲンの粉末を蒸留水中に入れ、0.1Nの塩酸溶液を滴下して酸性とし、pH ５付近でコラーゲンの均質な分散液を作成した。

次に布製人工血管基材にコラーゲン溶液を圧注入して、コラーゲン線維をポリエステル繊維に絡ませ、その後に凍結乾燥にてコラーゲン線維の多孔質な状態での人工血管被覆状態を得た。次にこれにゼラチン液を振りかけて、多孔質部分にゼラチンをしみこませ、高濃度の被覆状態を作った。そして再び凍結乾燥を行った。

つぎにこの様な状態となった被覆人工血管を接し135度、２４時間、真空下で熱架橋を行い、コラーゲンとゼラチンの不溶化を行った。このようにして、コラーゲンとゼラチンで被覆された人工血管を得た。

つぎにこの人工血管内腔に、ちょうど内面に接する様なサイズの断面を持つ、断面が円形のシリコーンひもを入れ、5-0 ポリプロピレン糸にて、人工血管とシリコーン樋本を固定した。この組み合わせの基材はエチレンオキサイドガスにて滅菌した。

２、人工血管基材への細胞の付着

作成した人工血管基材を成犬の腹部の皮下組織内に挿入した。このとき挿入に際して、2.5マイクログラム含有の VEGF 溶液およびセファロスポリン系の抗生物質とを被覆された基材に降りかけ、無菌的に植え込んだ。この植え込んだ資料は１週間から１０日後に付着した周囲組織とともに採取し、シリコーンひもを抜去した。この作業によって、布製人工血管を枠組みとした、細胞のからまた結合組織管を得た。

この管の一部は組織学的な検査のためにフォルマリンバッファー液によって固定した。

３，人工血管としての植え込み

作成した結合組織管を、それを取り出した同一のイヌの腹部大動脈に人工血管として植え込んだ。植え込みに当たっては、5-0 ポリエステル糸を用いて連続縫合で、端々吻合をおこなった。なお、術中に抗生物質の散布を手術創部分に散布したが、抗凝固薬はいっさい使用しなかった。

４，人工血管の取り出しと試料観察

術後３日目から１２０日目に至るまで、順次、時間の経過を追って動物から資料を採取した。得られた試料は血管の長軸方向に切り開き、内面を観察するとともに、フォルマリンバッファー液によって固定し、組織切片を作成して光学顕微鏡にて観察した。なお、組織切片の染色は HE 染色、ワンギーソン染色、フォンコッサ染色、アザン染色、およびファクターエイト染色のための PAP 法の各染色

を行った。

また、一部の試料はグルタールアルデヒドにて後固定したあと1%オスミウム液に浸け、さらにアルコール系列にて脱水し、液体炭酸ガスを用いて臨界点乾燥を行い、白金パラジュウムを蒸着して、走査型電子顕微鏡にて観察を行った。

結果

1、 人工血管基材の作成と細胞の付着

基材の作成と動物の腹腔内への挿入は容易であった。しかしながら、感染する例が１２例中に３例あり、一般の手術では感染は極めて希である我々の実験室においては、この数字は実に不名誉な高率感染であったため、慎重な手術が求められた。これらの感染例は評価の対象から外した。

皮下組織から取り出す際には、試料周囲には大量の血管の進入が肉眼レベルでも明らかにわかり、周囲はきわめて赤く、その部は出血しやすかった。この部の切り出しには電気メスを使用せざるを得なかった。細胞の付着は７日間で人工血管の網目が完全に被覆されるだけのフィブリンと細胞が絡まり、十分に付着していた。また１０日後ではしっかりした組織が付着していた。

光学顕微鏡による観察では、７日後の試料で、布製人工血管のポリエステル繊維はフィブリンと細胞に絡まられており、大この細胞はマクロファージ、赤血球、白血球、形質細胞などであった。さらにはこれらの細胞内に線維芽細胞が見られ、さらに毛細血管の形成と思われる血球を含む管腔が見られた。挿入後 10 日目には血管構造はハッキリとしており、多くの血管が人工血管のポリエステル繊維間隙に進入している状態がよく観察された。

2、 人工血管としての植え込み

人工血管の編み目はコラーゲンおよびゼラチンで完全に被覆されてはいたが、人工血管としての植え込み時には、これらのコラーゲンやゼラチンはほぼ完全に分解され吸収されていたと思われる。しかしながら、人工血管壁は進入してきた組織によって完全にシールされており、植え込み時に人工血管壁からの出血は全く認められなかった。

吻合に関しては、通常の布製人工血管の吻合と言うよりも、自家組織を縫いつけているといったハンドリングであって、人工血管と本来の血管との吻合がきわめて良好であって、その部からの出血は全くみられなかった。

3、人工血管の採取

人工血管の周囲はしっかりした結合組織に取り囲まれていた。しかし、その結合組織には次の様な変化が見られた。すなわち、通常の人工血管を植え込んだ場合には、緩やかな結合組織が周囲を覆うが、作製した人工血管の場合には、血管組織の多い、肉芽組織に近い状態の実質的な組織が人工血管を取り囲んでおり、この状態は植え込み後１ヶ月以上の経過例では全てに認められた。