MICROVENOUS ALLOGRAFTS IN RABBIT FEMORAL ARTERIES : EXPERIMENTAL STUDY OF FREEZE - DRIED ALLOVEINS

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I. INTRODUCTION

In the fields of clinical reconstructive microsurgery, there are numerous circumstances where vascular grafts are required. At present, autogenous vein grafts have been widely used and proven to be the most reliable in such instances[1-5]. However, there are some disadvantages involved in harvesting autogenous vein. So, other useful and desirable substitutes for autogenous vein have been searched for. Recently, readily available microarterial prosthesis made of polytetrafluoroethylene(PTFE) or polyurethane(PU) has been introduced as microvascular replacements ; but, many experimental studies have reported highly variable patency rate and its thrombogenetic property[6-11], and many surgeons agree that the long - term patency of synthetic grafts is less than that of venous autografts.

Alhough veins are floppy and more difficult to handle microsurgically than arteries, the venous allograft remains an attractive alternative for the reconstruction of small - caliber vessels. The early enthusiam regarding the use of venous allografts was based on the belief that vein wall elements were essentially nonantigenic[12]. Several experimental trials, however, have shown that venous tissue possesses normal antigenicity[13,14], which result in rejection and early occlusion of the vein when this is introduced into a nonhistocompatible host[15,16]. Several invesigators have attempted to reduced the antigencity and improve the patency rate of allovein by use of immunsuppressive agents and/or graft pretreatment, with conflicting results[12,17-24]. Chow et al[25], So and Chow[26], Pratt et al[27], and Raman and Hargrave[28] demonstrated the efficacy of rehydrated freeze - dried microarterial allo - or heterografts. Venous allografts do not depend on vasa vasorum for their nutrition, thus the problem of degeneration and aneurysm formation may be much less than was seen when arterial allografts were used[13]. This suggested possilbe applications of freeze - dried microvenous allografts and theoretical superiority to freeze - dried microarterial allografts.

The purpose of this study was to evaluate the efficacy of freeze - dried microvenous allografts to bridge the clinically significant arterial defects in rabbit model.

II. MATERIALS AND METHODS

1) Experimental Animals

Acknowledgments

1. 이 논문은 1990년도 문교부 지원 한국학술진흥재단의 지방대 육성 학술연구조성비에 의하여 연구되었음.
2. 1991년도 악안면성형재건외과학회 추계종합학술대회에 본 논문을 구연하였음.

Sixty adult rabbits, of either sex, were included in this study and divided into three groups. Group A(n=12, 24 grafts) had freeze-dried venous allografts inserted into the femoral arterial gaps of both sides; Group B(n=12, 24 grafts), fresh allovein grafts without any pretreatments; Control group (n=12, 24 grafts), autogenous femoral vein grafts. The rest twenty four rabbits(allovein donor group) were used for harvesting the veins.

2) Preparation of allovein

Total 48 segments of femoral vein measuring 2.5 cm in length were harvested from the both thighs of allovein donor group. The lumen of grafts were thoroughly flushed with heprinized saline(40 IU/ml) and immersed in physiologic saline. Twenty four fresh alloveins were immediately interpositioned in the right and the left femoral arterial gap(Group B). The rest of harvested vein segments were then freeze-dried at a temperature of −65℃ under a negative pressure of 200 mtorr, and stored for 2 to 7 days at bench[28](Fig. 1). Each freeze-dried graft segment was rehydrated for 60 minutes in physiologic saline prior to implantation(Group A).

3) Operative procedures

Anesthesia was induced with intravenous ketamine(8mg/kg) and maintained with halothane, nitrous oxide and oxygen via the tracheostomy site. Bilateral groin and thigh areas were shaved and cleansed. The femoral artery was exposed and excised after microvascular clamps were applied to the proximal and distal ends.

In group A, stored freeze-dried vein was reversed and vascular anastomosis was carried out using 10 to 14 interrupted stithces with 10-0 monofilament nylon sutures(Ethicon Co., Ltd.) under operating microscope(Karl Caps, magnification 20×1.6). During the microsurgical procedures, tissues were frequently irrigated with heparinized Ringer's solution. And 1% lidocaine hydrochloride solution was applied intermittantly to minimize spontaneous vascular spasm. The graft patency was checked for 10 minutes and then the wound was closed, and the opposite side was completed as described.

In group B, a similar technique was used except that fresh allovein was bridged.

For control group, autogenous femoral vein was harvested and grafted to the femoral artery of the same side using similar method.

4) Exploration and tissue processing

Explorations were carried out at one, two, three and four weeks postoperatively. Under general anesthesia, the previous incisions were reopened and the grafts were dissected free of surrounding tissues and their patency was assessed by visual and patency tests. Fisher's exact test[29] was used to determine if patency rates in control and allovein groups varied significantly($P<0.05$). Graft specimens were taken and perfused with heprinized saline to remove any blood. All specimens were split longitudinally and examined uner the operating microscope for any blood clot. After fixation with half Karnovsky's solution[30], one-half of the divided specimens were prepared for histologic evaluation and the other half for scanning electron microscopy. Specimens for light microscopy were embedded in Epon/Araldite and cut longitudinally into 1 micrometer-thick sections. The sections were stained with methylene blue. Some specimens were embedded in paraffin and stained with hematoxylin and eosin. Specimens for scanning electron microscope were post-fixated in 1% osmium tetroxide in 0.2M natrium cacoylate buffer, dehydrated in a series of ascending ethanol baths, critical point dried, and finally coated with gold in a vacuum evaporator.

5) Microlymphocytotoxicity test[31]

For 2-stage complement-dependent microlymphocytotoxicity assay, arterial blood sampling about 5cc at one, two, three and four weeks was done from animals that were to be explored at four weeks postoperative. Blood samples were centrifuged and

sera were diluted in heat - inactivated fetal calf serum at the ratio of 1 : 1, 1 : 2, 1 : 4 and 1 : 8. One microliter of prepared sera was added into each humidified Terasaki's plate. A single drop of mineral oil was added into each well. Lymphocytes were separated from heparinized whole blood of donor rabbits using polysucrose gradient centrifugation. Lymphocyte cell suspension was then washed and diluted to the cell density of 2×10^6cells/ml and 1 microliter of lymphocyte prepartion was added under the oil drop for prevent evaporation. The plates were incubated at 37℃ for 30minutes. Five microliters of fresh guinea pig serum were added to each well as a sourse of complement and the plates incubated at 37℃ for an additional 60minutes. Eosin was added to each wells and target cell lysis was assessed under inverted phase - contrast microscope.

Ⅲ. RESULTS

The patency rates of the vessels at different periods after anastomosis are shown in Table 1. The patency rate of the freeze - dried grafts was as high as 100% at 2nd week, 83.8% at 3rd and 4th week, a figure comparable with that for autogenous grafts (100% at all periods except 3rd week). With the non - freeze - dried fresh grafts, however, the patency rate fell to 50%(at 2nd and 4th week) and 66.7%(at 1st and 3rd week). On gross examination, there was no aneurysm formation or graft dilatation in freeze - dried allografts and autogenous vein grafts(Fig. 2), but one aneurysm was found in 3rd week non - freeze - dried allograft. The patency rates in groups of freeze - dried allograft and non - freeze - dried allograft at 14 days, in groups of non - freeze - dried allograft and autogenous vein graft at 14 days and at 28 days varied significantly($p < 0.05$). In freeze - dried allovein grafts groups, there were minimal surrounding fibrosis and the blocked grafts became partially disintegrated at four weeks, whereas fresh allovein grafts were surrounded heavily with thick fibrous tissue.

Histology

1) Freeze - dried allograft

One week. Histopathology showed a stage of destruction in which there was fibrinoid patchy necrosis of the vessel wall. The thin elastic laminae appeared as a dark staining line and there were mixed inflammatory cellular infiltrations in the subintimal layer with some medial fibrosis(Fig. 3).

Two weeks. Fragmentation of elastic laminae and organized fibrous tissue was observed. A new intima had started to grow over the old elastic laminae and patches were distributed through out the graft wall, which probably represented a degenerative reaction.

Three weeks and four weeks. Substantial development of the neointima which was separated by the original elastic lamina was seen, but the entire graft wall was not covered with new intima. Cellular inflammatory residue was almost disappeared in the subintimal layers(Fig. 4).

Table.1 Patency Rates of the Grafts at Different Periods after Operation

	Freeze - dried Allografts	Non - Freeze - dried Fresh Allografts	Autogenous vein Grafts(Control)
Immediate	100%(24 / 24)	100%(24 / 24)	100%(24 / 24)
7days	83.3%(5 / 6)	66.7%(4 / 6)	100%(6 / 6)
14days	100%(6 / 6)	50%(3 / 6)	100%(6 / 6)
21days	83.3%(5 / 6)	66.7%(4 / 6)	83.3%(5 / 6)
28days	83.3%(5 / 6)	50%(3 / 6)	100%(6 / 6)

2) Non - freeze - dried graft

The histologic findings were similar to those of freeze - dried allograft, except that the cellular reaction seemed to be more intense at an early stage. The intensity of this infiltration varied between grafts explored at the same time and along the length of individual grafts. However, it was generally most prounced at two and three weeks(Fig. 5,6).

3) Autografts

One and two weeks. Substantial denudation of endohelium in the graft wall which was covered with thin thrombus was seen. But some surface was lined by endothelium and an elastic laminae was seen. In the suture areas, some degree of inflammatory cellular infiltration was also noted(Fig. 7).

Three and four weeks. Autografts showed increased proliferation of endothelium and collagen matrix between the elastic laminae, along with subintimal hyperplasia. The elastic laminae was normal in character. The graft wall was largely lined with endothelial cells. Two to three smooth muscle cell consisted the media - like layer. The autografts showed the usual phenomenon of intimal hyperplasia, that was increased proliferation of endothelium and spindle cells, but was small quantity.

Scanning electron microscopy

1) Freeze - dried allografts

One and two weeks. The flow surface was covered with a fibrin layer with occasional entrapped red cells and platelet carpet, and normal vein structure was difficult to identify(Fig. 8).

Three and four weeks. There was re - endothelialization from both ends of the grafts. In certain areas a network was seen as if some surface cells had peeled off to expose the underlying collagen network(Fig. 9, 10).

2) Non - freeze - dried allografts

The SEM findings were similar to those of freeze - dried allograft(Fig. 11).

3) Autografts

One week. The endothelial configuration near the suture line was charaterized by cellular elements with elongated appearance, advancing from the normal endothelial layers, with their longitudinal axes lying along the direction of the blood flow. The stitches were not completely covered by endothelium(Fig. 12).

Two weeks. The endothelial repair was almost completed on the suture line as well as on the stitches, but in the center of grafts there were still some areas of endothelial denudation(Fig. 13).

Three and four weeks. Intima of grafts were almost completely lined by endothelial cells, but minor differences from one specimen to another could be detected. Endothelial orientation was irregular (Fig. 14).

Lymphocytotoxicity assay

Definite lymphocytotoxin response was produced in the fresh allovein recipients(Group B). The response was weak in the first week after grafting, and did not reach a peak until 3 weeks. Thereafter, lymphocytotoxin titers were reduced to the 4 weeks. In freeze - dried allovein recipients, there were only initial weak response(at 1 week). Autograft recipients did not show any antibody formation (Diagram 1).

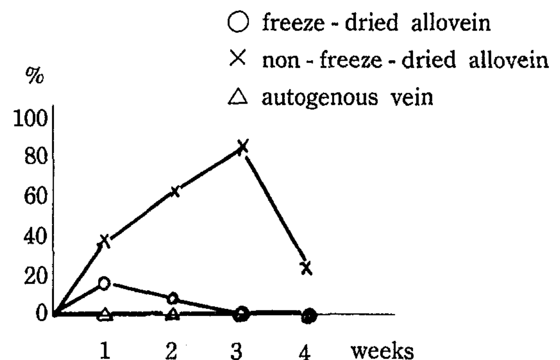

Diagram 1.
The Percentage of Target Cell Lysis by Rabbit Antiserum(1 : 1 dilution) Sensitized by Vein Graft

Ⅳ. DISCUSSION

Venous allografts would represent a theoretically attractive alternative for vascular reconstruction if rejection did not alter them and lead to occlusion. Unfortunately, these grafts undergo a characteristic sequence of histologic changes that result in vessel wall inflammation, intimal destruction, and in most cases thrombosis[6]. These changes were seen in this study and have been documented by numerous other observers[13,15-17]. Some attempts have been made to alter this sequence of events by host immunosuppression but results were inconclusive[12,13,15,19,20]. A review of the literature suggested that little work has been done on preserved biological microvascular grafts[18,21-28]. Moore et al[32] were perhaps the first to use freeze - dried caroid arteries in dogs. Despite the relatively big vessel caliber, the patency rate obtained was only 55%, which was far too poor to be of any clinical value. Since then there had been no publications on this subject until, in 1983, Chow et al[25] showed that freeze - dried arterial allografts were as reliable as autografts(84% success compared to 88%, respectively). More important, there was an absence of immune reaction which suggested that the freeze - drying process was capable of suppressing antigenicity. This study also indicates that freeze - drying is effective pretreatment method in reducing the antigenicity of venous allografts. The exact mechanism of actions on the immunosuppression by freeze - drying is unknown and beyond the scope of this experiment.

Although good results have been reported in short segments of freeze - dried arterial allografts by some investigators, Raman and Hargrave[28] cast doubts on the efficacy of freeze - dried microarterial allografts of clinically significant length with the suggestion that a lack of normal endothelium and loss of normal arterial wall viability could be derimental. But in this experiment, the patency rate of freeze - dried venous allografts was as high as 83.3% by the 4th week and seem to prove the theoretical superiority to freeze - dried arterial allografts. Although freeze - drying has elevated the patency rate of allovein significantly with preventing the graft rejection phenomena, but the endothelization was not completed by the 4th week. There was no difference in the endothelization process of freeze - drying and fresh allovein graft, but the autogenous vein grafts showed completed endothelization by the 3rd week postoperatively. This means that the patency inferiority of freeze - dried vein to that of autogenous vein graft is, somewhat, lack of endothelial cells. Even if the grafted allovein was survived with the aid of freeze - drying process and better patency rates were obtained than freeze - dried allo - arterial graft, the grafted vein is to be occluded ultimately without the normal endothelization. Here, I confronted new problems in using the freeze - dried allovein grafts. The ultimate goals of vascular grafts are to retain 100% patency rate simulating the original vessels morphology and hemodynamics. As this study revealed the endothelization delay in the freeze - dried allovein graft compared with autogenous vein grafts, the initial function of allovein was only the vascular conduit, not the biological grafts. In this regards, freeze - dried vein seems similar to vascular prosthesis.

Another problem to be resoluted was the sterilization of alloveins. Rationale in using freeze - drying was to negate any immune phenomenon that might be responsible for graft failure. However, the patency rates with 2 - day - stored freeze - dried allovein grafts were better than those of the 7 - day - stored grafts. This finding was probably due to the thrombosis resulted from infection or happen to meet. Generally, sterilization of preserved graft material is achieved using gamma iiradiation and ethylene oxide gas[28]. But sterilization process has the potential to alter complex protein configuration and render membranes leaky, and Raman and Hargrave[28] reported the gamma irradiation associated distortion of vessel wall archite-cuture.

So and Chow[26] reported 47.1% incidence of aneurysm formation in the freeze - dried microarterial heterograft model. The destructon of the vessel wall led to loss of normal compliance, elasticity of the vessel wall and the ability to withstand stretching, hence the high incidence of aneurysm formation. Aneurysm predisposes to thrombus formation and there may be a risk of delayed blockage of the graft. But no aneurysm and dilatation was obsereved in this experiment except one aneurysm of fresh allovein graft group. Oschner et al[33] suggested that the fresh venous allografts may have a lesser tendancy to degernerate and become aneurysmal than allogenic arteries because their thinner fibromuscular layer is less vulnerable to ischemic degerneration, being more readily nourished and survived by arterial blood of high oxygen tension. Vein wall itself is thinner than artery and can withstand more effectively in arterial blood pressure, so the venous graft has more efficacy in vascular allograft than arterial allograft.

From this observations, it would appear that the freeze - dried allovein grafts has many superiority than microarterial allografts. But a lack of normal endothelium could be detrimental. Although freeze - drying can control the antigenicity, expermental results were fall short of the autogenous vein grafts. Clinical application is doubtful with these outcomes, but more ellaborate study concerning the retaining normal graft wall viability and endothelization process could be the answer.

V. SUMMARY

Freeze - dried venous allografts of 2.5cm length were implanted to bridge the femoral arterial defects using adult rabbits as the experimental model. Segments of femoral veins were harvested and preserved for 2 to 7 days in vacuum - sealed clean test tube after freeze - drying at a temperature of −65℃ under a negative pressure of 200 mtorr. These allografts were rehydrated and then implanted in rabbit femoral arterial gaps(n=24), and compared with fresh(non - freeze - dried) allovein grafts(n=24) and autogenous vein grafts(n=24) at intervals ranging from immediately to four weeks. The patency rates at 4th postoperative week (freeze - dried allovein graft, 83.3% ; fresh allovein graft, 50.0% ; autogenous vein graft, 100%) indicated that freeze - drying treatment improved venous allograft survival. And microlymphocytotoxicity assay showed freeze - drying could adequately reduce the antigenicity of alloveins without aneurysm formation. But scanning electron microscopic study revealed a lack of endothelization. From this observation, it would appear that the freeze - dried allovein grafts had marked advantages over the freeze - dried arterial allografts, or over the fresh alloveins. But clinical application, in the light of better alternatives of autogenous vein graft, is doubtful.

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Figure legends

Fig. 1. Stored freeze - dried femoral vein with external diameters of 1.5 to 2mm.

Fig. 2. A freeze - dried interpositional vein graft(2.5cm in length) in the femoral artery of rabbit seen 3 weeks after operation. The anastomotic lines are shown with the arrows(FA, femoral artery ; G, freeze - dried allovein graft).

Fig. 3. Light microscopic appearance of a 1 - week - old freeze - dried venous allograft, exposed and partially fragmented elastic laminae(arrow head) and destroyed endothelium is noted(methylene blue - stained Epon/Araldite section, ×40).

Fig. 4. Light microscopic appearance of a 3 - week - old freeze - dried venous allograft. A, Dark stained elastic fibers are evenly distributed from proximal part of the recipient artery(A) to vein graft(V). A thick neointima has grown over the elastic laminae(arrow), B, Distal anastomotic area. C, Detail study of graft seen in Fig. 4. B, Vein wall is lined with smooth endothelial layer(methylene blue - stained Epon/Araldite section, A, ×100 : B, ×100 : C, ×200).

Fig. 5. Light microscpic appearance of a 1 - week - old non - freeze - dried venous allograft. A, Proximal anastomotic area showing fragmentation and some loss of elastic laminae(EL), and destroyed endothelium(arrow). Right side is recipient artery and shows endothelium(E). B, Enlarged view of Fig. 5. A(methylene blue - stained Epon/Araldite section, A, ×100 : B, ×200).

Fig. 6. A. Low - power light microscopic appearance of 2 - week - old non - freeze - dried venous allograft showing neointimal growth over the elastic lamina and patchy necrosis(arrow). There is no endothelial cells observed. B. Detail study of graft seen in Fig.6. A(methylene blue - stained Epon/Araldite section, A, ×40 :, B, ×200).

Fig. 7. Photomicrograph of 1 - week - old autogenous vein graft, shows endothelization from the proximal area(E,endothelial cell ; El, elastic laminae ; methylene blue - stained Epon/Araldite section, ×40).

Fig. 8. Scanning electron micrograph of the proximal area of the freeze - dried venous allograft, one week after operation. Note marked sloughing of endothelium with exposed subendothelial layer(SE) which was covered with platelet carpet(P) and initial endothelial growth(E). R, red blood cell ; L, leukocyte(×1,000).

Fig. 9. A & B, Scanning electron micrographs of the proximal area of the freeze - dried venous allograft, three weeks after operation. A, Endothelial proliferation on the arterial surface from the area proximal to the site of suture(S). Freeze - dried venous graft is located on the right - hand side(arrow). B, Higher magnification of the surface of the graft shows the collagen network with endothelial repair(lower left side). C & D, Scanning electron micrographs of the distal area of the freeze - dried venous allograft, three weeks after operation. C, Endothelial cells growing as a continuous sheet of elongated cells over the collagen network(arrow). Left side is the recipient femoral artery and right side is the distal part of freeze - dried venous allograft. D, Note nude areas are still present and covered with amorphous materials(A, ×40 : B, ×120 : C, ×40 : D, ×150 :).

Fig. 10. Scanning electron micrographs of the freeze - dried venous allograft, four weeks after operation. A, the proximal area of the freeze - dried venous allograft, shows nearly completed

endothelization. The luminal texture of recipient femoral artery(right side) and that of graft (left side) is very different. B, Higher magnification of the anastomotic area reveals endothelial cells flowed over the nylon. C, the mid portion of the graft, shows small area of endothelial disruption. D, enlarged view of figure C. E, F, Left side is the recipient femoral artery and right side is the distal part of freeze - dried venous allograft(A, ×100 : B, ×600 : C, ×200 : D, ×730 : E, ×48 : F, ×200 :).

Fig. 11. Scanning electron micrograph of the proximal area of the non - freeze - dried venous allograft, four weeks after operation. A, Anastomotic line is completely healed. B, Partial repopulation of intima by endothelial cells, but amorphous network and underlying connective tissue layers are seen(A, ×54 : B, ×780).

Fig. 12. Scanning electron micrograph of the proximal anastomotic area of autogenous vein graft, one week after operation. A, shows suture crater and proliferating endothelium. B, View of suture line, arrow points to area of new endothelial growth(A, femoral artery : G, vein graft : S, nylon suture)(A, ×72 : B, ×260).

Fig. 13. Healing of the anastomosis of autogenous vein graft, two week after operation. A carpet of regenerating endothelium(pseudointima) has covered the sutures and micrograph demonstrates irregular arragements of endothelial sheet(×150).

Fig. 14. Scanning electron micrograph of autogenous vein graft, four weeks after operation. A, The graft is fully endothelialized. The pattern of growth of the endothelium is irregular. B, sometimes overlapping layers of cells forming small valve - like pockets(A, ×240 : B, ×200).

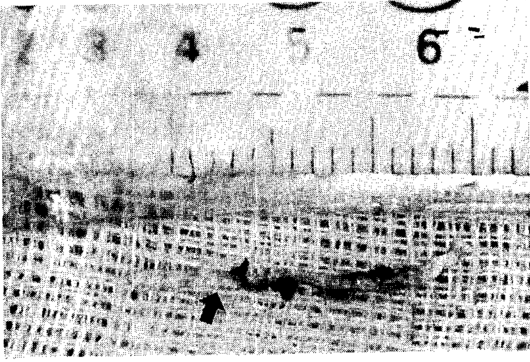

Fig. 1.

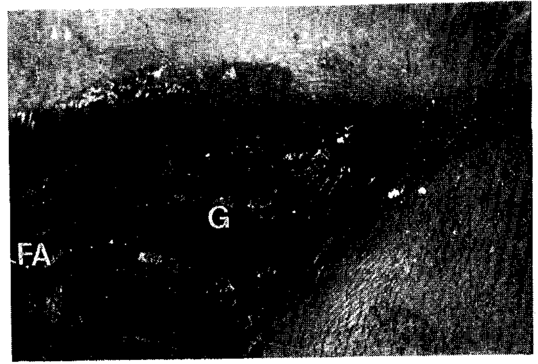

Fig. 2.

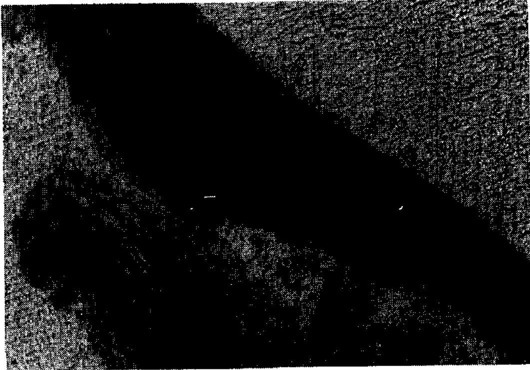
Fig. 3.

Fig. 4A.

Fig. 4B.

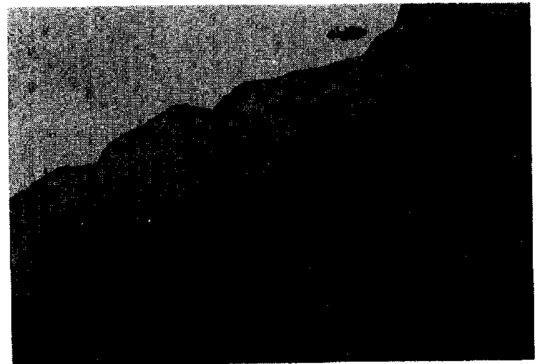
Fig. 4C.

Fig. 5A.

Fig. 5B.

Fig. 6A.

Fig. 6B.

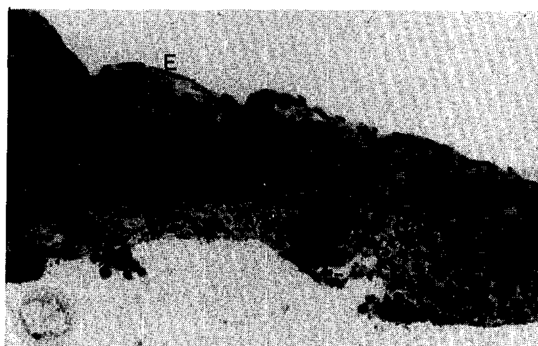

Fig. 7.

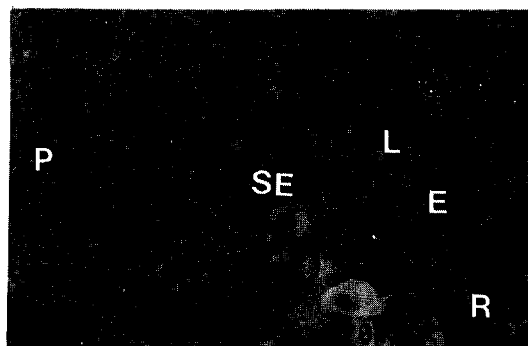

Fig. 8.

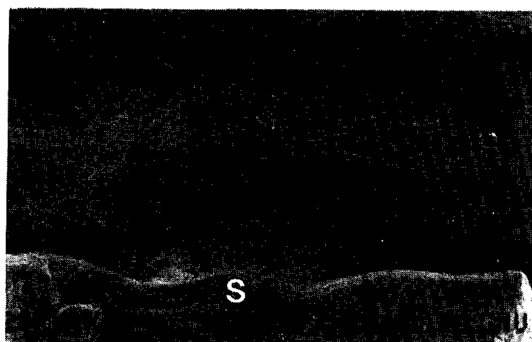

Fig. 9A.

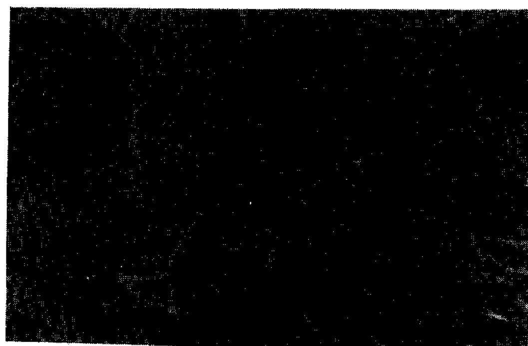

Fig. 9B.

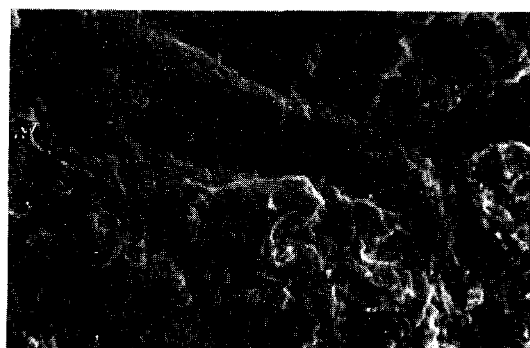

Fig. 9D.

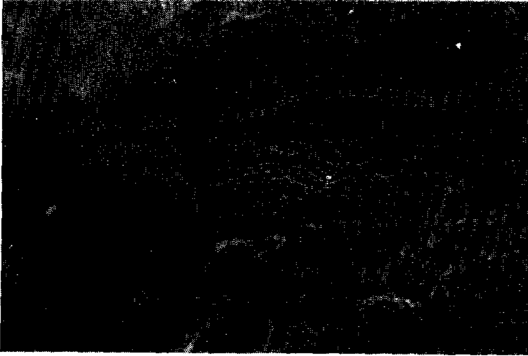
Fig. 10A.

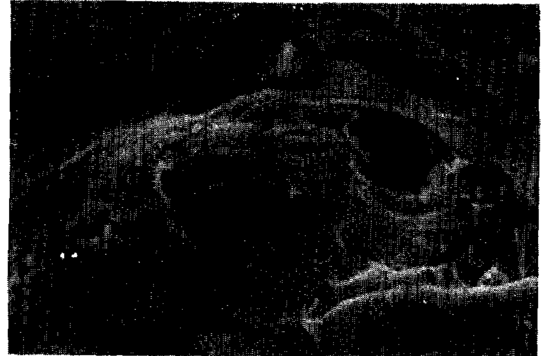
Fig. 10B.

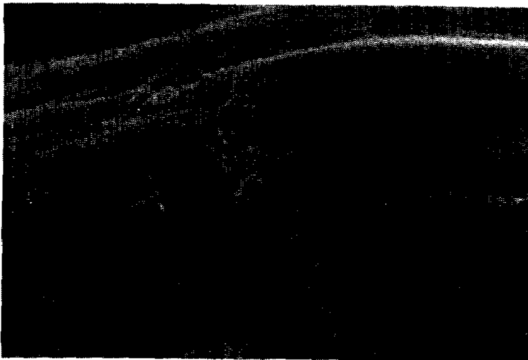
Fig. 10C.

Fig. 10D.

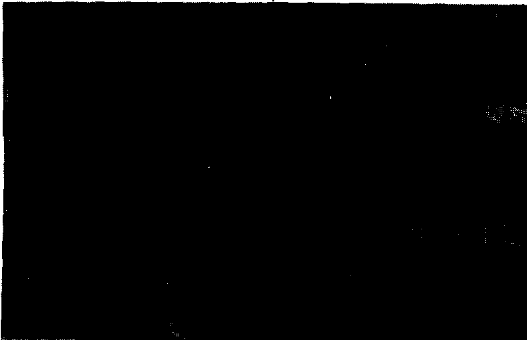
Fig. 10E.

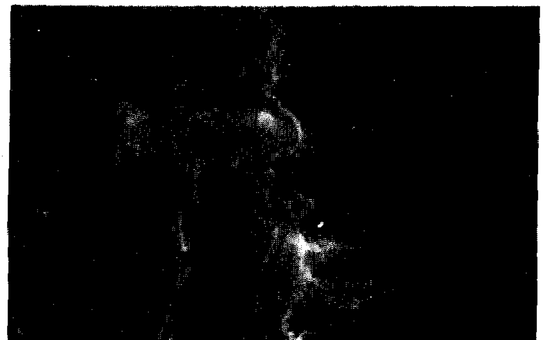
Fig. 10F.

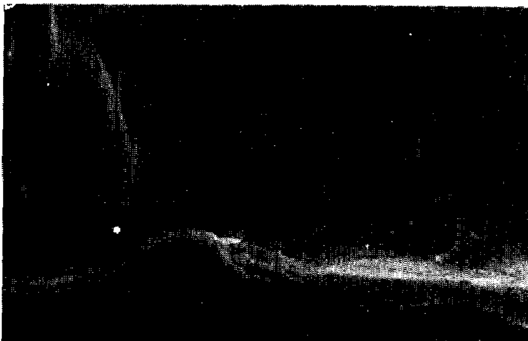
Fig. 11A.

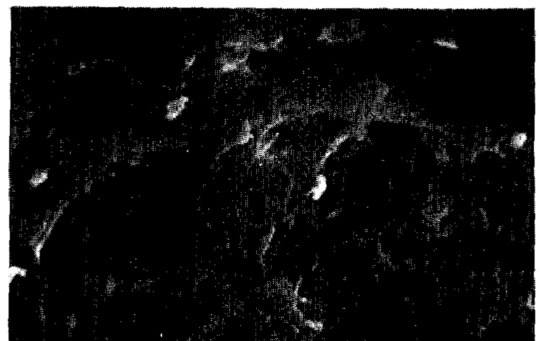
Fig. 11B.

Fig. 12A.

Fig. 12B.

Fig. 13.

Fig. 14A.

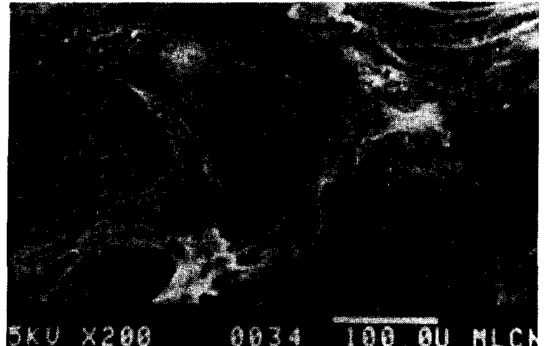

Fig. 14B.

동종 정맥 이식을 이용한 가토 대퇴동맥 결손부 수복 (동결건조 동종정맥이식의 실험적 연구)

전남대학교 치과대학 구강외과학교실

이 종 호

미세수술을 이용한 재건외과 분야에서 빈번히 혈관이식이 요구된다. 이러한 경우 자가정맥이 가장 널리 사용되고 있으며 그의 신뢰성도 인정되어 있다. 그러나 정맥 채취에 따르는 부가적인 수술이 요구되며 혈관 공여부에 또다른 결손을 초래한다. 동결건조동종정맥은 이러한 점을 보완하고 자가정맥을 대체할 수 있는 잠재성이 있다. 이에 동결건조동종정맥의 효율성을 알아보고자 2.5cm 길이의 가토대퇴정맥을 −65℃, 200 mtorr의 음압으로 동결건조시킨 다음 대퇴동맥 결손부에 동종이식하고(n=24), 신선한 가토대퇴정맥 동종이식군(n=24)과 자가정맥이식군(n=24)을 1주 간격으로 4주간 비교 관찰하였다. 2주 개존율은 동결건조동종정맥 이식군, 100%; 동종정맥이식군, 50%; 자가정맥이식군, 100%이었으며 4주 개존율은 동결건조동종정맥이식군, 83.3%; 동종정맥이식군, 50%; 자가정맥이식군, 100%로서 동결건조처치만으로 동종정맥이식의 생존율을 증가시켰다. 미세임파구세포독성검사에서는 동결건조정맥의 항원성이 상당히 낮아져 있음을 알 수 있었다. 그러나 동결건조정맥의 내막세포화가 주사전자현미경 및 광학현미경 소견상 자가정맥보다 지연됨이 관찰되었다. 이러한 결과를 종합해 볼 때 동결건조동종정맥은 아무런 처치를 하지않은 신선 동종정맥보다 현저한 장점이 있었지만, 자가정맥이식을 대신할 수 있는 보다 더 좋은 대체방법이라는 견지에서는 임상 적용이 어려울 것으로 사료되었다.