EXPERIMENTAL MICROVASCULAR ALLO
AND HETERO GRAFTS

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ABSTRACT

Experimental use of alcohol-preserved allo or hetero graft vessels to
interpose arterial or venous deficit which are about 1 mm in external diam-
eter and less than 2.5 cm long was performed. The preservation method is
simple and inexpensive and the preserved grafts could be used almost per-
manently.
The results were the same as one would expect from autografts.

INTRODUCTION

In microvascular replantation or transplantation surgery an interpositional
vascular graft is occasionally necessary due to lack of length of vascular pedicles.
In these occasions autogenous vein grafts have been the method of choice. How-
ever to obtain a proper-sized vein is often troublesome in time-consuming micro-
vascular surgery. The use of allo- or hetero-graft vessels which are preservable,
always ready to use and have clinically acceptable patency would solve these prob-
lems.

MATERIALS AND METHODS

I. Removal of Grafts (Table 1)

Albino rats and rabbits which were bred unrelated were used for the experi-
ments. Under intraperitoneal Nembutal anesthesia for rats and intravenous
Nembutal anesthesia for rabbits femoral arteries and veins, common carotid
arteries and external jugular veins were dissected free and removed atrametrical-
ly with the aid of operating microscope. The removal and implantation of the
Takao Harashina

**Table 1**

*Procedures including removal, preservation, reconstitution and implantation of graft*

<table>
<thead>
<tr>
<th>Removal of Grafts</th>
<th>Washing out the Lumens with Heparinized-Saline Solutions</th>
<th>Preservation in 70% Ethylalcohol at Room Temperature for more than 3 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstitution in Warm Saline Solutions for more than 10 minutes</td>
<td>Implantation of Grafts</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**

*Various graft materials which were tried to use and 0 indicates the materials which were proved to function well*

<table>
<thead>
<tr>
<th>Graft Material</th>
<th>Recipient vessel</th>
<th>Allograft</th>
<th>Heterograft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rat FA</td>
<td>0 rabbit FA</td>
</tr>
<tr>
<td>A</td>
<td>rat FA</td>
<td>rat FV</td>
<td>rat CA</td>
</tr>
<tr>
<td></td>
<td>rabbit FA</td>
<td>0 rabbit CA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rat FV</td>
<td>0 rabbit CA</td>
</tr>
<tr>
<td>V</td>
<td>rat FV</td>
<td>0 rabbit CA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rabbit FV</td>
<td>0 rabbit CA</td>
<td></td>
</tr>
</tbody>
</table>

Grafts were all done with non-sterile method.

II. Storage of Grafts

The lumen of the grafts were thoroughly washed out of bloods and clots with heparinized-saline solution (100 U/ml) under magnification. They were stored in 70% ethyl alcohol in sterile container at room temperature for more than three
weeks up to six months.

III. Implantation of Grafts

The grafts were reconstituted by immersion of warm saline solution for more than ten minutes before use.

The femoral arteries and veins of rats and rabbits were used as the recipient vessels. They were freed for appropriate distance from surroundings and two atraumatic vascular clamps were applied separately and the segments of vessels between the clamps were cut and discarded.

The both stumps of the grafts were refreshened with microscissors and the grafts were put into the defects and the anastomoses were done microsurgically. To repair rat's femoral arteries 11-0 monofilament nylon sutures (S & T, 7V 43) were used and 10-0 sutures (S & T, 10V 43) were preferred for other repairs. In doing anastomoses considerable technical difficulties were encountered as the grafts were much harder than fresh viable vessels.

No systemic anticoagulants nor antibiotics were used except for topical use of heparinized saline solution (100 U/ml) during anastomoses. 2% Xylocaine was used as antispasmodics after the completion of anastomoses.

Good blood flows were ascertained for twenty minutes after the restoration of circulation and skin incisions were closed and animals were returned to their cages.

A) Arterial reconstructions

To interpose rat's femoral arteries (average external diameter 0.9 mm) various materials were used as shown in Table 2. The materials except for rat's carotid arteries yielded poor results and 16 allo-grafts were done. (1.2–1.5 cm long). As heterograft materials rabbit's femoral arteries were used to interpose rat's femoral arteries. As the length of the grafts is limited to under 1.5 cm in rats, femoral arterial reconstructions were also done in rabbits with rabbits' carotid arteries (2.5 cm long).

B) Venous Reconstructions

To replace rat's femoral veins (average external diameter 1.2 mm) various materials were used as shown in Table 2. The veins proved to be poor materials because of excessive shrinkage during storage and rabbits' carotid arteries were used. (2.5 cm long).
C) Amputations and reimplantations of rat’s hind legs using allo- and hetero-grafts to interpose their feeding vessels

Complete mid-thigh amputations and reimplantations were done on seven rats. The implantation was begun by fixing the femur with intramedular K-wire and all the thigh muscles were grossly approximated with 4-0 catgut sutures. Shortening of the bone was not necessary on this occasion because of the use of interpositional vascular graft. Vascular continuities were restored using either hetero-graft alone or both allo- and hetero-grafts. Good blood flow was observed for twenty minutes after the restoration of circulation. No systemic anticoagu-lants nor antibiotics were used postoperatively.

RESULTS

There were few surgical deaths which usually occurred within 24 hours after surgery and they were excluded from the record. Most of the grafts were re-explored and inspected on the following day of the surgery. The final assessment of the graft patencies were done at three months in arterial reconstructions and three weeks in venous reconstructions.

The grafts were observed under magnification and dissected free from surroundings and removed for pathological studies.

<table>
<thead>
<tr>
<th>Graft Material</th>
<th>Patency</th>
</tr>
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<tbody>
<tr>
<td>rat CA</td>
<td>$\frac{1}{2} = 88%$</td>
</tr>
<tr>
<td>rabbit FA</td>
<td>$\frac{9}{17} = 55%$</td>
</tr>
<tr>
<td>rabbit CA</td>
<td>$\frac{5}{8} = 67%$</td>
</tr>
<tr>
<td>rabbit CA</td>
<td>$\frac{7}{17} = 100%$</td>
</tr>
<tr>
<td>rabbit CA</td>
<td>$\frac{5}{3} = 100%$</td>
</tr>
</tbody>
</table>

*A) Arterial reconstructions*

On the following day of the implantations both allo- and heterografts looked
Experimental microvascular allo and hetero grafts

almost indistinguishable from adjacent normal arteries. There were minimal inflammatory reactions.

At three months allografts did not develop thick scar formations and no difficulties were encountered due to adhesions in dissecting them out. The grafts were removed and the lumens were inspected by splitting them longitudinally. The luminal surfaces were always covered with smooth, glistening newly formed intima (Fig. 2), but these intimas were very easily peeled off with minimal mechanical trauma.

Out of 16 allografts in rats 14 mere found to be patent (144, 88%). Pathological findings reveal that the internal surface of the grafted rat artery was covered by the several layer of intimal cells. The inner surface was covered by continuous flattened endothelial cells.

Elastic fibers of the media were stacked and no smooth muscle cells was recognized in between. Thin adventitia was also re-covered without any inflammatory reaction except for a small amount of lymphocytic infiltration. (Fig. 3)

Allografts in rabbits showed one occlusion out of 3 but this one failure was apparently technical one.

Heterografts developed thick, dense scar tissues around them and those grafts which were patent at the time of inspection inevitably underwent universal dilatations and/or aneurysmal formations. (Fig. 4)

The aneurysms measured 0.5 cm to 1.5 cm in diameter. The adhesions to the surroundings were so thick that it was very difficult to dissect them out. 11 hetero grafts were done and the first 5 were found to be occluded and the last 6 were consecutively patent though they always developed aneurysm. The probable reason for the failure of the first 5 is that the implantation of heterograft were technically difficult due to their discrepancy in external diameter and wall thickness.

Histologically an aneurysmal formation was common in this group, followed to the disruption of the elastic bundles. These aneurysms were usually filled with thrombus and the wall was fibrous tissue but elastic bundles. The intima adjacent to the aneurysm was markedly thickened with frequent calcium deposition as well as cholesterin crystals. (Fig. 5)

B) Venous reconstructions

Both allografts in rabbits and heterografts in rats did not show severe inflammatory reactions either on the following day or three weeks after surgery and the adhesions were minimal. The grafts looked white compared with normal adjacent veins. Inspection of the internal surfaces revealed smooth shining new
intimas which were also very fragile. (Fig. 7)

7 heterografts were done in rats and 3 allografts in rabbits and all were patent.

Histologically recovery of the intima was similar to those of the allograft arteries, but the thickness was half to two third, possibly in accordance with low venous pressure. A narrow edematous slit separated the intima and elastic media. A characteristic finding in this group was a strong inflammatory reaction against the elastic layer. An infiltration of lymphocytes and plasma cells from the adventitia was prominent and they separated the each elastic fibers. Fibroblasts were also present but the capillary penetration was a rare finding. Not infrequently a disruption of the elastic bundles was present and replaced by a fibrous and/or granulation tissue throughout the whole layer. Newly formed fine elastic fibers were complementary produced in the intima in such lesion. (Fig. 8)

C) Amputations and reimplantations of rats’ hind legs using allo- and heterografts to interpose their feeding vessels

Seven replantations were done and five completely survived and the grafts, both arterial and venous, were all patent at the time of reexploration ranging from 7 days to 3 months.

These legs which failed showed severe cyanosis and congestion on the following day of the replantation and the reexploration revealed still functioning arterial grafts but occluded venous grafts. One graft showed thrombotic occlusion probably due to remaining clots in the lumen and the other thrombosis was caused secondarily by kinking of normal adjacent vein.

DISCUSSION

Vascular replacement is one of the most important and interesting problem in vascular surgery. Clinically synthetic materials such as Dacron and Teflon have been used for medium- or large-sized arteries and autogenous vein grafts have been the method of choice for arteries smaller than popliteal artery. It is a well known fact that synthetic grafts, of which internal diameter is less than 5 mm, are not available for clinical use. Venous reconstructions are limited to only large veins such as Vena cava since there exists little clinical demand for them and results are usually not satisfactory.

Fresh or preserved\textsuperscript{12,9} allo- or hetero-grafts of vessels appeared before floodlights in the early ninety fifties and even blood vessel banks were founded but they were soon abandoned due to their fatal complications\textsuperscript{1,7,15} such as aneurysmal formation or rupture and now present only historical interest. Experimentally
Experimental microvascular allo and hetero grafts

cutaneous allo- or hetero-grafts were successfully used to replace as small vessels as dog's femoral arteries and it was considered that these techniques could be extended to the field of microvascular surgery.

Interpositional vascular grafts in microvascular surgery should preferably preservable, always ready to use and the preservation technique must be simple, easy and inexpensive. Needless to say grafts must possess antithrombogenic property and no or little antigenicity. Flexibility which is required for medium- or large-sized grafts in clinical use is not necessary.

**Graft Material**

As autograft materials in clinical purpose veins are used to replace arterial defects because proper sized arterial grafts could not be obtained as donor vessels. In the field of microvascular surgery both arteries and veins are used experimentally but only veins are used clinically because of their easier availabilities.

In this study both preserved arteries and veins were used and it was found that only arteries yielded satisfactory results for either arterial or venous replacement. The probable reason is that during storage veins excessively shrink and almost lose their lumens and do not fully regain them even after restoration of flows especially in low-pressured venous system. On the contrary preserved arteries always maintain their lumens because of less shrinkage and function well even for venous system. It was also found that the donor vessels which were considerably larger than the recipient vessels in external diameter yielded good results.

**Preservation Method**

Vascular preservation method consists of vital and non-vital techniques. Non-vital method include frozen, dried-frozen, glutaraldehyde, alcohol, formalin preservation and so on. Among these alcohol preservation may meet above described criteria as only 70% ethyl alcohol and sterile container are necessary for the storage and sterile techniques are not necessary when grafts are obtained.

The use of alcohol for vascular preservation was first described by Paolucci in 1950 and later extensively studied by Kimoto and his associates and a few clinical cases were reported by them. They conclude that alcohol, through its action of dissolution of lipoproteins and denaturization of other proteins, might render the material to be grafted serologically inert. They also found that the structures except for elastic fibers of media in alcohol treated arteries would be replaced with host tissues by three months and they work only as structural frameworks.
Preservation Period

Kimoto et al. recommended more than two weeks of preservation period and two weeks may be adequate for grafts to become serologically inert.

It seems that the maximum period that grafts could be safely used is almost permanent as successful interpositions were done by Kimoto et al. with one year old grafts and by present author with six months old micrografts.

Graft Patency

In microsurgical composite tissue transfers permanent patency of feeding vessels may not be necessary. So in this study graft patencies were assessed at three months in arteries and three weeks in veins. From the patencies obtained in present study it may be concluded that alcohol-preserved small vessel grafts are almost completely antithrombogenic. There were few grafts which showed thrombotic occlusion shortly after the restoration of flows and exploration of these grafts revealed that the remaining clots in the lumens were responsible for thrombus formation. So the donor vessels must be with no or as little as possible tributaries and the vascular lumens must be thoroughly washed out of their contents. Common carotid arteries are preferable as donor vessels since they have no branches and external diameters are not different either proximally or distally.

Moore17 et al., in their experimental interpositions to replace dog’s carotid and femoral arteries with alcohol preserved grafts, obtained 55% overall patency. The external diameters of those vessels range from 3 to 4 mm but their anastomoses were not performed microsurgically. So it may be purely technical which decides patency rate.

Length of Grafts

As far as the relationship between length of grafts and patency are concerned, there was no reduction of patency rate in 2.5 cm long grafts in rabbits compared with 1.5 cm long grafts in rats. Some8,16 say that there is no correlation between length of grafts and patency in larger arterial interpositions but it seems quite reasonable that the longer the grafts the more thrombus formation especially in low-pressured venous system.

How long graft could be successfully used in the field of microvascular surgery is still open to question.

Antigenicity

Many people referred6,14,20,21,25 to antigenicity of allo- or hetero-graft vessels, either fresh or preserved, and some3,26,27 tried to alter rejection phenomena with immunosuppressive drugs, always in vain. As far as alcohol-treated grafts are
concerned Takahashi\textsuperscript{24} experimentally showed the elevation of humoral titer after implantation of pig's abdominal aorta to dog.

It may be concluded that alcohol preservation can suppress early reaction to certain degrees but can not completely extinguish late rejection phenomena from the fact that early patency are excellent with either allo- or hetero-grafts but hetero-grafts always undergo degenerative changes leading to aneurysmal formation within three months.

\textit{Aneurysm}

The vascular allografts in\textsuperscript{1,3,23} clinical use were abandoned due to their fatal complications, aneurysmal formation and rupture. Usually it took more than few years for the grafts to become aneurysmal. In the field of smaller arterial replacement no aneurysmal formations were reported. Moore\textsuperscript{17} \textit{et al.} in their experiments to replace dog's femoral or carotid arteries with alcohol-preserved allo-grafts, found no aneurysmal dilatation within one years placement. In present study arterial grafts were left in place for three months and allografts never showed aneurysmal dilatation but hetero-grafts inevitably underwent degenerative changes, universal dilatation and/or aneurysmal formation.

Aneurysmal formation of feeding arteries may not produce a serious problem in free flap transfers as the tumors will be covered with soft, fatty free flaps and they could be easily excised without endangering flap viabilities when necessary. But it will be a big problem in finger replantations.

\textit{Infection}

According to Kimoto\textsuperscript{11} \textit{et al.}, since these preserved allo- or hetero-grafts are foreign bodies for the hosts, presence of infection is a strong reason to contraindicate their uses. In this experiment all procedures such as removals of grafts and implantations of grafts were done with non-sterile method and no antibiotics were used and still infection was not a problem in any of the cases.

\textbf{SUMMARY}

Experimental use of alcohol-preserved allo- or hetero-graft vessels to interpose arteries or veins which are less than 2.5 cm long was presented and this is the first successful report of using non-viable vascular grafts in the field of microvascular surgery.

The preservation method is simple and inexpensive and the preserved grafts could be used almost permanently. For graft materials arteries are preferrable rather than veins for either arterial or venous reconstructions because of their
less shrinkages during storage.

To obtain good patency donor vessels should be larger than host vessels in external diameter and sound microsurgical techniques are of prime importance.

The results showed that these alcohol-treated vessels possessed almost perfect antithrombogenic property. The graft patencies were so excellent as to justify their future clinical uses except for heterografts which were used to interpose arterial defects and these heterografts inevitably underwent degenerative changes leading to aneurysmal formation within three months.

ACKNOWLEDGEMENT

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REFERENCE

13. Marrangoni, A. G. and Cecchini, L. P.: Homotransplantation of arterial seg-


Fig. 1 The allograft which was implanted in the arterial defect three months previously. Preserved rat’s carotid artery to rat’s femoral artery. The graft looks almost indistinguishable from adjacent normal recipient arteries. There are slight inflammatory signs and adhesions to surroundings are minimal.

Fig. 2 The internal surface of the graft shown in fig. 1. The lumen is covered with smooth glistening newly formed intimas but these neo-intimas are very easily peeled off with mechanical trauma.

Fig. 3 Pathological finding of the graft shown in fig. 1 and 2. H-E stain 200×.

Fig. 4 The aneurysm which was developed from the heterograft three months after implantation. Preserved rabbit’s femoral artery to rat’s femoral artery. There are thick scar formations and adhesions around the graft.
Fig. 5 Pathological finding of the aneurysm shown in fig. 4. H-E stain 100×.

Fig. 6 The heterograft which was implanted in the venous defect three weeks previously. Preserved rabbit's carotid artery to rat's femoral vein. The graft looks white compared with adjacent normal recipient veins. Scar formations of surrounding tissues are not so severe and adhesions are also mild.

Fig. 7 The internal surface of the graft shown in fig. 6. The lumen are all covered with smooth, shining and fragile neo-intimas.

Fig. 8 Pathological finding of the graft shown in fig. 6, and 7. H-E stain 200×.