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Experimental microarterial grafts: freeze-dried heterografts versus autografts

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Summary—Segments of the femoral artery from New Zealand white rabbits were removed and freeze-dried. They were then used as a graft for a defect in the femoral artery of a Sprague-Dawley rat. The femoral artery on the other side of this recipient rat had a similar grafting procedure using a segment of its own femoral artery, thus acting as a control. The patency rate of the freeze-dried heterografts was comparable to the autografts, but the former showed a high incidence of aneurysm formation as a result of immune rejection as supported by histopathological and scanning electron microscopy studies.

In clinical microvascular surgery, grafting of blood vessels is sometimes necessary (Alpert *et al.*, 1978; Gould *et al.*, 1979). Although the reliability of autologous microvascular grafts has been established (O'Brien *et al.*, 1979a and b; Das *et al.*, 1980), there has been a continuous search for substitutes such as preserved allografts and synthetic grafts. Synthetic grafts have been disappointing (Caffee, 1980), but the use of freeze-dried (Moore *et al.*, 1956) and alcohol preserved grafts (Harashina, 1978) has met with some success. Recently, we published our findings with freeze-dried microarterial allografts (Chow *et al.*, 1983) and found them reliable. We performed a second group of experiments using human placental vessels as heterografts (Chow *et al.*, 1985) but met with a high failure rate and evidence of immune reaction. To continue the search, we performed experiments using freeze-dried rabbit arteries as heterografts. It is the purpose of this paper to show that such grafts present potential problems despite a good patency rate.

Materials and methods

The femoral arteries of New Zealand white rabbits were exposed by longitudinal incisions bilaterally. The distal part of the arteries were chosen to obtain a smaller calibre to match that of the femoral artery in the Sprague-Dawley rat. Usually four segments, each about 8 mm long and 1–1.3 mm wide, could be harvested from each side. They were then irrigated with heparin-saline and immediately freeze-dried at −70°C. The grafts were stored in sealed vacuum bottles at room temperature, ready to be transplanted into rat femoral arteries as heterografts. Storage time of the grafts ranged from 42–364 days (average = 200 days). One hour before grafting the heterograft was rehydrated in physiological saline solution.

All anastomoses were performed by one surgeon using 10/0 nylon (Ethicon 2870) with the Carl Zeiss OPMI-7D microscope. About 10–12 interrupted sutures were used for each anastomosis. Patency both in the antegrade and retrograde direction was tested immediately, at 1 hour, 7 to 14 days and 1 to 3 months after operation. At different intervals after surgery the femoral arteries were removed for histopathological studies and examination under the scanning electron microscope. Altogether, 20 grafting procedures were performed on one leg of the rats, to be compared to a concomitant series of autografts performed on the contralateral leg of the same rat.

The results have been subjected to statistical analysis with the standard t-test using 0.05 significance level. Using this test, all critical values (t) falling outside the bracket between −1.96 and +1.96 were considered significant.

Results

From Table 1 we can see that the two groups of grafts achieved the same patency, *i.e.* 100% up to 2 weeks. However, one heterograft was lost at 3 weeks; all the other vessels remained patent until 3 months, giving a patency rate of 100% in the autograft and 94.1% in the heterograft. There was no

Table 1 Patency rates of freeze-dried heterografts and autografts at different times after operation

	Immediate	*1 hour*	*7–14 days*	*1–3 months*
Freeze-dried heterografts	100% (20/20)	100% (20/20)	100% (18/18)	94.1% (16/17)
Autografts	100% (20/20)	100% (20/20)	100% (18/18)	100% (17/17)

Table 2 Incidence of aneurysm formation at different times after operation

	7–14 days	*1–3 months*
Freeze-dried heterografts	22.2% (4/18)	47.1% (8/17)
Autografts	0% (0/18)	0% (0/17)

significant difference in the patency rates of the two groups ($t = -0.02985$) although the heterograft group had a significantly greater incidence of aneurysm formation, *i.e.* 47.1% at final exploration ($t = -2.47339$) (Table 2).

Scanning electron microscopy studies

In the autograft group, normal re-endothelialisation occurred, and was completed in about 3 months.

In the freeze-dried heterograft group, two phases of change could be identified. Initially the flow surface was covered with a fibrin layer with occasional entrapped red cells (Fig. 1). Thrombotic phenomenon was common. In a later stage, there was re-endothelialisation from both ends of the graft (Fig. 2) and the final appearance was similar to that of the autograft group.

Histopathological studies under light microscopy

The autografts showed the usual phenomenon of

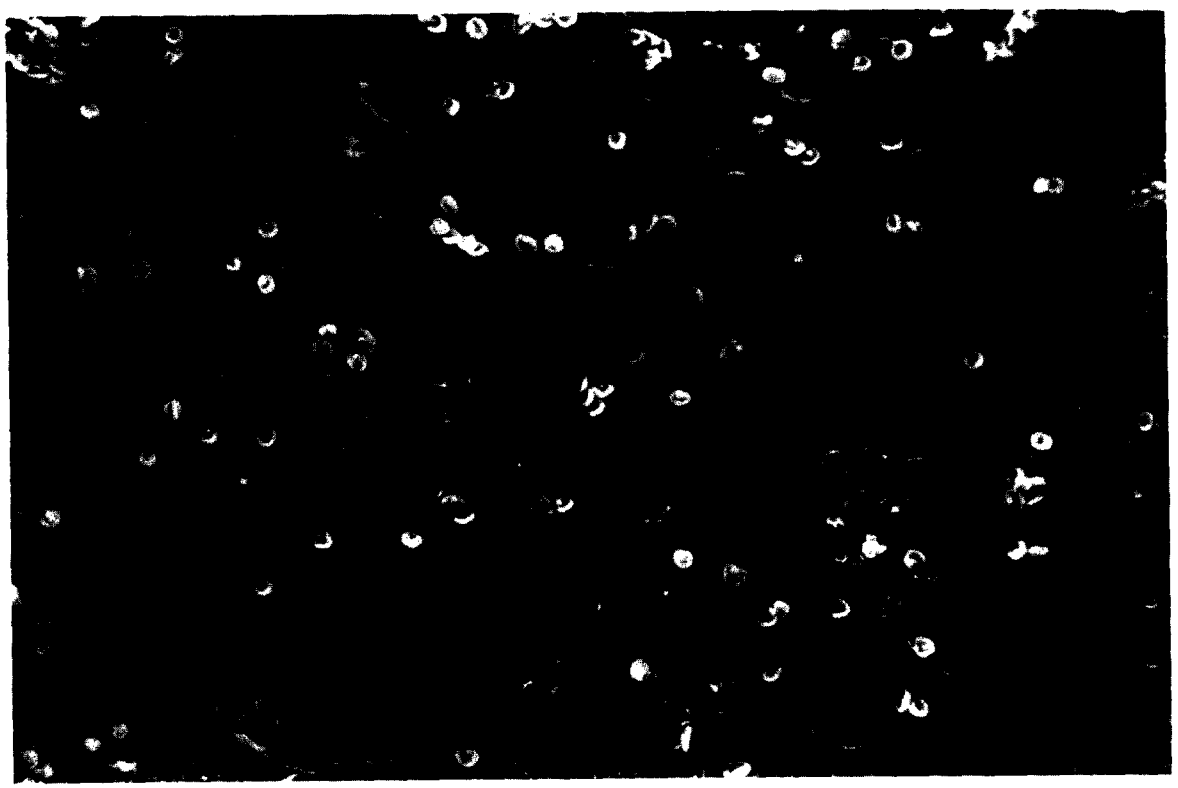

Fig. 1

Figure 1 –Scanning electron microscopic appearance of a 2-week-old heterograft showing the fibrin-lining with entrapped red cells.

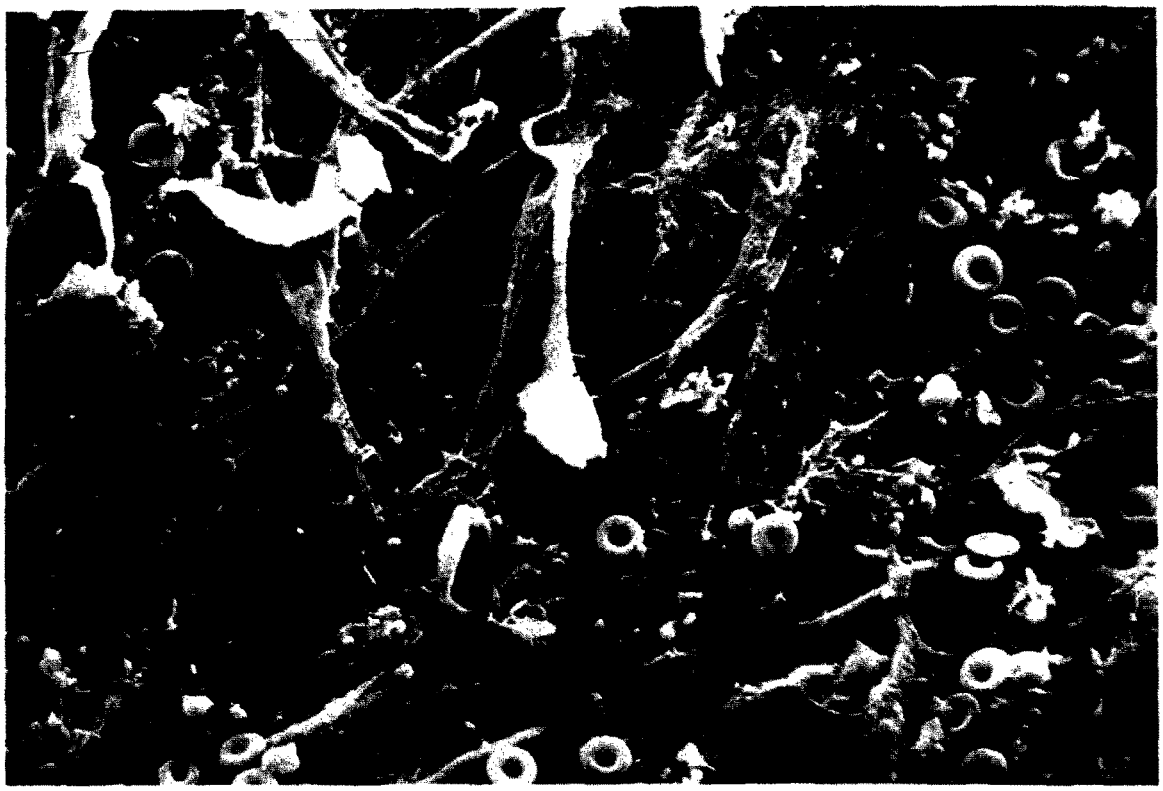

Fig. 2

Figure 2—SEM appearance of a 3-month-old heterograft showing active endothelial cells covering the flow surface.

intimal hyperplasia and reaction around the sutures only.

In contrast, the freeze-dried heterografts showed two phases of change corresponding to the findings in SEM studies. Early phase: a stage of destruction in which there was fibrinoid necrosis of the vessel wall, fragmentation of elastic laminae and mixed cellular infiltration consisting of polymorphs, mononuclear cells and eosinophils. On the surface, the endothelium was destroyed (Fig. 3). All these features were compatible with acute immune rejection.

Late phase: a stage of re-organisation in which the two elastic laminae and smooth muscle fibres were completely replaced by collagen tissue. The flow-surface finally became covered with endothelial cells, correlating well with the SEM findings.

Discussion

A review of the literature suggests that little work has been done on preserved biological microvascular grafts. Moore *et al.* (1956) were perhaps the first to use freeze-dried or alcohol-preserved carotid arteries in dogs. Despite a relatively big vessel calibre, 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 1978, Harashina reported his use of alcohol-preserved microvascular grafts. His allografts and heterografts met with 87.5% and 54.5% success rates respectively, but he failed to mention the size of his grafts nor could he demonstrate the biological stability of his grafts histologically.

For these reasons we launched a series of experiments on preserved microvascular grafts. We showed (Chow *et al.*, 1983) that 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. To continue the search, we began to experiment on freeze-dried heterografts, starting off with human placental vessels (Chow *et al.*,

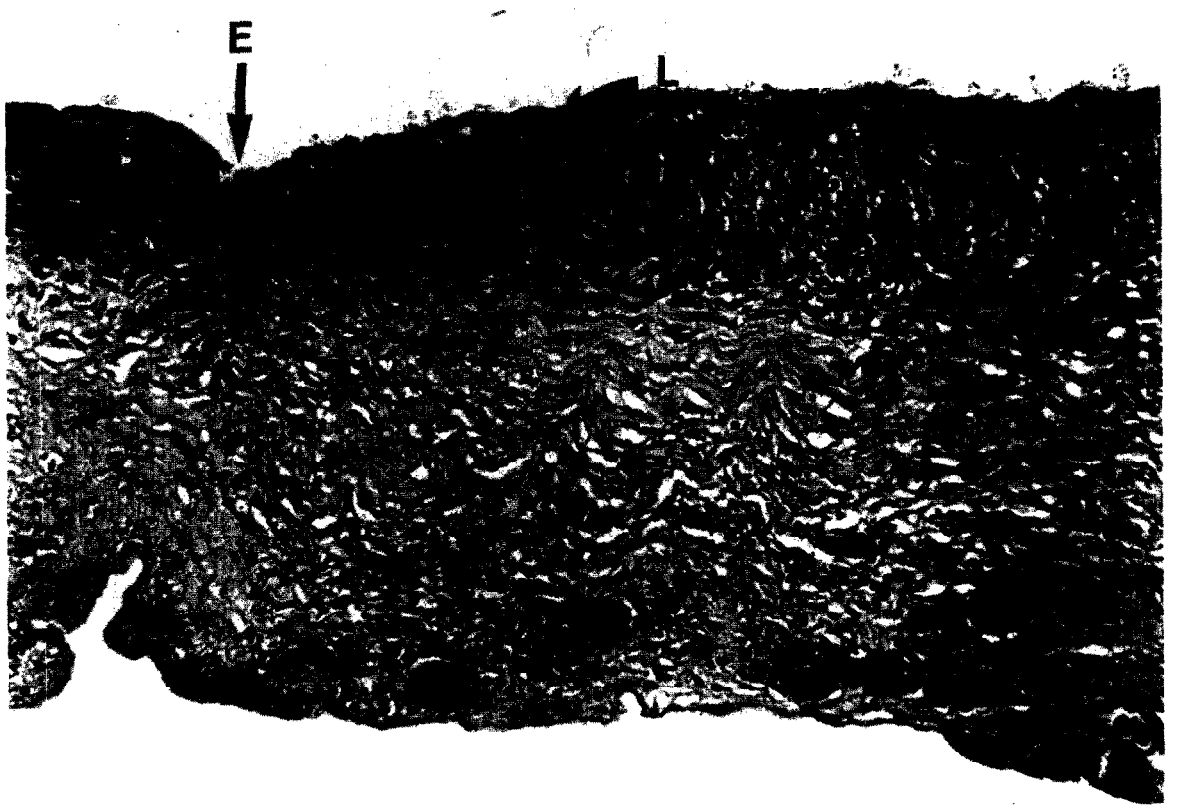

Fig. 3

Figure 3—Low-power light microscopic appearance of a two-week-old heterograft showing mixed cellular infiltration, fragmentation of elastic laminae (arrows L), and destroyed endothelium (arrow E).

1985) on the assumption that, since they are of foetal nature, they might have low antigenicity and thus a higher chance of success. The results, however, were very disappointing: a 55% patency rate at 3 months and florid histological evidence of immune reaction.

The second phase of experimentation on freeze-dried heterografts constitutes the present study. By transplanting vessels to more closely related species (rabbit-to-rat as opposed to human-to-rabbit in the phase one study), we hoped that better results could be obtained. Indeed the patency rate was very satisfactory (94.1% at 3 months) but, despite this, it is evident that the freeze-drying process cannot prevent immune reaction to the heterografts. The destruction of the vessel wall led to loss of elasticity and the ability to withstand stretching, hence the high incidence of aneurysm formation. Aneurysm predisposes to thrombus formation and there is a risk of delayed blockage of the graft which was exactly what happened in our failed case (Fig. 4). Furthermore, the presence of aneurysms in superficial vessels is unacceptable in clinical practice. From this observation, it would appear that the freeze-dried heterografts have doubtful clinical application in the light of better alternatives. Perhaps a combined synthetic and biological substitute will be the answer.

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Fig. 4

Figure 4—Gross appearance (3 ×) of a 4-month-old heterograft showing aneurysm formation (big arrow) and graft blockage proximal to it (small arrows). A = artery; V = vein; AN = anastomotic sites.

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