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Healing of microvenous PTFE prostheses implanted into the rat femoral vein

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SUMMARY. **44 PTFE prostheses (Gore-Tex®; ID 1 mm) were implanted into rats' femoral veins by means of the sleeve anastomotic technique and were evaluated at regular intervals from 1 h up till 24 weeks after implantation by means of light and electron microscopy to study in detail their healing process.**

All prostheses, except one at 1 week and one at 24 weeks after implantation, were patent at the time of removal. Upon implantation, the luminal surface of the prostheses became covered with a thin clot layer. From 1 week onwards, endothelial cells originating from the anastomotic sides grew in across the anastomoses. In addition, small capillary-like orifices were present at the anastomotic sites, from which endothelial cells also seemed to originate. At 2 weeks, in several areas in the mid-region of the prostheses, the fronts of regenerating endothelial cells had reached each other, and about 80% of the luminal surface was covered by endothelium and at 3 weeks, the prostheses were completely covered by an endothelial layer.

These results demonstrate that PTFE microvenous prostheses heal exclusively by means of rapid ingrowth of endothelial cells originating from both sides at the anastomoses.

In clinical microsurgery, a readily available synthetic microvascular prosthesis of various sizes that can be used successfully would be very useful. Thus far, several experimental studies have demonstrated the value of polytetra-fluoroethylene (PTFE) prostheses as a microarterial conduit (Caffee, 1980; Tizian, 1981; Ganske *et al.*, 1982; O'Brien *et al.*, 1985; van der Lei and Wildevuur, 1988, 1989). PTFE prostheses when used as a microvenous conduit have rarely been successful, due to thrombotic occlusion (Parsa and Spira, 1979; Cuadros and Hughes, 1986; Shen *et al.*, 1988).

The study of microvenous PTFE prostheses has mainly focused on patency rates and implantation techniques (Parsa and Spira, 1979; Cuadros and Hughes, 1986). We recently found evidence that the sleeve anastomotic technique (Fig. 1A) for implanting microvenous prostheses leads to significantly less

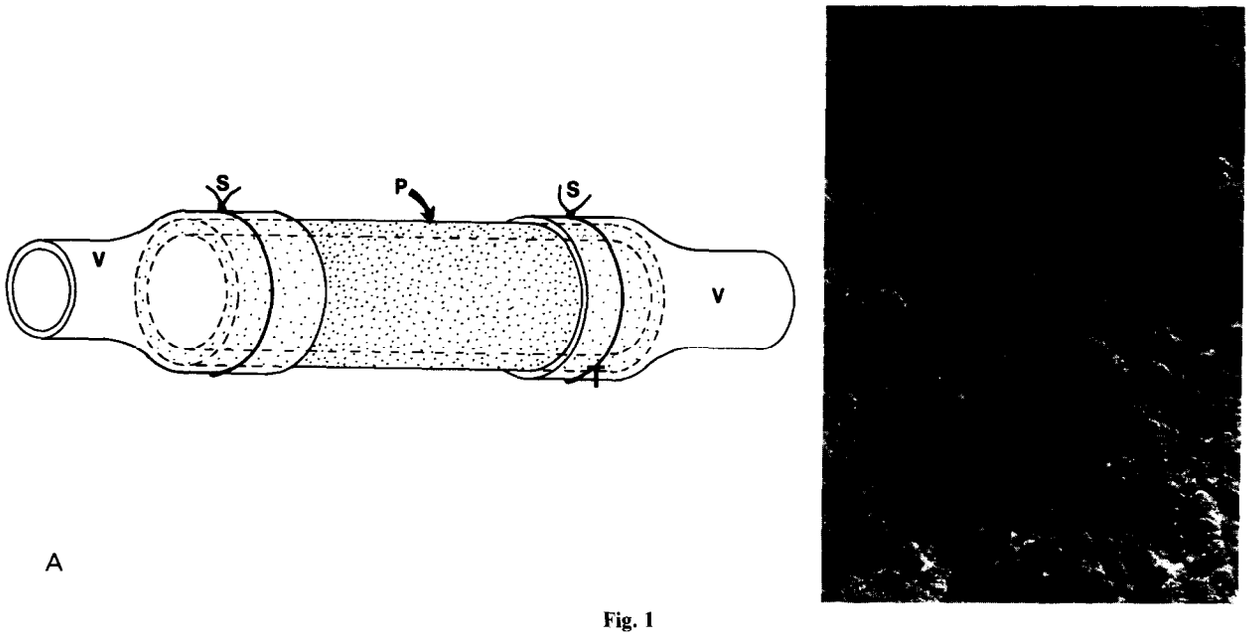

Fig. 1

Figure 1—(A) Schematic drawing of the sleeve anastomotic technique for implanting a PTFE prosthesis (P) into the rat femoral vein (V). (B) A scanning electron micrograph (SEM) of the graft surface of a PTFE prosthesis 1 day after implantation in the rat femoral vein. Note the deposition of thrombus-like material on the graft surface. S suture. (Magnification B 800 ×).
(A) from Robinson *et al.*, 1990, J. Reconstr. Microsurg. 6: 287–292, reprinted with permission).

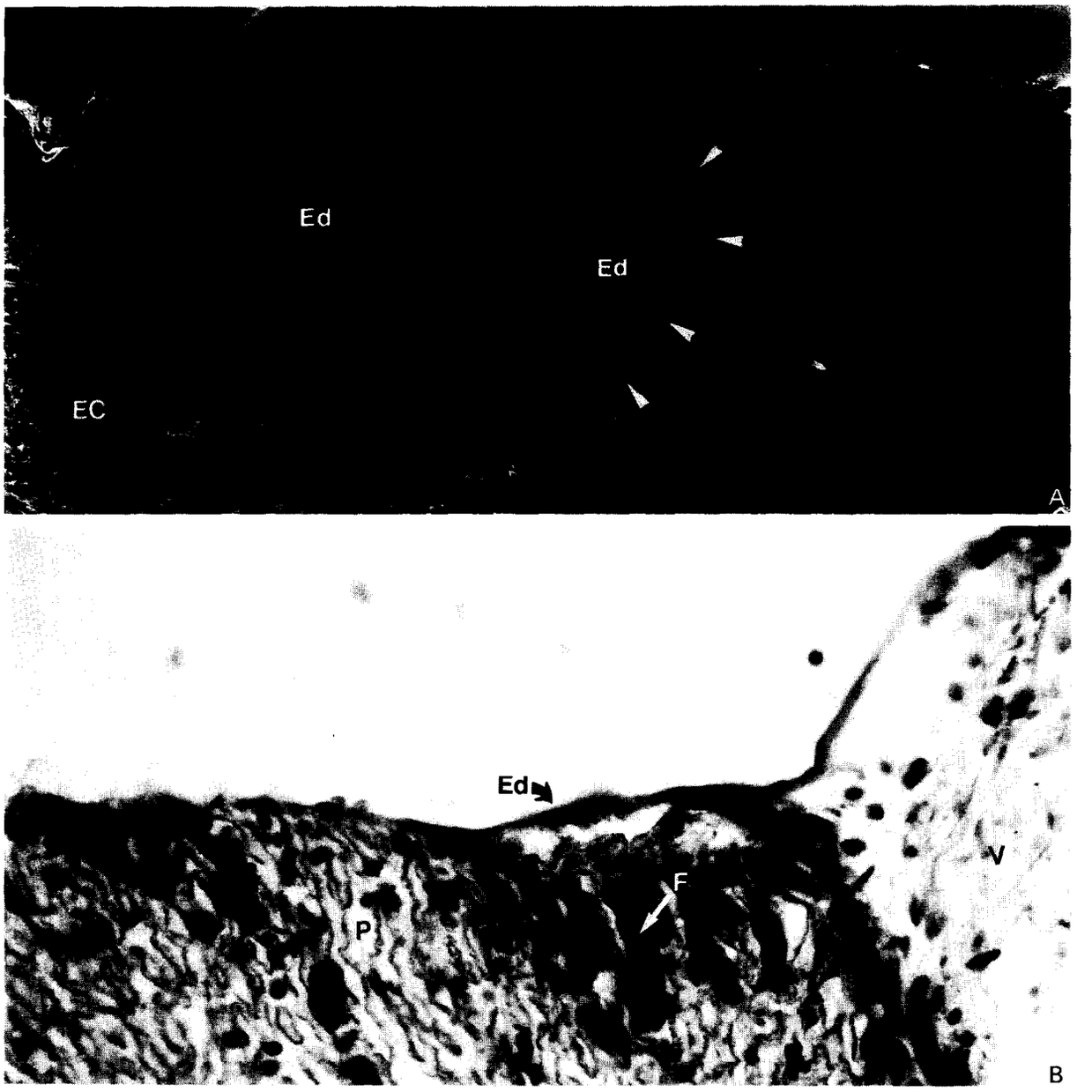

Fig. 2

Figure 2—(A) SEM of proximal anastomotic region at 1 week. Endothelial cells (Ed) grow in as a continuous sheet over the graft surface (arrows mark front of endothelial cells). Note the presence of an endothelial channel (EC) from which endothelial cells seem to originate. (B) Light micrograph of distal anastomotic region at 1 week. Note the smooth conjunction from vein (V) to prosthesis (P) and the presence of some fibroblast-like cells (F) in the interstices of the graft wall. Arrow marks ingrowing endothelial cell (Ed). (Magnification A 220 × ; B 350 ×).

"surgical iatrogenic" damage of the anastomotic areas and subsequently results in higher patency rates (Robinson *et al.*, 1989, 1990; van der Lei *et al.*, 1991).

Since PTFE material potentially can be applied clinically (Dale *et al.*, 1984; van der Lei *et al.*, 1989), it was the aim of this study to evaluate the sequential healing mechanisms of microvenous PTFE prostheses when implanted by means of the sleeve anastomotic technique; evaluation of the dynamics of this healing process could contribute to further development and application of PTFE microvenous prostheses.

Materials and methods

Male Wistar rats (n = 22) weighing 250 to 350 g were premedicated with atropine (0.25 mg kg^{-1} body weight, administered intramuscularly) and anaesthetised with 1 % halothane. After disinfecting the groins with 10 % povidone-iodine solution, the femoral triangles were incised along the inguinal folds and using microsurgical techniques, both femoral veins were carefully dissected free. A PTFE prosthesis (Gore-Tex®, length 5–7 mm, internal diameter 1 mm, fibril length 30 μm, wall thickness 0.2 mm; n = 44) was

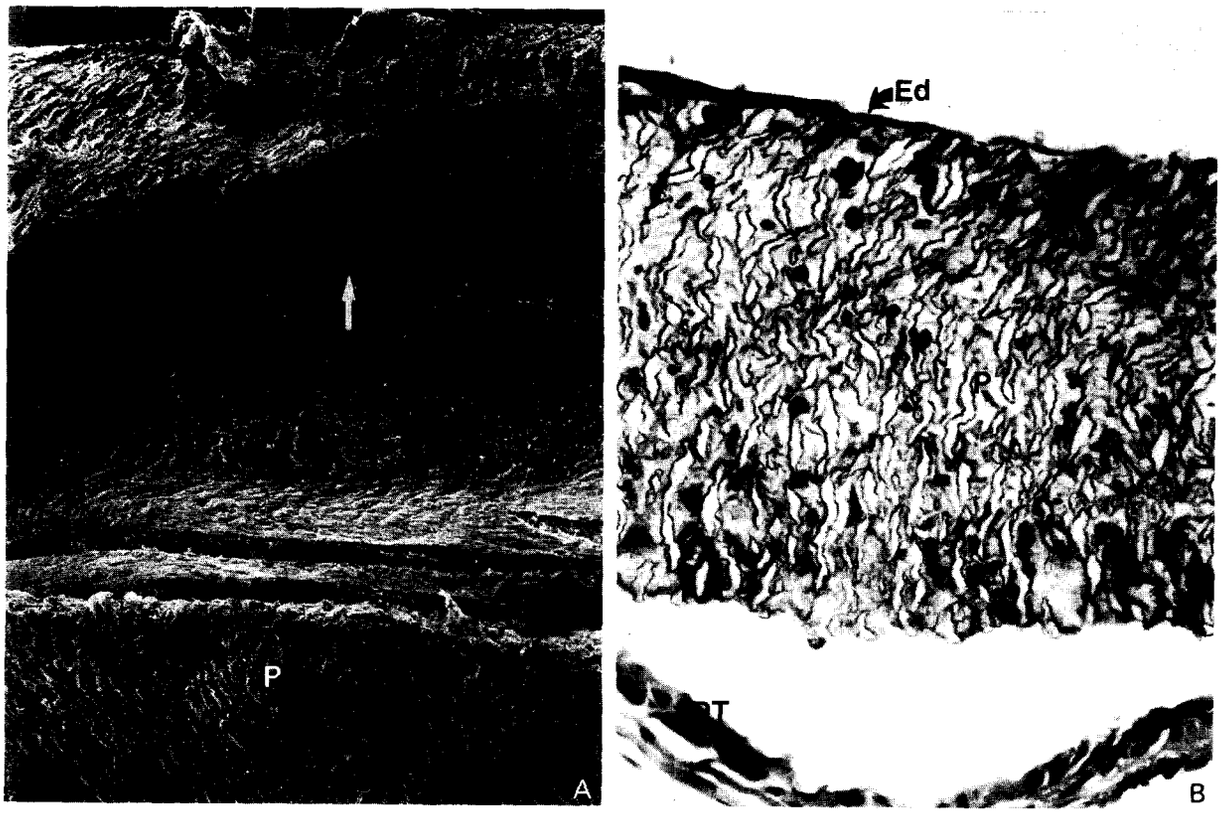

Fig. 3

Figure 3—SEM (A) and light micrograph (B) of the mid-portion of a PTFE prosthesis (P) after 2 weeks. The proximal and distal fronts of endothelial cells have reached each other (arrow). Note that the endothelial cells (Ed) lie directly on the graft surface, and the scarce ingrowth and attachment of perivascular tissue (PT) into the wall of the prosthesis (P). (Magnification A 100 × ; B 350 ×).

implanted in each femoral vein by means of the sleeve technique, as has been described in detail elsewhere (Robinson *et al.*, 1989; see Fig. 1A). After implantation, 0.2–0.5 ml 2% lidocaine was topically applied to relieve spasm and the clamps were removed. No anticoagulants or antiplatelet agents were used.

The prostheses were inspected and harvested after 1 day (n = 4), 3 days (n = 4), 1 week (n = 6), 2 weeks (n = 6), 3 weeks (n = 6), 6 weeks (n = 6), 12 weeks (n = 6), and 24 weeks (n = 6).

Graft patency was determined by direct macroscopic inspection. The prostheses were removed, flushed with saline solution if patent, and fixed in 2% phosphate-buffered glutaraldehyde, and finally routinely prepared for, and evaluated by means of, routine light and scanning electron microscopy (SEM).

Results

All prostheses were initially patent upon release of the clamps. The mean cross clamping time was 19 min (range 13–24 min) for implanting a PTFE prosthesis. No infection or wound dehiscence was found during follow-up, and all rats survived the experimental period.

All prostheses, except one harvested after 1 week and one harvested after 24 weeks, were patent (overall patency rate 95%). Examination of the occluded prosthesis at one week showed a fresh thrombus already being infiltrated by fibroblast-like cells; the occluded prosthesis at 24 weeks showed an organised thrombus.

Macroscopically, little tissue reaction could be seen around the prostheses. Even after 24 weeks of implantation, the perigraft tissue could easily be stripped off the prosthesis.

After 1 day of implantation there was some deposition of thrombus on the prosthetic surface (Fig. 1B). After 3 days, the prosthetic surface still had a similar appearance. After 1 week, endothelial cells were observed across the anastomoses (Fig. 2A, B). They were in part continuous with the endothelium of the vein. By now, small capillary-like orifices, so-called "endothelial channels", were also already present in the anastomotic areas from which endothelial cells seem to grow in and over the graft surface (Fig. 2A). Some fibroblast-like cells were observed in the interstices of the prosthetic graft wall (Fig. 2B).

At 2 weeks, in most of the PTFE microvenous prostheses, the two fronts of regenerating endothelial cells (from proximally and distally) had reached each other, and more than 80% of the graft surface was endothelialised (Fig. 3A, B).

At 3, 6, 12 and 24 weeks, all microvenous prostheses were completely covered by an endothelial lining (Figs 4, 5). Some smooth muscle-like cells could occasionally be found underneath the endothelial layer. Endothelial channels could still be found in the anastomotic zones (Fig. 4A, B). Stenosis of the lumen was never observed at the anastomotic sites (Fig. 5B).

Throughout the periods of evaluation, perigraft

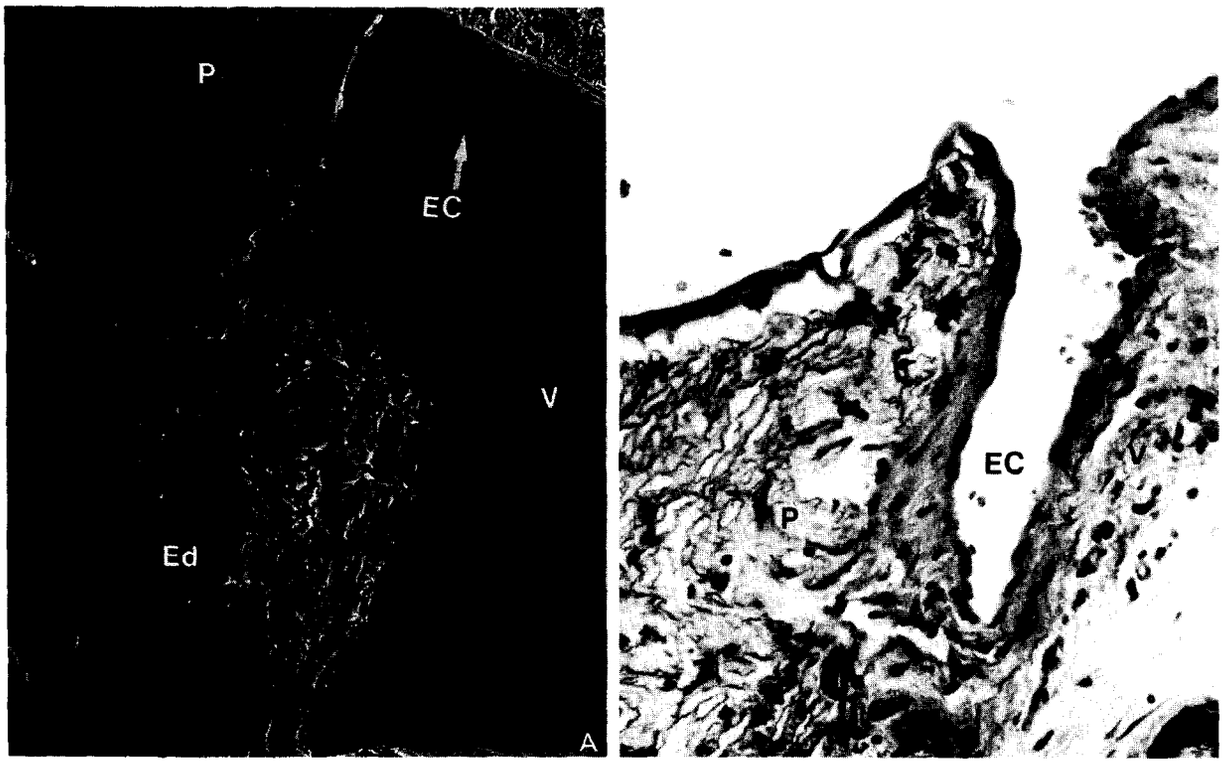

Fig. 4

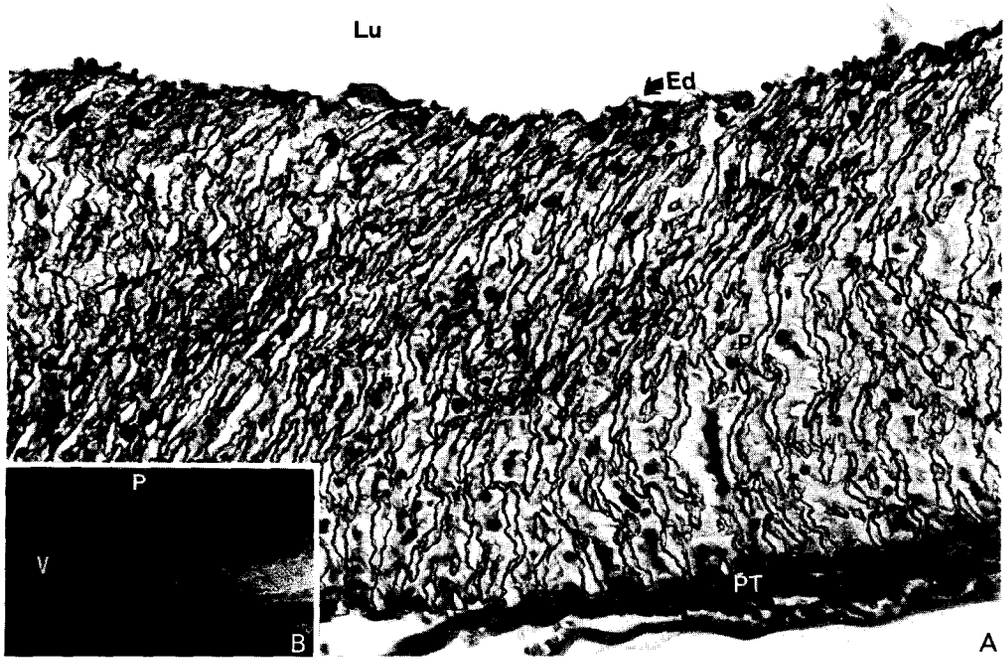

Fig. 5

Figure 4—SEM (A) and light micrograph (B) of the distal anastomotic region of a PTFE prosthesis (P) after 6 weeks, V—vein. Note the complete stable endothelial cell (Ed) coverage of the prosthesis (P) and the presence of a well-established endothelial channel (EC). (Magnification A 250 × ; B 400 ×). **Figure 5**—Light micrograph (A) and SEM (B) of a PTFE prosthesis (P) after 24 weeks. V—vein. Note again the complete stable endothelial cell coverage that is present in the prosthesis (P), and the absence of narrowing or stenosis of the lumen (Lu) or thrombus accumulation. Ed endothelial cells. (Magnification A 350 × ; B 15 ×).

Table Patency rates of microvenous prostheses when implanted into the rat femoral vein

Study	*Year*	*Prosthesis*	*I.D. (mm)*	*Length (mm)*	*Technique*	*Number of grafts*	*Patency*
Parsa and Spira	1979	PTFE	1	5–10	end to end	7	0 after 15 min
Lauritzen	1983	PGA	1	8–10	sleeve	12	6 after 12 months
Cuadros and Hughes	1986	PTFE	1.5	5	end to end	4	0 after 4 min
					end to end + av shunt*	18	18 after 40 to 70 days
Robinson *et al.*	1989	PU	1	5–7	end to end	16	16 after 1 to 20 min
						16	1 after 30 min to 24 h
					sleeve	8	6 after 24 h
Robinson *et al.*	1990	PU	1	5–7	end to end	12	0 after 1 day to 3 weeks
					sleeve	34	31 after 1 day to 6 weeks
van der Lei *et al.*	1991	PTFE	1	5–7	end to end	8	1 after 3 weeks
					sleeve	24	23 after 1 day to 12 weeks
Present study	1992	PTFE	1	5–7	sleeve	44	42 after 1 h to 24 weeks

* temporary av shunt. PTFE, polytetrafluoroethylene. PGA, polyglycolic acid. PU, polyurethane

tissue ingrowth into the walls of the PTFE prostheses remained scarce (Figs 3B, 5A). There were no indications that perigraft tissue had contributed to luminal graft healing.

Discussion

There are several possible routes of healing of PTFE (micro)venous prostheses:

1. ingrowth of endothelial cells and smooth muscle cells from the anastomotic sides (O'Brien *et al.*, 1985; van der Lei and Wildevuur, 1988, 1989);
2. endothelial cells or multipotential cells conveyed by the blood stream and seeded on the graft surface, as has been demonstrated for arterial prostheses (Stump *et al.*, 1963; Bossart *et al.*, 1982);
3. ingrowing microvessels, from either the vasa vasorum of the host vessels' stumps, or from the perigraft's invading tissue (Clowes *et al.*, 1986; Kogel *et al.*, 1989).

Kogel *et al.* (1989), investigating different fibril lengths of PTFE prostheses for venous reconstruction in beagles, have clearly demonstrated that healing of PTFE venous prostheses with a fibril length of 60 μm or greater is fast and complete via transmural vascularisation. The cells from these microvessels organise the inner clot layer that is present on the surface of the lumen of the prostheses. The presence of a clot layer is of special importance for the healing of the (venous) PTFE prostheses (van der Lei and Wildevuur, 1989; van der Lei *et al.*, 1991), functioning as a natural matrix for regenerating cells, just as in repair of normal vessels, and providing several growth promoting factors, such as platelet-derived growth factor (Ross *et al.*, 1974), low-density lipoproteins (Fisher-Dzoga *et al.*, 1974) and fibrin (Chemitz and Christensen, 1984). All of these mechanisms of healing have also been described for PTFE prostheses implanted in the arterial circulation (Clowes *et al.*, 1986; van der Lei and Wildevuur, 1988, 1989).

In the present study, healing of the microvenous PTFE prostheses occurred exclusively by rapid endothelial ingrowth from the anastomotic sides, in a combination of pannus ingrowth of endothelial cells originating from the host intima, and ingrowth of endothelial cells from the stumps of the host vessels via vasa vasorum near the anastomotic areas. In these areas we observed numerous orifices of capillary-like branches (see Figs. 2A, 4A, B) as described by Kogel *et al.* (1989). Healing by transmural perivascular ingrowth or seeding of cells from the circulation was not observed; capillaries were never observed growing in or through the graft interstices; only limited numbers of fibroblasts were present in the interstices (Fig. 5A), and isolated islands of endothelial cells were never observed. Both transmural perivascular ingrowth and adherence of cells from the circulation are impaired by the impervious structure and inertness of PTFE prostheses with a fibril length of 30 μm, and thus cannot contribute to endothelialisation.

The observation of an almost complete endothelial lining on the surface of the PTFE prostheses at 2 weeks, and the onset of endothelial cell ingrowth at 1 week, indicates a venous endothelial growth rate in PTFE prostheses of about 0.5 mm/day. This growth rate is comparable to that observed by Ishimaru *et al.* (1981). They estimated a growth rate of venous endothelium in PTFE prostheses implanted in the canine inferior vena cava of 0.3–0.5 mm/day.

The patency rate observed in the present study additionally underlines the importance of the anastomotic technique for implanting microvenous prostheses. A relatively atraumatic anastomotic technique such as the sleeve technique causes minimal iatrogenic surgical trauma at the anastomotic sites and leads to good patency rates, in contrast to the end-to-end technique, as observed by several other authors (see Table). Only the additional use of a temporary arteriovenous fistula (Cuadros and Hughes, 1986), the use of anti-platelet agents (Friedman and Hamilton, 1983), or the use of finer or coated suture material (Eddy *et al.*, 1986) can improve the patency rates of PTFE microvenous prostheses implanted by means of the conventional end-to-end technique.

In conclusion, the present study clearly demonstrates the potential of PTFE prostheses as a microvenous conduit. These experimental observations warrant further investigation, especially on longer venous segments, hopefully leading to reliable clinical microsurgical applications of PTFE microvenous prostheses.

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