In vitro Endothelialisation of Arteriovenous Loop Grafts for Haemodialysis

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Objectives: To evaluate the feasibility in a pilot study of in vitro endothelialisation of PTFE grafts used as interposition arteriovenous fistulas in uraemic patients.

Methods: Autologous saphenous vein endothelial cells were harvested and cultured on PTFE grafts in seven patients undergoing maintenance haemodialysis. The patients had several previous failures of vascular access sites. The patients were followed with duplex ultrasound, clinical examination and in one case an explanted graft was examined.

Results: At the end of follow-up four of the seven patients had patent grafts. One patient occluded the graft immediately postoperatively and another after 3.5 months. The former patient received a second endothelialised graft. In two further patients revision of the outflow was performed. In two patients a functioning graft was excised, in one case because of bleeding of a venous aneurysm and in one case because of suspected infection. The former which was excised 5 weeks postoperatively revealed that 85% of the surface was covered by endothelial cells.

Conclusions: This pilot study shows that in vitro endothelialisation of PTFE grafts used for haemodialysis is possible in uraemic patients. In this highly problematic patient group the results are promising with endothelial cell coverage after 5 weeks of implantation.

Introduction

In order to overcome the thrombogenicity of synthetic vascular grafts, endothelial cell seeding has been suggested. This was initially done as a one stage procedure, seeding the graft at the time of implantation. Even when the graft is seeded with cells at a high density, there is a critical period before coverage with a non-thrombogenic monolayer of endothelial cells occurs. There is also a critical initial amount of cells needed in order to eventually obtain a complete endothelial cell monolayer. One stage seeding has been associated with difficulties and has mostly produced disappointing results. In order to overcome the difficulties with one stage seeding and to eliminate the period between seeding and development of a confluent cell-layer, two methods have been developed. One alternative is to seed massive amounts of microvascular cells obtained from adipose tissue. It has, however, been suggested that these cells are of mesothelial origin and there is also a risk that fibroblasts or smooth muscle cells present as contaminants outgrow the endothelial cells.

An alternative is to harvest a small number of endothelial cells which are grown in culture and eventually seeded on the graft where they produce a confluent layer before implantation of the graft. This method has been termed in vitro endothelialisation (IVE). In order to decrease the time it takes to produce a completely covered graft, agents that increase cAMP have been used for the culture. This method using the patient's own serum as a growth medium has been adapted for IVE of ePTFE grafts.

Patients undergoing haemodialysis need a vascular access site with a large blood flow. The primary procedure is to arterialise a vein by creating an arteriovenous (AV) fistula at the wrist. Should this procedure fail, most centres use an interposition graft between the brachial artery and an antecubital vein. The graft, which is shaped in loop configuration, is punctured during the dialysis. The grafts often develop stenoses either within the graft or, more commonly, at the venous outflow.
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Table 1. Summary of patient characteristics.

<table>
<thead>
<tr>
<th>Patient no</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>72</td>
<td>74</td>
<td>49</td>
<td>64</td>
<td>53</td>
<td>69</td>
<td>66</td>
</tr>
<tr>
<td>Years on haemodialysis</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2.5</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>No. of previous fistula/grafts</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>No. of revisions of access sites</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>No. central dialysis catheter</td>
<td>&gt;4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>&gt;5</td>
<td>1</td>
</tr>
</tbody>
</table>

In the present study we report the experience of IVE of ePTFE interposition loop graft AV-fistulas in uraemic patients. This patient group was selected for our initial clinical attempts with IVE grafts because they represent a problem because of numerous thrombotic complications. Furthermore the risk for the patient in the event of graft occlusion is not life-threatening.

Patients

This report concerns seven patients on maintenance haemodialysis (five women and two men) ranging in age between 49–74 years. The patients all had multiple previous recurrent problems with their haemodialysis access sites including numerous thrombotic complications. In addition they had also been treated with central dialysis catheters several times in order to enable haemodialysis when the ordinary fistula or graft had failed. As a reflection of the problematic nature of these patients the average number of revisions for their access sites was three, including thrombectomies and revisions of the venous outflow. They had been on haemodialysis treatment for an average of 2.3 years (range 1–4). A summary of their previous medical history regarding access sites is given in Table 1. The weekly dialysis time was 9–12 h. The dialysers used were polysulphon hollow fibre (Fresenius). The dialyser area ranges from 0.7 to 1.3 m². Single pass dialysis systems were used with bicarbonate as the dialysis buffer. The blood flow during dialysis was 155–300 ml/min. All patients were treated with recombinant human erythropoietin 40–50 units/kg body weight administered intravenously three times weekly after dialysis. The haemoglobin concentration ranged from 62 to 113 g/l.

Informed consent was obtained and the study was approved by the Local Ethics Committee.

Methods

Culture of endothelial cells

A segment of the saphenous vein, or in some cases the external jugular vein, approximately 5 cm long was carefully excised under local anaesthesia as a source of endothelial cells. The vein was filled with collagenase (0.1% Worthington, Freehold, NJ, U.S.A.) and the harvested cells were cultured in minimal essential medium (MEM; Gibco, BRL, Paisley, Scotland) with 40% autologous serum. In order to decrease culture time, cAMP elevating compounds – iso-butyl-methyl-xantin (IBMX; Sigma, St Louis, MO, U.S.A.) and cholera toxin (Sigma) were added as previously described.12 The cells were grown for four passages using the same conditions and after the fourth passage they were transferred to a 6 mm diameter, ePTFE graft of 25 cm length. The graft was primed with autologous serum, after which the cells were seeded in four quadrants.13 After 1 day the graft was carefully bent into a horse shoe configuration and incubation was continued for another day. The average number of cells seeded into the graft was 7 million corresponding to approximately 200 000 cells/cm². The cells were grown in the graft in MEM with 30% autologous serum without IBMX or cholera toxin. The whole procedure was performed under sterile conditions and culture medium was sent for analysis of bacterial contaminants before the graft was cleared for implantation.

Surgical procedure

The loop graft was inserted usually under general anaesthesia and in a few cases under brachial plexus block. The brachial artery and a suitable vein at the elbow level were dissected free. The largest vein available was used irrespective of whether this was a superficial or a deep vein. A transverse counter incision was made approximately 20 cm distal to the incision.
at the elbow level. The loop graft was then tunnelled pulling both ends of the graft simultaneously in order to keep the U-shape of the graft intact during the tunnelling procedure thereby minimising trauma to the inner surface. After positioning of the loop graft, both ends were cut and the inner surface of the graft was kept moist by flushing it at constant intervals with Ringer’s solution. An end-to-side anastomosis was usually performed first to the side of the brachial artery using CV-7 Gore Tex suture. The venous anastomosis was performed similarly. Blood flow was allowed to start gradually by slowly releasing manual compression on the brachial artery after completion of both anastomoses. During the whole procedure extreme care was taken in order not to damage or bend the graft and to keep the inner surface moist. Both ends of the graft were cut since they had been used for handling during the endothelialisation and therefore did not have a cellular coverage. The cut ends of the graft were saved and stained with cresyl violet to examine if endothelial covering was present at the anastomotic sites.

Colour duplex ultrasound

Duplex scanning was performed with a colour-flow duplex imager (Acuson 128, Mountain View, CA, U.S.A.). A linear imaging transducer of 5 MHz or 7.5 MHz was selected in conjunction with a 3.5 MHz or 5 MHz pulsed Doppler.

The follow-up program included ultrasonographic examinations 1, 3, 6, 9 and 12 months postoperatively. Six of the patients were subjected to this program. Four of them also underwent a preoperative ultrasonographic assessment of the venous system of both arms. The follow-up duplex examinations included 

B-mode imaging of morphology and vessel diameter, colour-flow Doppler to localise flow abnormalities and pulsed Doppler to measure maximal and mean blood flow velocities. The scanning included the brachial artery from the mid upper arm to the proximal anastomosis, the whole length of the graft and the efferent venous vessels from the venous anastomosis to the mid subclavian vein. Angle corrected flow velocities (m/s) were measured at a 50–60 degree angle between the ultrasonic beam and the length of the blood vessel. A localised increase of flow velocity of $\geq 2.5$ times that in the closest preceding segment was used to denote a significant stenosis, at the proximal anastomosis a velocity ratio of $\geq 3.5$ was used. 

Volume flow measurements were performed by calculating the product of vessel cross sectional area and the time average blood flow velocity (TAV). TAV was measured for 5–8 complete cardiac cycles. The mean of three measurements of vessel diameter from the B-mode image was used for area calculation. Volume flow was measured in the mid brachial artery, approximately 10 cm above the loop graft anastomosis. Volume flow was also estimated in a non-curved segment of the graft.

Immunohistochemical and scanning electron microscopy analyses

Segments from excised grafts from the arterial and venous ends and the middle portion of the graft were prepared for scanning electron microscopy (SEM) as described by Schroetter et al. Immunohistochemical demonstration of von Willebrand related antigen and the basement membrane component collagen type IV was performed as previously described.

Results

Surgery was primarily successful in all patients but one where the loop graft occluded immediately postoperatively. This patient was later reoperated with a second IVE graft. At present, four of the seven patients have patent grafts with an observation time of 6 months in one case, 10 months in two cases and 1 year in one case. Apart from the patient where an occlusion occurred immediately postoperatively, three other graft failures have been recorded. One patient occluded her graft 3.5 months postoperatively. In two cases the loop graft had to be removed because of bleeding. In one woman rupture of an aneurysm in the outflow vein occurred and the graft had to be removed 5 weeks postoperatively. SEM of segments from the arterial and venous ends and the mid portion of the explanted graft revealed that approximately 85% of the surface was covered by endothelial cells. The endothelial nature of the cellular lining was confirmed by a positive staining for von Willebrand antigen. Antibodies raised against collagen type IV also produced a positive staining indicating presence of a basement membrane. In one further patient bleeding occurred at the venous outflow, it was, however, difficult to exactly identify the bleeding source but for fear of an infection causing the bleeding, the graft was removed. No positive bacterial culture was obtained from the latter two patients.

Among those grafts that are patent, three have been
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Table 2. Colour duplex evaluation of seven patients operated with endothelialised loop grafts.

<table>
<thead>
<tr>
<th>Patient no</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No significant stenosis</td>
<td>No significant stenosis</td>
<td>&lt;50% stenosis in proximal graft</td>
<td>&lt;50% stenosis in proximal graft</td>
<td>&lt;50% proximal graft</td>
</tr>
<tr>
<td>QB = ?</td>
<td>Vc = 2.2 m/s</td>
<td>Qb = 950 ml/min</td>
<td>Vc = 2.3 m/s</td>
<td>Qb = 1170 ml/min</td>
<td>Vc = 2.5 m/s</td>
</tr>
<tr>
<td>&gt;50% stenosis at distal anastomosis</td>
<td>Progression of stenosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>QB = 690 ml/min</td>
<td>Vc = 1.7 m/s</td>
<td>Qb = 870 ml/min</td>
<td>Vc = 1.3 m/s</td>
<td>Qb = 710 ml/min</td>
</tr>
<tr>
<td>No significant stenosis</td>
<td>Two outflow vein stenosis</td>
<td>Progression of vein stenosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qb = 1050 ml/min</td>
<td>Qb = 870 ml/min</td>
<td>Vc = 2.1 m/s</td>
<td>Qb = 710 ml/min</td>
<td>Vc = 1.3 m/s</td>
<td>Qb = 785 ml/min</td>
</tr>
<tr>
<td>Vc = 2.1 m/s</td>
<td>Vc = 2.1 m/s</td>
<td>Vc = 2.1 m/s</td>
<td>Vc = 2.1 m/s</td>
<td>Vc = 1.5 m/s</td>
<td></td>
</tr>
<tr>
<td>3*</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QB = 850 ml/min</td>
<td>Vc = 1.5 m/s</td>
<td>Qb = 95 ml/min</td>
<td>Vc = 0 m/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vc = 1.8 m/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No significant stenosis</td>
<td>Stenosis in 1 of 3 outflow veins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qb = 1100 ml/min</td>
<td>Qb = 875 ml/min</td>
<td>Vc = 2.1 m/s</td>
<td>Qb = 785 ml/min</td>
<td>Vc = 1.6 m/s</td>
<td>Qb = 785 ml/min</td>
</tr>
<tr>
<td>Vc = 2.1 m/s</td>
<td></td>
<td>Vc = 2.1 m/s</td>
<td>Vc = 2.1 m/s</td>
<td>Vc = 1.5 m/s</td>
<td>Vc = 2.1 m/s</td>
</tr>
<tr>
<td>5</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qb = 95 ml/min</td>
<td>Vc = 2.1 m/s</td>
<td>Qb = 710 ml/min</td>
<td>Vc = 1.6 m/s</td>
<td>Qb = 875 ml/min</td>
<td>Vc = 2.1 m/s</td>
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<td>Vc = 1.8 m/s</td>
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<td></td>
<td>Vc = 2.1 m/s</td>
<td>Vc = 2.1 m/s</td>
</tr>
<tr>
<td>6</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>No significant stenosis</td>
<td>Occluded graft</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Patient no. 3 received one graft which occluded immediately postoperatively. The graft listed in the table is the second graft in this patient.

QB = flow in the brachial artery, Vc = maximal velocity in a non-curved and non-stenotic segment of the graft.

subjected to interventions. In one case a false aneurysm at a puncture site was operated 8 months after the original operation. In one case with increasing outflow resistance a stenosis of the outflow vein was diagnosed following surgery. This was treated by percutaneous transluminal angioplasty. In order not to puncture the seeded graft, the vein was punctured proximal to the stenosis and the balloon catheter was introduced in a retrograde fashion. Venoplasty was successful. In one further case with stenosis of the venous outflow and a false aneurysm, surgery was again performed 9 months after the initial surgery. At this operation a false aneurysm was repaired and the venous outflow was reconstructed by interposition of a new segment of an IVE graft. The new graft was positioned between the venous end of the graft and the outflow vein proximal to the previous anastomosis. The grafts that are presently patent are all functioning well and supporting adequate haemodialysis. Blood flow at dialysis was 330 ml/min, which resulted in a venous pressure ranging between 180 and 220 mmHg.

Duplex results for each patient are shown schematically in Table 2. In general, B-mode imaging showed a smooth surface along the graft walls. In two patients, however, small areas with irregular wall structure developed at the near wall at the puncture sites. Mean flow velocity in a non-curved and non-stenotic graft segment (Vc) was 1.9 m/s (range 1.5–2.4) and volume flow in the brachial artery (QB) was 905 ml/min (range 785–1030) at the end of follow-up. Volume flow in the graft was in general 50–100 ml less than in the brachial artery. There was a clear relation between progression of stenoses and decrease of volume flow.

Discussion

The present pilot study examines the feasibility of IVE in general and for use in loop grafts in uraemic patients in particular. The report shows promising results in a patient group with many thrombotic problems. It should be remembered that many of the patients in the present study had multiple previous failures often with loop grafts occluding in the early postoperative period after previous attempts.
There are several reasons to believe that the coating of the grafts with endothelial cells is beneficial for the patency. A confluent endothelial cell layer is non-thrombogenic provided that the cells have not changed their phenotype. An intact endothelial cell layer is probably also a prerequisite to prevent myointimal hyperplasia which usually occurs at the anastomotic sites. The potential advantage with an *in vitro* endothelialised graft is therefore two-fold: it contributes to decreased thrombogenicity and may also prevent myointimal hyperplasia occluding the outflow of the graft. Endothelial cells have several built-in non-thrombogenic characteristics and it has been shown previously that cells seeded on ePTFE with the method used in this study are capable of producing prostacyclin and tissue plasminogen activator. They furthermore support inhibition of thrombin and are able to activate protein C upon challenge with thrombin.

Intraoperatively seeded grafts have not been shown to result in better patency than ordinary grafts. Zilla *et al.*, however, have reported better patency for femoropopliteal IVE grafts than for ordinary grafts. In that report, however, fibrin glue derived from donor blood with the addition of a protease inhibitor was used to support adhesion of the endothelial cells instead of autologous serum as used in the present report. Furthermore, another culture medium without cAMP-elevating compounds, but with the addition of exogenous growth factors, was used in that study. In the present study growth of the cells *in vitro* was supported by autologous serum and the fact that the patients were uraemic did not seem to influence the effect of the autologous serum as a growth promoting agent. If these different growth conditions influence cellular function after implantation is not known.

Values from flow measurements in these grafts were in the same orders as those presented earlier from studies of AV-fistulas and conventional loop grafts. For example, one study found flow rates of 750 ml/min in 31 PTFE grafts (6 mm)27. In this small number of patients we found flow measurements useful in the evaluation, especially to assess the haemodynamic effect of stenoses and the timing for surgical intervention. As shown earlier, careful ultrasonographic examination of haemodialysis access systems will reveal a high number of abnormalities. In our study, as in others, these abnormalities were most frequently found in the venous/efferent part of the access system.28,29 Our impression from limited observations is that wall changes within these PTFE graft was a rather uncommon finding, in contrast to what has been observed earlier in conventional synthetic PTFE loop grafts.

In conclusion, the present pilot study shows promising results. From a theoretical point of view and based on earlier literature reports with a similar method, it is likely that IVE is beneficial and contributes to better graft patency. However, a randomised study is required to prove this and we have recently initiated such a study. If it can be shown that IVE contributes to better graft patency, such a study may also answer the question of whether IVE should be used as the standard procedure or be reserved for patients with a high risk of thrombotic graft occlusion.

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**References**

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15 BRISCE M, CIMINO JE, APPEL K, HURWICH BJ. Chronic hemo-
dialysis using venipuncture and a surgically created arterio-


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