Expanded polytetrafluoroethylene graft for bypass surgery using the excimer laser–assisted nonocclusive anastomosis technique

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Object. Patients with complex craniocerebral pathophysologies such as giant cerebral aneurysms, skull base tumors, and/or carotid artery occlusive disease are candidates for a revascularization procedure to augment or preserve cerebral blood flow. However, the brain is susceptible to ischemia, and therefore the excimer laser–assisted nonocclusive anastomosis (ELANA) technique has been developed to overcome temporary occlusion. Harvesting autologous vessels of reasonable quality, which is necessary for this technique, may at times be problematic or impossible due to the underlying systemic vascular disease. The use of artificial vessels is therefore an alternative graft for revascularization. Note, however, that it is unknown to what degree these grafts are subject to occlusion using the ELANA anastomosis technique. Therefore, the authors studied the ELANA technique in combination with an expanded polytetrafluoroethylene (ePTFE) graft.

Methods. The experimental surgeries involved bypassing the abdominal aorta in the rabbit. Ten rabbits were subjected to operations representing 20 ePTFE graft–ELANA end-to-side anastomoses. Intraoperative blood flow, follow-up angiograms, and long-term histological characteristics were assessed 75, 125, and 180 days postoperatively. Angiography results proved long-term patency of ePTFE grafts in all animals at all time points studied. Data from the histological analysis showed minimal intimal reaction at the anastomosis site up to 180 days postoperatively. Endothelialization of the ePTFE graft was progressive over time.

Conclusions. The ELANA technique in combination with the ePTFE graft seems to have favorable attributes for end-to-side anastomoses and may be suitable for bypass procedures.

KEY WORDS • cerebral revascularization • expanded polytetrafluoroethylene • excimer laser–assisted nonocclusive anastomosis • rabbit

Most cerebral aneurysms are treated either microsurgically with a clip or endovascularly with embolization techniques; however, when lesion size, location, or shape makes surgical clip application or endovascular embolization impossible, trapping must be considered. A balloon test occlusion is indispensable for deciding whether a cerebrovascular bypass is necessary.21,22 Bypasses involve either an intracranial-to-intracranial or an extracranial-to-intracranial connection. Establishing a bypass may be associated with a considerable risk of ischemia, directly proportional to the time of temporary occlusion. The more proximal the anastomosis is to the circle of Willis, the greater the area of possible brain ischemia.

The high-flow ELANA technique allows for unions with large-caliber recipient vessels without the need for temporary occlusion. The consequent risk of ischemia is therefore reduced. This bypass technique necessitates a vascular graft interposition.23,24 An autologous graft is the ideal bypass conduit in arterial reconstruction, for which either a venous graft, such as a saphenous vein, or an arterial graft, such as a radial artery, may be considered. For the ELANA procedure a venous graft is preferred over an arterial graft for technical handling in terms of the thickness of the platinum ring–vessel complex and its rigidity for laser catheter introduction. Systemic disease may further complicate the clinical utility of autologous grafts. As a result surgeons must rely on prosthetic grafts as an alternative conduit in arterial bypass procedures. Factors such as the amount of blood flowing through an artificial blood vessel, the heparin coating on the artificial grafts, and especially the applied technique of microsurgical anastomosis with its resulting flow dynamics influence the patency and long-term results of

Abbreviations used in this paper: ELANA = excimer laser–assisted nonocclusive anastomosis; ePTFE = expanded polytetrafluoroethylene.
vascular grafts. Intimal hyperplasia most distinctively occurs at the anastomosis site, and less so along the vessel shaft. Thus, before clinically using ePTFE grafts more extensively in combination with the ELANA technique, an experimental study analyzing the long-term effects of patency, especially at the anastomosis site, should be performed. A rabbit abdominal aorta model was chosen because the organ’s dimensions and associated blood flow correspond best to human intracranial vessel conditions around the circle of Willis and the branching vessels.

Materials and Methods

The study protocol was approved by the committee for the conduct of animal experiments of the Kanton of Bern, Switzerland. Animals were kept in cages conforming to the animal protection regulations. Animals were routinely checked with regard to behavior, motor function, and nutrition status.

Preparation of the Graft Vessel

A 3-mm-diameter ePTFE graft was cut to a length of 10 cm in a maximally extended condition (W. L. Gore and Associates, Flagstaff, AZ). A platinum ring (Elana BV, Utrecht, The Netherlands) with a diameter of 2.6 mm was sutured with 9-0 Prolene (Ethicon, Johnson & Johnson, Spreitenbach, Switzerland) to the inside of the ePTFE graft, starting with an outside-inside and then an inside-outside stitch, with the knot on the outside. Eight stitches were inserted to fix the platinum ring to the ePTFE graft (Fig. 1), and this step was performed on both ends of the vessel. A longitudinal incision of 2 cm in the middle of the vessel was made for the subsequent introduction of the laser catheter. Thus, the laser catheter reached both rings at a right angle to the aorta once the graft was sutured to it (Fig. 2). The ePTFE graft, whose wall was 0.5 mm thick, was then gas-terilized.

Operative Procedure

Ten Burgundy rabbits weighing 3.9 to 4.4 kg were subjected to the ELANA technique combined with an ePTFE graft. The same surgeon (M.R.) performed all operations. One rabbit was pregnant and one suffered postoperative paraplegia, most likely due to spinal ischemia resulting from closure of a spinal artery with the hemoclip, and these animals were killed immediately after surgery. Each operation consisted of two ELANA–ePTFE graft anastomoses. The ELANA technique has been previously described, and the following descriptions are specific to the combined ePTFE graft–ELANA technique (Fig. 3). Animals were sedated, intubated, and received ventilation. Anesthesia was induced initially using an intravenous combination of ketamine (65 mg/kg body weight) and xylazine (4.5 mg/kg body weight) and then isoflurane as an anesthetic agent inhaled throughout the operation. The abdomen was shaved and disinfected. After placing sterile covering, a midline abdominal incision was made. The intestines were laid to the left and covered with body-tempered saline-imbued gauze surrounded by sterile aluminium foil. By doing so the inferior vena cava and abdominal aorta were exposed and gently dissected to separate the aorta from surrounding tissue. The vena cava was separated from the aorta by using a distractor, without direct compression of either blood vessel. The arteries to the kidneys were localized. A proximal and a distal end-to-side anastomosis site were chosen. With the aid of a microscope, the ePTFE graft with the platinum ring was sutured to the aorta first distally and then proximally by using 9-0 Prolene. A total of eight stitches were made on each anastomosis. The 12 and 6 o’clock stitches on the lateral side of the aorta were made deeper through the vessel layer, whereas the other stitches were made more superficially, to flatten the vessel to the platinum ring. After inserting the eight stitches, tightness was assessed under the microscope by using an irrigation syringe. If necessary, additional sutures were applied using 9-0 nylon (Ethicon, Johnson & Johnson). After fixation of the graft, the aorta was irrigated with a diluted solution of papaverine (Streuli, Uznach, Switzerland) and allowed to rest for a few minutes. During this time the excimer laser (CVX 300, Spectranetics International BV, Leusden, The Netherlands) was started and calibrated using a commercially available laser catheter (Elana BV) for perforation of the aorta. The catheter was then introduced into the previously made longitudinal cut to first reach the distal anastomosis site. Using an adapted vacuum pump (Leybold Pump modified by University Medical Center Utrecht, The Netherlands) the aorta inside the platinum ring was aspirated against the laser catheter, thus creating tight contact between the laser fibers and the aorta. After 2 minutes of stable vacuum, the laser was activated to 40 Hz and 30 mJ/mm² for a maximum of 5 seconds. With slight traction on the ePTFE graft and simultaneous pressure on the catheter, the aorta was perforated. If the perforation was complete on visual inspection or if backflow was profuse in the ePTFE graft, the laser was switched off. A standard temporary Yasargil clip (Aesculap, Tuttingen, Germany) was used to prevent backflow through the first anastomosis. Heparin (200 IU; Fresenius Medical Care, Manno, Switzerland) was then administered intravenously. Small leakages at the anastomosis site were treated using hemostatic gauze (Tabotamp; Ethicon, Johnson & Johnson). The second proximal anastomosis was then performed using the same technique. A second intravenous heparin injection of 200 IU was administered after having performed the second proximal laser perforation. A second temporary Yasargil clip was applied to prevent flow through the second anastomosis. The longitudinal incision in the ePTFE graft was then sutured using 8-0 Prolene (Ethicon, Johnson & Johnson). After creating the ePTFE graft–ELANA bypass, blood flow was measured in the abdominal aorta proximal to the upper anastomosis by using a quantitative blood flow device with a 3-mm Charbel Probe (both products Transonic Systems, Inc., Ithaca, NY). The bowel retractors were then removed, and the intestines were replaced and irrigated with warm saline. Anesthesia was intermittently increased before closure, given that tension on the intestines is painful. The abdomen was closed using a continuous suture technique. The skin was sutured with tight intracutaneous sutures. The

Fig. 1. A: Photograph showing the 3-mm-diameter ePTFE graft and platinum ring (arrowhead). B: Schematic demonstrating the suturing of the vessel to the platinum ring with 9-0 Prolene (arrow). A total of eight stitches were inserted to tighten the platinum ring to the vessel. C: Photograph revealing the end result of the inside fixation. The remnant of the vessel overlapping the sutured platinum ring (arrowhead) is cut as close to the platinum ring as possible with microscissors.
animal was then placed in a bin with a warm bottle and was extubated. A single shot of benzylpenicillin (150,000 IU; Intervet, Skovlunde, Denmark) was injected intramuscularly. Rabbits received an intramuscular injection of 250 mg metamizolum (Aventis Pharma, Zürich, Switzerland) for 3 days as a postoperative analgesic.

Angiographic and Histological Studies

The rabbits were intramuscularly anesthetized, and the right subclavian artery was catheterized using small tubing (602-205; Dow Corning, Midland, MI). Thereafter, abdominal biplanar angiography (matrix 1024/H11003, CAS500; Toshiba, Ltd., Tokyo, Japan) was performed to assess the patency of the bypass by using Iopamiro (Bracco SA, Mendrisio, Switzerland). Animals were then killed for excision of the abdominal aorta bypass. The vessels were pressure-fixed with formaldehyde through the subclavian artery and a cut in the femoral artery. The abdominal aorta including the bypass were extracted and fixated in formaldehyde and later embedded in methylmethacrylate resin over a 30-day period for slow hardening to obtain slices of the tissue with the platinum ring. Tissue blocks with a slice thickness of 150/9262m were then cut using a diamond band saw (Exakt Apparatebau, Norderstedt-Hamburg, Germany). Thereafter, the slices were polished down to a thickness of 50/9262m. After pretreatment with paragon solution, the slices were stained with H & E. To prevent shadow formation during microscopy on the remaining thickness of the ePTFE graft and platinum ring, the histological slices were illuminated from both above and below the slide. The middle section of the bypass was previously cut at a conventional slice thickness and analyzed separately with H & E.

Results

The operation was well tolerated. Among the operated rabbits, eight were used to assess the anastomosis. The first two rabbits were evaluated on postprocedure Day 1. Thereafter, the time points at postprocedure Days 75 (two rabbits), 125 (two rabbits), and 180 (two rabbits) were studied.

Blood Flow Evaluation

The mean blood flow value (measured using the Tranonic quantitative blood flow device) through the ePTFE graft–ELANA bypass after clipping the abdominal aorta between the two anastomoses was 53.3 ± 7.6 ml/minute (mean ± standard deviation, eight rabbits).

Angiographic Results

Digital subtraction angiography results demonstrated complete patency of the bypass at all postoperative time points (Days 1, 75, 125, and 180; Fig. 4), and contrast medium passed unimpeded in the dynamic views. Significant obstruction or plaque formation at the anastomosis sites or in the ePTFE graft were excluded. The variation in the angiographic image of the ePTFE graft resulted from the different development of retroperitoneal fat and postoperative peritoneal adherences.

Histological Results at the Anastomosis Site

Intimal proliferation was minimal at the anastomosis at all time points in the ePTFE graft, and there was no progressive effect over time. In the host vessel (aorta) no plaques or intimal hyperplasia were observed (Fig. 5). Endothelialization was progressive over the observed time points. At 180 days postprocedure, endothelialization was complete.

Aortic Wall Flap Rate

Twenty laser perforations were performed in the 10 rabbits, with recovery of a flap in 15 cases (75%). In five laser perforations no flap was recovered. Missed retrieval of the flap occurred in rabbits scheduled for postoperative evaluation on Days 1 (one animal), 75 (two animals), and 180 (two animals). However, there was no association with obstruc-

Fig. 3. Schematics representing steps in the conventional ELANA technique in combination with the artificial ePTFE graft. A: Artificial ePTFE graft (arrow) with sutured platinum ring on the inside of the shaft sutured to the abdominal aorta (asterisk). B: Laser catheter is introduced into the ePTFE graft. C: Catheter tip is positioned within the platinum ring so that the laser fibers are in contact with the wall of the recipient artery. Vacuum suction is then applied through the catheter, ensuring firm fixation of the wall of the recipient artery to the laser fibers. D: When the laser is activated a disc of arterial wall, or flap, is punched out of the recipient artery. E: When the catheter is withdrawn, the flap is also withdrawn because of the continued vacuum suction. The anastomosis is now complete, leaving a rim of medial and adventitial layers exposed to the bloodstream within the anastomosis inside the lumen of the platinum ring.
morbidty and mortality rates are high. Therefore, the centralization of microsurgical expertise in specialized centers and the development of new techniques to minimize risks for both interventional and microsurgical procedures are necessary. Included in these strategies are cerebral bypass techniques without temporary occlusion to minimize the risk of ischemia. Furthermore, newly developed techniques such as laser tissue soldering may reduce microsurgical procedure time and thus morbidity.13

The Optimal Interposition Graft

The optimal interposition graft may be defined by the long-term results and would thus be the one with the best patency profile. Yet many factors are involved, such as whether the bypass is performed in the venous or the arterial system.12,15 A healthy autologous graft usually will have a good long-term profile. Whatever the nature of the graft, injury to the graft and host may lead to an intimal reaction at the injury site, with possible intimal thickening and a resultant occlusive lesion; therefore, these factors remain the major concerns with respect to long-term results. Based on data from animal experiments the long-term results reported for artificial Dacron and PTFE grafts range from weeks to months.3,8,9,19 Bassiony and colleagues1 have characterized the precise location and severity of intimal thickening in a canine end-to-side PTFE anastomosis. Two types of anastomotic intimal thickening were described: first, at the suture line in the graft, which was probably related to vessel compliance mismatch; and second, in the host arterial floor, which was related to oscillating shear. In fact, a multitude of factors seem to be responsible for the expression rate of intimal hyperplasia. Interplay between endothelium, intimal smooth-muscle cell proliferation and fibroblast in-growth has been demonstrated.4–6 Clowes and associates5 made the lack of endothelialization or chronic endothelial injury responsible for intimal proliferation and thus smooth-muscle cell proliferation. In grafts fully covered by endothelium, intimal smooth-muscle cell proliferated less than in grafts lacking a complete endothelial covering. These authors further reported that intimal proliferation was most prominent in the first 3 months after operation in PTFE vessels and that thereafter there was a slow but progressive healing of the grafts by additional and possibly complete in-growth of endothelium.4–6 Approximately 6 months after bypass surgery the cellular organization within the graft represented a recapitulation of the organization within the normal artery. Invariably, the intima was composed of smooth-muscle cells covered by a luminal monolayer of endothelial cells, fibroblasts in perigraft tissue, and graft matrix external to smooth-muscle cells. Endothelial cells grow from the anastomosis toward the center of the graft, and full coverage appears possible depending on the length of the graft.4 Therefore, intimal proliferation may stabilize at some point, possibly in a state of equilibrium between proliferation and cell death; otherwise every artificial PTFE graft would lead inevitably to occlusion. The exact reasons why these positive developments occur (or do not) are not fully understood, but local rheological factors depending on the anastomosis technique probably play an important role. The ELANA technique in general as described by Streefkerk, et al.,16,17 and in combination with a ePTFE graft as described

Discussion

Recent changes in the treatment of cerebrovascular pathophysiologies such as the introduction of endovascular techniques has reduced the number of microsurgically treated cases. Moreover, the remaining cases consist of those that are difficult to treat with either the microsurgical or the combined endovascular–microsurgical approach, and thus
in the present study appears favorable for these rheological conditions, because at 6 months postoperatively no angiographic or histological intimal hyperplasia was observed.

**Flap Retrieval Rate and Possible Implications**

In 75% of the arteriotomies in the present series of animals the flap of arterial wall tissue cut out with the laser catheter was retrieved using the catheter; this percentage is similar to that in a large clinical series. In the remaining cases no tissue was found adhering to the laser catheter on its removal from the graft. An incomplete laser cut can cause the flap to be pulled off the catheter, that is, the flap remains attached to the artery wall. A loose piece of tissue that could migrate with the bloodstream and cause embolization can be ruled out: even in the failure mode of a total loss of vacuum in the catheter, a cut out flap is kept pressed against the catheter given that blood pressure always exceeds ambient pressure. In the largest relevant clinical series (> 300 surgeries) to date there was no evidence of new postoperative embolization-induced neurological deficits that could be attributable to a loose flap, which corroborates our theory. It is not clear whether the inspection and removal of the flap under short temporary occlusion is worth the extra risk of thrombosis of such manipulation. Data from the largest series thus analyzed to date (unpublished data) have shown that the presence of a flap at the anastomosis did not prove to be a predictor of bypass patency, although this hypothesis is being further investigated. To better understand the processes involved and thus to be able to avoid the incomplete laser cut, the laser ablation procedure is being endovascularly analyzed in an experimental setup in which a camera will be used to film the creation of the arteriotomy in realistic circumstances.

**Clinical Implications**

The optimal graft for the ELANA technique is an autologous venous graft, but this type may not always be possible. When using the radial artery as an alternative graft vessel, the platinum ring–vessel complex is thicker than that with the saphenous vein and is thus less suitable for the ELANA
Expanded polytetrafluoroethylene graft for the ELANA bypass

technique. For this reason and also for development of sutureless anastomosis techniques, alternative vessels must be studied.

Although the conventional ELANA technique has proven to be a safe technique in a clinical situation and now in combination with the ePTFE graft in the rabbit, a one-to-one comparison between the two is not feasible. Long-term results depend not only on the anastomatic site as described earlier but also on the long-term behavior of the intima along the length of the ePTFE graft. Although the intimal proliferation in the shaft of the ePTFE graft never exceeded the thickness of the ePTFE graft, our ePTFE grafts never exceeded the length of 10 cm. A comparison with an extracrani-al-to-intracranial bypass would thus imply another experimental setup using a longer graft, and one must consider that in an extracrani-al-to-intracranial bypass the proximal anastomosis is not performed using the ELANA technique. Note, however, that the results in our study are comparable with a strict extracrani-al-to-intracranial ePTFE graft–ELANA bypass in which a length of 10 cm is conceivable. These strict extracrani-al bypass operations account for approximately 50% of the performed ELANA bypasses. Further analysis including a larger population and an observation period longer than 1 year is indicated before transmitting the results into clinical application.

Secondary thrombosis is feared in any vascular bypass. In the present study we did not observe secondary thrombosis, even without any specific treatment, a phenomenon that may be species-specific. In a cross-species analysis of coagulation activity in rabbits and humans prothrombin time was 9.9 compared with 11.7 seconds; activated partial thromboplastin time, 21.4 compared with 34 seconds; and thrombin time, 16.8 compared with 17.3 seconds, respectively. The most significant difference was the 50 times higher factor V values in the rabbits. In addition to these coagulation factors, thrombogenesis in an artificial graft depends on the amount of blood flow through the artificial bypass. Blood flow in a clinical high-flow ELANA anastomosis is expected to be greater than 100 ml/minute to take over the blood supply to the bypassed brain. This rate is higher than what was measured in the ELANA bypass in the rabbit. Therefore, thrombogenesis in an ePTFE graft in a human is at least not more likely than one in a rabbit considering these two aspects. In the clinical context the ELANA procedure is performed while aspirin and heparin are administered, and these agents would be used for the ePTFE graft combined with the ELANA technique.

Conclusions

The combination of the ELANA technique with an artificial ePTFE graft produces a patent anastomosis together with the angiographically and histologically proven absence of obstruction for up to 6 months postsurgery. The local tissue reaction during the observed postoperative period can be considered as healing rather than the usual reported intima hyperplasia that occurs with the artificial PTFE grafts. This anastomosis technique with its specific hemodynamic conditions may play an important role in the induction of intimal healing. The ELANA technique in conjunction with the ePTFE graft appears to be beneficial in end-to-side anastomosis. Although the long-term results must be extended to more than 1 year before extrapolating to any clinical application, the results indicate a promising addition to the ELANA technique already in use in humans and possible future developments in sutureless anastomosis techniques.

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