Fat- and Bone Marrow-impregnated Small Diameter PTFE Grafts*

E. Frechette1,2, Y. M. Dion1,2, A. Cardon3, N. Chakfé4 and C. J. Doillon†1,2

1Department of Surgery, Laval University, Ste Foy, QC, Canada, 2Biomaterials Institute of Quebec, Pavillon St-François d’Assise, CHUQ, Quebec City, QC, Canada, 3Department of Vascular Surgery, Centre Hospitalier Régional et Universitaire de Rennes, Rennes, France and 4Department of Cardio-vascular Surgery, Les Hôpitaux Universitaires de Strasbourg, Strasbourg, France

Objectives: to evaluate an alternative and simple technique which consists in impregnation of a synthetic prosthesis with either autogenic omental fat or bone marrow. These tissues have been selected based on previous works and because they contain multiple cellular and extracellular compounds with biological healing properties (i.e. angiogenesis, endothelialisation, etc.).

Design: PTFE grafts of Group 1 were impregnated with fatty tissue, those of Group 2 with bone marrow and those of Group 3 served as controls.

Materials: nine mongrel dogs divided among these three groups. PTFE grafts are 3 mm in diameter.

Methods: in each animal, both iliac arteries were submitted to an end-to-side ilio-iliac bypass. At 3 months, pathology assessment was performed.

Results: group 1: all grafts were thrombosed and intimal hyperplasia was found occluding the anastomotic sites. Group 2: 4/6 grafts were patent and their mid-portion presented a thin neointima which did not totally cover the anastomotic sites. Group 3: 2/5 grafts were patent and their mid-portion as well as the anastomotic sites were covered with neointima which was hyperplastic in some areas.

Conclusions: addition of bone marrow cells may contribute to improve the quality of the healing process.

Keywords: Vascular prosthesis; Small diameter vessel replacement; Bone-marrow; PTFE, Omental tissue; Healing.

Introduction

Clinical use of small-calibre synthetic arterial grafts has shown lower patency rate than that of autologous arteries1 or saphenous vein.2 The two major causal factors are (i) enhanced thrombogenicity of the synthetic material due to reduced flow through these small-calibre grafts, and (ii) development of intimal hyperplasia particularly at the anastomoses, leading to late obstruction and thrombosis.3-5

Graft seeding techniques using endothelial cells have been proposed to overcome the thrombogenic properties of synthetic luminal surfaces of small-diameter vascular prostheses. Several methods of endothelialisation have been offered, like direct seeding or sodding of endothelial cells within synthetic graft.6-9 However, results showed poor endothelial cell retention under blood-flow stress.10 Experimental graft impregnation with adhesive molecules (i.e. oligopeptides or glycoproteins) appeared to improve endothelial cell retention.11-12 However, clinical results were inconsistent and unpredictable.13,14 The isolation of a large number of endothelial cells and the cultures of endothelial cells are required for lining grafts with cells prior to surgery. Therefore, the use of these techniques is restricted.15-17

Recently, autologous tissue fragments, including omental or subcutaneous cells, vein fragments or bone marrow cells have been proposed as alternative biological materials.18-22 Preliminary results with tissue fragments-impregnated polyester prostheses were promising with improved endothelialisation, and decreased myointimal proliferation with subsequent long-term patency. Fatty tissue contains numerous capillaries. It is postulated that capillary-derived endothelial cells will promote angiogenesis within graft wall.21 In bone marrow, stem cells can differentiate into endothelial cells,23 and enhance endothelial cell lining by the release of cytokines.20,21 Moreover, use of these materials seems to present other advantages such as rapid availability, without expensive and time-consuming endothelial cell isolation and culture.

The technology involving the use of autologous tissue fragments or bone-marrow cells could well influence the future of small-diameter prostheses (i.e.
2–3-mm diameter). In addition, end-to-side anastomoses which reproduce the clinical reality more adequately than end-to-end anastomoses should probably be preferred in such experiments. In the present study, the effect of omental fat emulsion and that of bone-marrow cell seeding have been compared in a canine iliac artery bypass model.

**Materials and Methods**

**Technique of graft implantation**

Surgery was performed under sterile conditions on nine adult mongrel dogs weighing within 16 and 28 kg. The experiments were conducted according to the guidelines of the Canadian Council for Animal Care and after approval by the Local Institutional Animal Care Committee. Each dog received daily 160 mg of acetylsalicylic acid orally 48 hours before the surgery to remove non-adherent fatty tissue. The grafts were soaked in DMEM containing heparin for 5 minutes prior to implantation.

The graft (W.L. Gore & Associates, Flagstaff, AZ, U.S.A.) was a 5-cm straight tube with a 3-mm diameter. Its internodal distance was 30 μm. The proximal anastomosis (end-to-side) was performed on the iliac artery at the level of the aortic trifurcation. The distal anastomosis (end-to-side) was done 5-cm distal to the first one. A running suture was done with 7-0 PTFE monofilament (W. L. Gore & Associates, Flagstaff, AZ, U.S.A.). The iliac artery was ligated between the sites of the anastomoses using a polypropylene thread. Both right and left iliac arteries were bypassed in each dog.

Dogs were randomly divided into 3 groups. In Group 1, fragments of autogenic omental fat were used to impregnate grafts prior to implantation. In Group 2, autogenic bone-marrow cells were used to impregnate grafts. In the Third Group (control group), the grafts were not impregnated with biological material.

**Doppler imaging**

Peak velocities at different levels (aorta, proximal and distal anastomoses, inside the graft, distal to the graft) were measured using Doppler ultrasound.

**Group 1 (omentumal fat)**

During surgery and before arterial clamping, about 0.5 g of fatty tissue was collected from the omentum and inserted in a 3-ml syringe. Another syringe was filled with 2 ml of culture medium (Dulbecco’s Modified Eagle’s Medium, DMEM, Sigma Chemical Co., St Louis, MO, U.S.A.) containing 90 μg heparin and connected to a 3-way valve to which was also fixed the syringe containing fatty tissue. Homogenisation of the fatty tissue was obtained by alternately passing the content of one syringe into the other while gradually closing the valve. The homogenate was then injected under manual pressure (syringe piston) into a 7-cm segment of the PTFE graft, one end of which had been ligated. Sufficient pressure was exerted so as to visualise the appearance of some of the homogenate on the external surface of the whole graft. At this stage, graft impregnation was considered valid. The graft was then extensively irrigated with culture medium to remove non-adherent fatty tissue. The graft was then implanted.

**Group 2 (bone marrow cells)**

Bone marrow was retrieved from the iliac bone with a paediatric puncture needle previously rinsed with a citrate solution. One ml of bone marrow suspension was then injected into the graft according to a technique similar to that described for Group 1. The graft was then implanted.

**Group 3 (control)**

PTFE grafts were soaked in DMEM containing heparin for 5 minutes prior to implantation.

**Scanning electron microscopy (SEM)**

Prior to implantation, short segments of impregnated grafts were proceeded with for SEM. At sacrifice, samples from patent grafts were also retrieved for SEM observation. Samples for SEM were fixed in 2.5% glutaraldehyde solution (in phosphate-buffered solution, PBS) and washed in cacodylate buffer (0.1 M in PBS). After dehydration with increasing concentrations of ethanol, samples were immersed in hexamethyldisilazane (Polysciences Inc., PA, U.S.A.). Once dried, samples were sputter-coated. Samples were observed using a Jeol 35CF scanning electron microscope at 15 kV.
were measured (image point Doppler ultrasound system, HP Sonos, Hewlett Packard, Toronto, Canada) immediately and at 1, 2 and 3 months after surgery.

**Graft explantation and histologic analysis**

Under anaesthesia, dogs were sacrificed 3 months after surgery. Prior to sacrifice, 5000 I.U. of heparin were administered intravenously and the animal was exsanguinated. The aorta and the common iliac arteries and the grafts were excised and fixed in a 4% fresh paraformaldehyde solution (in PBS at pH 7.2). Gross examination was performed. Explanted grafts were cut in three segments: (i) proximal anastomotic site, (ii) mid-part of the prosthesis, and (iii) distal anastomotic site. Anastomotic sites included portions of the native vessels. Fixed tissue specimens were then processed for serial histological sections (every 150–200 μm) transversally to the axis of the anastomoses (proximal and distal) or the prostheses (middle). Serial tissue sections were stained by Hematoxylin Phloxin Saffron and double stained by Mason’s trichrome and Weigert. Using an image analysis system (NIH Image, Bethesda, MA, U.S.A.), the luminal surface area was measured on each serial tissue section in the mid-portion of patent prostheses. The surface area measurements were respectively reported to those occupied by the lumen of the PTFE itself. This ratio was considered as the relative degree of stenosis observed on patent grafts.

**Results**

**Scanning electron microscopy**

Before implantation, fat-impregnated PTFE grafts (Group 1) were partially covered with a thin layer of biological material (Fig. 1). Bone-marrow-impregnated grafts (Group 2) were totally covered with numerous cells and a biological film. By 3 months (Fig. 1C), endothelial cells were present on the whole surface of the luminal surface of patent bypasses in Group 2, and in some areas in Group 3.

**Doppler results**

Immediately after surgery, Doppler imaging showed all grafts to be patent. Because of the small graft diameter, velocity values were high (average of 294 cm/s) compared to those found in the aorta (99.7 ± 28.4 cm/s). In Group 1, four out of the six grafts exhibited stenosis at mid-portion by 1 month and were occluded by 2 months, while the remainder presented a proximal stenosis with a slight distal stenosis. The
two latter stenoses became subtotal by 3 months. In Group 2, four out of the six grafts appeared normal while the other two showed moderate proximal stenosis by 1 month for one and occlusion by 2 and 3 months for the others. In Group 3, occlusion was found in one case by 2 months; subtotal stenoses were found at the proximal anastomoses in three grafts by 1 month which were found occluded by 3 months; two grafts appeared normal at the end of the observational period.

Macrosopy

In Group 1, all grafts were occluded at 3 months by a dense fibrotic tissue with an old thrombus. In Group 2, four grafts were patent, one presented a moderate stenosis, and the other was occluded. In Group 3, one prosthesis was infected and therefore eliminated from the study. One graft was normal and the other moderately stenotic; the other three grafts were occluded by old thrombus with some fibrotic tissue.

Histology

Occluded grafts of all groups exhibited the same pathology. The proximal anastomoses were occluded by a highly vascularised fibrotic tissue (Fig. 2) and, as serial tissue sections were away from the proximal anastomotic sites, old thrombus was present. In a few cases, occluding fibrotic tissue was observed at the anastomotic sites without clot formation. At the midportion of the prostheses, thrombi filled the lumens (Fig. 2), except in one case in Group 1, one case in Group 2 and two cases in the control group. In those latter conditions, the lumens were found patent at midportion with no endothelial cell lining or formation of new tissue on the inner surface of the grafts (i.e. neointima). At the distal anastomotic sites of these occluded grafts, both fibrotic tissue and thrombus occluded the lumen in all the groups.

In the four patent grafts of Group 2, a new layer of tissue (i.e. neointima) inconsistently covered the luminal surface of the proximal and distal anastomotic sites (Figs 2 and 3). Endothelial shape-like cells were lining the neointima. In the areas free from neointima, the luminal surface of the PTFE graft remained uncoated. Neointima covered predominantly the surface of the prostheses away from the distal or proximal anastomoses, when compared to that of the anastomotic sites. Neointima containing proliferative smooth-muscle cells (i.e. hyperplasia) was seen at the level of one proximal and one distal anastomosis, at the toe of one proximal and one distal anastomosis; and at the floor of one proximal anastomosis. The mid-portions of the patent grafts of Group 2 were completely covered with neointima of moderate thickness (Figs 2 and 3) which reduced luminal surface by 26.9±3.4%. Neointima had a smooth surface covered with endothelial cell lining. Endothelial cells were positive for factor VIII-related antigen as observed by immunohistochemistry. In addition, the neointima seemed to tightly bind the PTFE internal surface.

Conversely, in the patent grafts (2/5) of the control group (Group 3), neointima with endothelial-shape-like cells was found at the proximal and distal anastomotic sites. However, the neointima, which did not exceed 30 μm in thickness, was limited to an area close to the suture line in most cases, and did not extend away from the anastomotic sites. The rest of the anastomoses were devoid of endothelial cells or neointima. Concerning hyperplasia at the anastomotic sites, it was observed at the heel and at the toe of two proximal and of one distal anastomotic site, and at the floor of one proximal and of one distal anastomotic site. At the mid-point of the graft, thick neointima was found with endothelial-shape-like cells (factor VIII-positive cells) in one graft, and in another case the PTFE graft was covered with a thin layer of blood (Figs 2 and 3) with no endothelial cell lining. The average of the luminal surface was reduced by 13.4±0.8% at mid-portion of the patent grafts of Group 3.

Discussion

Efforts to bring small diameter vascular prostheses to clinical use have focused on luminal cell lining in order to obtain a biological barrier to thrombus formation and intimal hyperplasia. Among polymers used to produce small-diameter prostheses, PTFE seems to offer optimal biomechanical and haemo-compatible properties. PTFE-impregnation with bone marrow cells or fatty tissue could be an alternative to direct endothelial-cell seeding or sodding. We felt it important from a clinical point of view to perform end-to-side anastomoses which present stressful haemodynamic conditions, and since this type of anastomosis is preferentially used in peripheral and coronary bypass surgery.

Following implantation in our canine model, PTFE grafts impregnated with omental fat led to rapid occlusion by fibrotic tissue. Conversely, bone marrow
impregnation of the grafts appeared to improve the quality of the healing process. Specifically, the healing pattern resulted in a smooth covering of the luminal surface by endothelial cells as demonstrated by the presence of factor VIII. Decreased thrombogenicity could be consequently expected in 66% of the grafts of Group 2. Non-impregnated grafts of Group 3 were expected to be thrombogenic. However, the small numbers of animals in each group suggests caution in interpretation of the results.

In our experience, fatty tissue failed to improve patency of the 3-mm PTFE grafts. This could be due to intraluminal growth of omental tissue but this was not substantiated by histology. However, thrombogenicity of the homogenate is significant since Group 1 demonstrated no graft patency by 3 months, a result inferior to that of the control group. Grafts’ impregnation with fatty tissue might be more appropriate for porous grafts as reported by Noishiki and colleagues\textsuperscript{18,22} using Dacron or high-porosity PTFE, probably due to wound tissue ingrowth. However, Dacron prostheses are not available in small diameter (≤3 mm), and high-porosity (i.e., 90 μm) PTFEs are subjected to dilation and rupture. On the other hand,
Fat- and Bone Marrow-impregnated PTFE Grafts

on the luminal surface. By histological assessment, this neointima consisted of a dense fibrotic tissue with no sign of a hyperplastic process which is observed, however, in the patent grafts of the control group. A recent study using in situ hybridisation demonstrates the absence of PDGF expression in this neointima (personal communication). This suggests that bone-marrow cells could enhance the wound-healing process. Newly formed tissue is rapidly laid down onto the surface of this synthetic material. On the other hand, the anastomotic sites, and more specifically the distal site, were exposed to critical haemodynamic stress with increased risks for hyperplasia development. In our end-to-side model, no hyperplasia was seen at the floor of the recipient artery at the distal site in Group 2, and minimal hyperplasia at the heel in Group 2, compared to that found in the control group. PTFE grafts at the anastomotic sites showed partial coverage with neointima compared to that seen in the middle part of the graft itself. This phenomenon might be due to (i) surgical manipulations during the impregnation and/or suture phases that could induce detachment of marrow cells; and (ii) haemodynamic stresses induced by an end-to-side anastomosis. Our findings observed in Group 2 with an end-to-side anastomosis are close to those of Noishiki et al. who observed a decrease in myointimal hyperplasia after implantation of bone-marrow-impregnated grafts in end-to-end anastomoses. Although 40% of the grafts were patent in the control group at 3 months, one could expect occlusion to occur in the rather near future since the original thrombogenicity of the graft had not been altered.

Fall out endothelialisation has been shown to occur in human. More recently, Shi et al. have clearly shown that endothelial cells lining a vascular prosthesis can be derived from the marrow because a subset of bone-marrow cells have the ability to differentiate into endothelial cells. On the other hand, reduction in progression of intimal hyperplasia may be achieved if a stable endothelial-cell lining can be formed close to or at the time of graft implantation.

In contrast to endothelial-cell seeding that needs extensive preoperative preparation in terms of cell culture and in terms of graft preparation using adhesive peptides or protein coating, acceleration of the vascular healing process could be achieved by directly introducing bone-marrow cells into the microstructure of PTFE. The technique of impregnation of autologous tissues, more specifically bone marrow, that we used

Fig. 3. Histologic sections of patent grafts. In patent grafts (2/6) of Group 3 (control), the luminal surface of the graft was irregularly covered by a thin layer, mostly composed of blood elements with some foci of hyperplasia (A). In patent grafts (4/6) of Group 2 (bone marrow), neointima covered the whole surface at mid-portion (B) and partially at the anastomotic sites (C). (× 30).
and which was originally reported by Noishiki and colleagues, could easily be performed during surgery. This is an additional advantage compared to other alternatives. Refinement of the technique, such as introducing bone-marrow cells into the microstructure of PTFE, could further improve the graft patency rate compared to virgin PTFE grafts. A larger number of animals and implanted grafts would be necessary in order to statistically validate our results.

Acknowledgements

This research has been supported by the Heart Stroke Foundation of Quebec, Canada. PTFE grafts and sutures were kindly provided by W. L. Gore & Associates.

References