Development of Intimal Hyperplasia in Six Different Vascular Prostheses

P. Y. Ao, W. J. Hawthorne*, M. Vicaretti and J. P. Fletcher*

Department of Surgery, The University of Sydney, Westmead Hospital, Westmead, NSW, 2145, Australia

Objectives: to compare six vascular prostheses for the development of intimal hyperplasia (IH) in a sheep model.

Material and methods: prostheses tested were gelatin sealed Dacron (GSD), fluoropassivated Dacron (FPD), Fluropassiv™ (FD), expanded polytetrafluoroethylene (ePTFE), carbon-lined expanded polytetrafluoroethylene (CL-ePTFE) and vascular access graft (VAG). Sixty-two adult female Merino sheep (35–45 kg) were used. Elliptical graft patches were implanted into the left common carotid artery using one of the six graft types: GSD (n = 10), FPD (n = 10), FD (n = 12) VAG (n = 10), ePTFE (n = 10), or CL-ePTFE (n = 10). Four weeks later grafts were removed for histopathological assessment and measurement of the degree of IH obtained on a computerised image analysis system.

Results: IH indices were significantly less for FPD (0.191 ± 0.095, p < 0.05), FD (0.199 ± 0.081, p < 0.05), ePTFE (0.213 ± 0.078, p < 0.05) and CL-ePTFE (0.161 ± 0.066, p < 0.01), compared to the GSD group (0.287 ± 0.077). The VAG group (0.257 ± 0.091) showed no difference compared to GSD. There was no significant difference between the FPD, FD, ePTFE and CL-ePTFE grafts.

Conclusion: this study indicates that less IH occurred in the two ePTFE grafts and two fluoropolymer coated Dacron grafts than in gelatin sealed Dacron or polyurethane grafts.

Key Words: Intimal hyperplasia; Vascular prostheses; Polyester; Polytetrafluoroethylene; Polyurethane.

Introduction

In peripheral vascular surgery, implantation of a synthetic vascular prosthesis is necessary for the restoration of distal blood flow when suitable autologous vein grafts are unavailable. Synthetic vascular prostheses are prone to development of intimal hyperplasia (IH), a common complication that can lead to the progressive occlusion of implanted grafts.1–3 IH is a tissue reaction, which is thought to be caused primarily by injury to the endothelial layer of the blood vessel.4–5 The lesion is usually located between the endothelium and the internal elastic lamina of the involved artery or on the inner surface of the implanted graft, consisting of an abnormal migration and proliferation of vascular smooth muscle cells (SMCs) and deposition of extracellular matrix. Compared with autologous veins, synthetic vascular grafts lack an endothelial lining, having instead a fibrous capsule with a fibrin or collagenous inner surface, which is exposed to blood flow. This creates a relatively thrombogenic environment which may be susceptible to IH. Some studies indicate that the presence of polyester fibres activate cells and complement components which are involved in the IH process.6 Polyester and polytetrafluoroethylene materials remain as popularly used arterial blood conduits. Aided by advances in technology, the current generation of vascular prostheses have been developed to mask the highly reactive surface of synthetic prostheses by using different substances such as gelatin, albumin, collagen and carbonate to coat the graft. These grafts maintain the essential characteristics of classical synthetic grafts such as knitted and woven designs, variable porosity and good bioresilience with some reported advantages over the older versions of vascular prostheses, including increased antithrombogenicity7 and possible inhibitory effects on IH development.8 Although the healing characteristics of vascular prostheses have been reported extensively,1–3,9 there is limited information regarding the effect on the development of IH caused by the manipulation of adding agents such as fluoropolymer and carbon to the grafts. The present study aimed to evaluate and compare the development of IH in six different prosthetic vascular grafts comprised of three families. These were: (a) polyester grafts, gelatin sealed Dacron–Gelsoft® (GSD), two types of fluoropolymer coated Dacron – an earlier version “fluoropassivated” Dacron (FPD)
Materials and Methods

Vascular prostheses

Six types of prosthetic vascular graft were selected for this study. They are described under the following classifications:

1. Polyester prostheses. The grafts used in this class included the GSD, FPD and FD. GSD is a knitted Dacron prosthesis that is impregnated with gelatin. FPD and FD are two new modified models of GSD where the surfaces of these grafts are treated with a novel fluoropolymer before sealing with gelatin. These grafts were manufactured by Vascutek Ltd., Inchinnan Renfrewshire, Scotland, U.K.

2. PTFE prostheses. The ePTFE and CL-ePTFE were the two grafts used in this class. The ePTFE graft was manufactured by W. L. Gore & Associates Inc., Flagstaff, Arizona, U.S.A. The CL-ePTFE graft is a new generation ePTFE impregnated with pyrolytic carbon bonded to the inner surface and was manufactured by Impra, Inc., Tempe, Arizona, U.S.A.

3. Polyurethane prostheses. The V AG graft belongs to this class of graft. The V AG graft is made from a polyuretheneurea biomaterial (BPS-215M) with a surface-modifying additive (silicone). The wall of the V AG graft is capable of bearing multiple punctures and is intended for use in patients who require long-term periodic access to their vascular system. It was manufactured by Thoratec Laboratories Corporation, Nepean, Canada.

Surgical procedure

Sixty-two adult Merino sheep weighing 35–45 kg were used. The study was approved by the Animal Care and Ethics Committee at Westmead Hospital with all animals housed and cared for under the guidelines of the Australian National Health and Medical Research Council. The sheep were premedicated with intramuscular xylazine (Ilium Xylazil 1 mg/kg, Troy Laboratories Pty. Ltd., Sydney, Australia) and atropine (10 µg/kg, Astra Pharmaceuticals Pty. Ltd., Sydney, Australia). General anaesthesia was induced with thiopentone (16 mg/kg, Abbott Australasia Pty. Ltd., Sydney, Australia) and maintained on inhalation of 1–2% halothane (Zeneca Ltd., Macclesfield, U.K.) in oxygen after intubation. The graft materials were cut into a fusiform patch of 5 cm length and 0.8 cm width before implantation. The graft patch implantation was performed in sheep according to our previously established protocol. In brief, the left common carotid artery was exposed via a longitudinal incision (6–10 cm in length) in the left side of the neck and 5000 IU of heparin (David Bull Laboratories, Sydney, Australia) was given intravenously. A patch of vascular prosthesis (GSD n = 10, FPD n = 10, FD n = 12, V AG n = 10, ePTFE n = 10, or CL-ePTFE n = 10) was sutured into the common carotid artery using a continuous 6.0 polypropylene suture after making a longitudinal arteriotomy. The wound was closed in two layers with continuous 2.0 poliglecaprone to muscle and 2.0 polypropylene to skin. The sheep were given analgesia (Buprenorphine, 0.01 mg/kg, Reckitt & Colman Products Ltd., Hull, U.K.) on waking, allowed to recover indoors overnight then released to pasture after inspection the following morning.

Specimen collection

At the end of the 4-week study period, animals were sacrificed with intravenous sodium pentobarbitone (Euthal 85 mg/kg, Delta Veterinary Laboratories Pty. Ltd., Sydney, Australia) after xylazine premedication. The patch grafts were harvested together with adjacent artery and immediately irrigated with 10% buffered formalin. The grafts were examined grossly for signs of infection, patency, disruption and perigraft tissue incorporation. They were then immersed in 10% buffered formalin for at least 24 h before sectioning. Eight transverse sections were taken at equidistant intervals along the graft. The specimens were dehydrated and paraffin blocks prepared. One section (5 µm thick) from each of the eight specimen blocks was stained with haematoxylin and eosin.

Cell type analysis

Further paraffin sections were stained with avidin-biotin complex immunoperoxidase technique for
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Fig. 1. Diagram illustrating the methodology for measurement of IH index: the area of intimal hyperplasia (IH) formed on the inner surface of the graft and the width of the graft in the transverse section of the grafted arterial segment measured by an image analysis system (Optimas, USA). IH index = the area of IH/the width of the graft.

IH assessment

Each haematoxylin and eosin stained cross-section was observed through a camera mounted on a microscope connected to a computerised image analysis system (Optimas, Optimas Corporation, Seattle, U.S.A.). Each of the transverse sections was measured using the image analysis system for both the area of intimal thickness developed on the internal surface and the width of the graft. A ratio (IH index) of the area of intimal thickness (mm²) on the internal surface of the graft to the width of the graft (mm) was obtained from each transverse section (Fig. 1).

Statistics

Data processing and analysis was accomplished with a computer software program (Microsoft, Excel version 5). All data were expressed as mean ± standard deviation (SD). Two-way analysis of variance (ANOVA) was used to compare the IH indices between groups. A p value of less than 0.05 was accepted as being statistically significant.

Results

Four sheep were excluded from the current study due to unrelated complications. They included: one sheep which died from an anaesthetic complication in the FD group, one infected graft from the CL-ePTFE group, and two occluded grafts, one each from the FD group and the CL-ePTFE group due to faulty suturing technique. A total of 58 sheep were available for final analysis. There were 10 animals in each of the GSD, VAG, ePTFE, FPD and FD groups and eight in the CL-ePTFE group. During the 28-day observation period, all sheep remained healthy with no sign of ill health or distress.

The six prostheses had a similar macroscopic appearance, which comprised an outer capsule surrounding the whole grafted arterial segment, with considerable perigraft tissue incorporation. The outer capsule felt hard and looked similar to scar tissue, and appeared thicker in the GSD, FPD and FD grafts than those of the VAG, ePTFE and CL-ePTFE. The inner surfaces were smooth and glistening, with sparsely localised flattened red thrombi.

Upon microscopic examination (×12.5) the inner surfaces of all six prostheses were entirely covered by a layer of thick fibrous tissue. The distribution of IH in the six different prostheses followed a unique pattern in which IH was universally prominent on the internal surface of the graft and most marked on both the artery and graft edges at the site of the anastomosis, but was less apparent away from the suture line. The thickened intima usually extended across the anastomosis, producing a smooth transition between graft and artery (Fig. 2). The IH was easily identified, as its structural appearance was different to that of the prosthetic graft, which stained less intensely than the media of the native artery.

The extent of IH varied considerably between the different graft materials (Fig. 3). Observation under higher magnification light microscopy demonstrated that the cellular components of the IH lesion were similar in each of the six types of vascular grafts. SMC-like cells dominated the field with relatively fewer inflammatory cells such as lymphocytes, eosinophils and macrophages. Fibrotic extracellular matrix was extensive between these cells. A monolayer of endothelial coverage was generally seen on the internal surfaces of the prostheses but was associated with occasional disruption. The adjacent carotid artery had a less thickened intima, which was covered by a continuous layer of endothelium. Upon examination of the cross-section view, all of the six grafts revealed infiltration of granulation-like tissue to various extent within the matrix of the graft fibres (Fig. 4). The ingrowth of tissue in the graft interstices mainly consisted of SMCs and sparsely distributed inflammatory cells. The implanted prostheses were surrounded by
Fig. 2. Photomicrograph of transverse section of Gelsoft patch graft four weeks after implantation showing the characteristic distribution of intimal hyperplasia, which maximised in areas near the anastomoses, producing a smooth transition between graft and artery. Arrows indicate intimal hyperplasia; A, arterial wall; G, Gelsoft graft. (Original magnification ×12.5).

foreign body giant cells, showing a foreign body reaction to the graft material. The adventitia was fibrotic with mild chronic inflammation.

In terms of the morphological appearance of the graft material some differences as well as tissue reaction were observed between the different types of prostheses. The microscopic appearance of the GSD, FPD and FD grafts were quite similar, with a bluish amorphous pool matrix with doubly-refractile material. Some isolated gel-like material was occasionally found in the GSD graft, which might indicate incomplete resorption of the gelatin substance. The wall of these three types of grafts was structurally disturbed and invaded by surrounding tissue. The ingrowth tissue occupied the spaces between fibres, forming an unevenly distributed pattern (Fig. 4A–C). Compared to the PTFE and polyurethane, the polyester prosthesis had fewer T lymphocytes (CD3) and dendritic cells within the synthetic materials, but the perigraft inflammatory response in this group was stronger with prominent formation of foreign body cells (giant cells). In the PTFE prostheses, both the ePTFE and CL-ePTFE grafts revealed a doubly-refractile wavy stranded appearance. The CL-ePTFE grafts had a thin layer of carbon, which stained as aggregated black spots on the internal surface. The cellular infiltration was more extensive within the carbon layer than in the rest of the graft. Unlike the Dacron grafts (GSD, FPD and FD), the structure of the ePTFE and CL-ePTFE grafts was generally intact with a lesser extent of perigraft tissue ingrowth but was infiltrated by pronounced immunocompetent cells (Fig. 4D & E). The Thoratec VAG grafts of polyurethane prostheses revealed a reticulated structure, which stained poorly and was non-refractile (Fig. 4F). It was infiltrated by a moderate amount of immunocompetent cells evenly throughout the whole of the graft material. The perigraft inflammatory response in the VAG graft was highly variable, with the greatest perigraft accumulation of immunocompetent cells in some cases. EC coverage was seen in all of the six types of prostheses but unevenly distributed pattern (Fig. 4A–C). Compared to the PTFE and polyurethane, the polyester prosthesis appeared to be more apparent in the FPD, FD, ePTFE, CL-ePTFE and VAG grafts compared to the GSD grafts. The best endothelial coverage was seen in the CL-ePTFE graft.

The IH index (mean ± S.D) of each group is summarised in Table 1. The GSD group had an IH index of 0.287 ± 0.077; the VAG group, 0.257 ± 0.091; the ePTFE group, 0.213 ± 0.078; the FPD group, 0.191 ± 0.095; the FD group, 0.199 ± 0.081 and the CL-ePTFE Group,
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Discussion

The events involved in the healing process around vascular prostheses in humans and animals have been well documented.\textsuperscript{13-15} IH has been established as a major cause of occlusion of small and medium size arterial prostheses.\textsuperscript{16} Although the intricate pathophysiological mechanisms involved in the formation of IH are not fully understood, it is believed that IH is the ultimate result of the complex interaction of both cellular and enzymatic systems which are influenced by multiple factors. Regardless of the type of vascular anastomosis or type of graft implantation, damage to the vascular endothelium has been recognised as an important factor in initiating IH development.\textsuperscript{5} The patch graft model has a greater margin of anastomosis between the graft and the adjacent artery. This provides substantial intimal injury and allows multiple sampling points for intimal hyperplasia assessment. Therefore, we consider that this model is more acceptable than the interposition model. Other mechanisms such as haemodynamics, shear stress\textsuperscript{17,18} and flow velocities,\textsuperscript{19} are also implicated in this pathological activity, but such effects are not examined in our current study. Furthermore, much evidence has suggested that IH can be potentially affected by the properties of prosthetic grafts themselves. Synthetic

0.161 ± 0.066. There was no significant difference between the GSD and VAG groups. Compared with either of the GSD or VAG groups, significantly lower IH indexes were obtained for the ePTFE group (p<0.05), the FPD group (p<0.05), the FD group (p<0.05) and the CL-ePTFE group (p<0.01). However, there was no significant difference between FPD, FD, ePTFE and CL-ePTFE.

Fig. 3. Photomicrographs of transverse sections of six different vascular prostheses showing various degrees of intimal hyperplasia. (A), GSD graft; (B), FPD graft; (C), FD graft; (D), ePTFE graft; (E), CL-ePTFE graft; (F), VAG graft. Arrows indicate intimal hyperplasia. (Original magnification × 63).
Fig. 4. Photomicrographs of transverse sections of six different vascular prostheses showing the infiltration of granulation-like tissue in the matrix of the graft fibres and disturbance of the graft structure. (A), GSD graft; (B), FPD graft; (C), FD graft; (D), ePTFE graft; (E), CL-ePTFE graft; (F), VAG graft. Arrows indicate infiltrated tissue. (Original magnification × 160).

Table 1. Mean IH indices of the six vascular prostheses.

<table>
<thead>
<tr>
<th>Graft type</th>
<th>Number</th>
<th>Mean IH index</th>
</tr>
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<tbody>
<tr>
<td>Polyester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelsoft® gelatin sealed Dacron (GSD)</td>
<td>10</td>
<td>0.287 ± 0.077</td>
</tr>
<tr>
<td>Fluoropassivated Dacron (FPD)</td>
<td>10</td>
<td>0.191 ± 0.095</td>
</tr>
<tr>
<td>Fluropassiv™ (FD)</td>
<td>10</td>
<td>0.199 ± 0.081</td>
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<tr>
<td>Polytetrafluoroethylene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gore-Tex® polytetrafluoroethylene (ePTFE)</td>
<td>10</td>
<td>0.213 ± 0.078</td>
</tr>
<tr>
<td>Impra® carbon-lined polytetrafluoroethylene (CL-ePTFE)</td>
<td>8</td>
<td>0.161 ± 0.066</td>
</tr>
<tr>
<td>Polyurethane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoratec® vascular access graft (VAG)</td>
<td>10</td>
<td>0.257 ± 0.091</td>
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* Data are expressed as mean ± SD.

† p<0.05 vs Gelsoft® gelatin sealed Dacron.

‡ p<0.01 vs Gelsoft® gelatin sealed Dacron.

grafts are more prone to IH than autologous saphenous vein grafts. The implantation of a synthetic vascular prosthesis produces a mismatch of mechanical properties and elasticity to the native artery, creating a high stress at the anastomosis, which may be responsible for the development of IH. Other factors which may affect IH development include the type of graft material, porosity and substances used for im-
pregnated of the graft. The potential to manipulate the mechanical and biological properties of the graft to minimise IH formation has become a driving force for continued efforts to produce an ideal vascular prosthesis.

Our previous and current studies demonstrate that IH is a universal phenomenon associated with implantation of synthetic vascular prostheses. Examination of the IH lesion revealed that SMC-like cells, which are indistinguishable between mature SMCs and myofibroblasts, are the dominant cellular component embedded in the extracellular matrix. This morphologic characteristic is comparable with the classical pathology of IH developed in Dacron and ePTFE grafts implanted in humans, documented by other authors. The SMCs are not indigenous cells to the site of IH. The origin of these migratory cells is currently undetermined. Three possible sources have been proposed: firstly, ingrowth of tissue derived from the cut edge of the adjacent artery, secondly, fallout of circulating cells, thirdly, transinterstitial ingrowth through the porous prosthesis. In this study, the distribution of IH was similar in the six prosthetic grafts, with IH being generally greater on the areas near the anastomotic edges and gradually thinner at points more distal (Fig. 2). This characteristic distribution of IH suggests that the tissue is primarily derived from the native artery and migrates across the anastomosis to cover the whole inner surface of the patch graft. On the other hand, this study has also shown that perigraft tissue invades into the graft, suggesting that the transinterstitial tissue ingrowth at least in part might contribute to the development of IH. The extent of neovascularisation inside the graft was shown to correlate to the porosity of the prostheses (the porosity of the three graft types: polyester > polyurethane > PTFE). More importantly, the degree of IH in the GSD, ePTFE, CL-ePTFE and VAG prostheses revealed correlated relationships to their porosity (Table 1), suggesting the role of transgraft tissue ingrowth in the development IH.

The GSD graft is a knitted Dacron prosthesis which has been impregnated with absorbable protein (Gelatin) in order to achieve zero porosity at the time of operation. The gelatin sealant hydrolyses from the graft over a period of 14 days by non-enzymatic mechanisms after implantation. The gradual degradation of the gelatin allows for unimpaired tissue incorporation into the interstices of the knitted fabric skeleton of the graft to provide a good healing conduit. This feature may provide the grounds for the significant amounts of IH formation in the GSD grafts as demonstrated in the current study. The FPD and FD grafts are modified versions of the GSD graft, in which a novel fluoropolymer is coated onto the inner surface of the polyester graft before sealing with gelatin. They maintain the basic structure and biomechanical characteristics of GSD. This study showed that both FPD and FD had significantly less IH compared with GSD (p<0.05, Table 1), suggesting the possible role of fluorine in inhibition of IH development. The mechanism behind this effect is not known. It may be linked to the multiple effects of fluorine on inflammatory response, EC growth and thrombogenicity. Guidoin et al. compared the healing behaviour of the fluoropassivated and the non fluoropassivated grafts in a canine model and found that fluoropassivated grafts gave a milder inflammatory response with a more complete and mature degree of endothelialisation. Our results partially concur with their study: we showed a similar degree of perigraft inflammatory response in the three polyester grafts but a better endothelial coverage in the FPD and FD grafts compared to the GSD grafts. This suggests that the inflammatory treatment applied to the surface of the polyester grafts might suppress IH by promoting EC growth. In addition, fluoropassivated graft has been reported to have better antithrombogenicity than non-fluoropassivated Dacron graft that could inhibit the early events in IH formation. The CL-ePTFE graft is an ePTFE graft impregnated with pyrolytic carbon on its inner surface. Deposition of pyrolytic carbon on a prosthetic surface has been demonstrated to enhance haemocompatibility and EC growth. The CL-ePTFE grafts have also shown decreased platelet accumulation and thrombogenicity compared to standard ePTFE. Bacourt recently reported the results at 2 years of a randomised study comparing CL-ePTFE and ePTFE graft patency. The CL-ePTFE graft had better patency than standard ePTFE graft for below-knee popliteal and distal bypass. In our current study, despite a better endothelial coverage found in the CL-ePTFE grafts, the results failed to reveal a statistically significant difference between the two grafts in terms of the development of IH, although the CL-ePTFE group (0.161 ± 0.066) had a lower IH index than ePTFE (0.213 ± 0.018). This may be due to the small number of animals in each experimental group.

This study showed an interesting association between the degree of IH and the immunoinflammatory response. The smaller amount of IH was found in the groups of ePTFE and CL-ePTFE associated with more prominent intragraft infiltration of immunocompetent cells and less perigraft inflammatory response compared with the GSD group. More interestingly, the
CL-ePTFE graft, which had the lowest IH index among the six graft types, was found to have more immunocompetent cell accumulation within the carbon layer than the rest of the graft body. Furthermore, the VAG grafts revealed a considerably higher IH index than all of other graft types except the GSD grafts, which was associated with a moderate amount of immunocompetent cells within the graft and more prominent perigraft inflammatory compared to the polyester and PTFE grafts. These associations strongly suggest that the development of IH is inhibited by intragraft infiltration of immunocompetent cells and is promoted by perigraft inflammatory response. However, the same association was not shown in the groups of FPD and FP, in which the effects of the immuno-inflammatory response on the development of IH may have been compromised by the inhibitory effect of fluorine. It is not understood why the patterns of immuno-inflammatory response differ between these graft materials.

Pressure perfusion fixation with glutaraldehyde prior to graft removal is an effective method to obtain constant morphometric quantitation of IH. However, this method was not used in this study since the measurements of IH were only taken on the surface of the implanted graft. This graft material is a fixed structure, which is not prone to change in size or structure following its explantation. In addition, we have found that the immediate irrigation of the graft and artery segment with 10% buffered formalin prior to paraffin blocking minimises morphological changes.

In conclusion, this study shows that the two fluoropolymer coated Dacron grafts (FPD and FD) and the two PTFE grafts (ePTFE and CL-ePTFE) produce significantly less IH in compared to GSD and VAG. The development of IH is inhibited by intragraft infiltration of immunocompetent cells and is promoted by perigraft inflammatory response. The effect of synthetic vascular prostheses on the development of IH may be dependent on the chemical property, porosity and impregnated substances of the graft. Synthetic vascular grafts with an inner coverage of fluoropolymer and pyrolytic carbon may have an inhibitory effect on the development of IH.

Acknowledgments

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