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HEALING OF PROSTHETIC ARTERIAL GRAFTS

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Abstract

Numerous synthetic biomaterials have been developed as vascular substitutes. In vitro, ex vivo and in vivo studies have demonstrated that in animals, selected materials, i.e., Dacron and ePTFE (expanded polytetrafluoroethylene) grafts, are successfully incorporated in both the large and the small caliber host arteries through a process which is generally referred to as graft healing. Morphologically, this process consists of a series of complex events including fibrin deposition and degradation, monocyte-macrophage recruitment and flow-oriented cell-layer generation, this last event being the complete endothelialization of the arterial substitute.

In contrast to experimental animals, the flow surface of synthetic vascular grafts remains unhealed in humans, particularly in the small caliber conduits. Healing in man consists of graft incorporation by the perigraft fibrous tissue response with a surface covered by more or less compacted, cross-linked fibrin.

It is therefore obvious that: i) marked differences in graft healing exist between animals and man; and ii) the usual mechanisms of graft endothelialization are partially ineffective in man.

In order to guarantee the patency of synthetic vascular grafts for human small artery bypass, new strategies and approaches have recently been attempted. In particular, the endothelial cell seeding approach has been successfully accomplished in animals and is being experimented in human clinical studies.

The problems and results of this biological approach are outlined in this paper.

KEY WORDS: Transmission electron microscopy, Scanning electron microscopy, Prosthetic Grafts, Human, Animals, Graft Healing, Endothelial Cell, Seeding.

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Introduction

The incorporation of synthetic vascular grafts in the host artery has been studied for a long time by many investigators (8, 71, 74, 76). Features suggesting an efficient graft healing have been outlined (12, 13, 19, 30, 62, 69, 74). It was subsequently suggested that a neointimal coverage on the implanted prosthetic material could significantly improve the clinical performance of high-flow and even more of low-flow small caliber grafts (7, 28, 79, 81). In particular, graft endothelialization is currently considered one of the essential factors influencing patency or occlusion of prosthetic grafts (7, 44, 81). However, the promising experimental results have not yet been confirmed in man (1, 7, 15, 24, 56, 76).

This study deals with the healing of synthetic vascular grafts in animals and in humans. Particular attention will be paid to the recent techniques of seeding vascular grafts with endothelium.

Spontaneous Graft Healing in Animals

During the 1950s and early 1960s, Wesolowski (74, 77) screened more than 300 different materials in the growing pig and the adult dog. As a result of a number of observations, a relative scale of biological acceptability was established, in which synthetic materials occupied an intermediate position. During the last few years, all but Dacron materials were rejected for different reasons. Thereafter, new biomaterials (e.g., expanded polytetrafluoroethylene, ePTFE) have been introduced. These are the only plastic materials used in large and small diameter grafts in humans.

Dacron grafts

Morphological studies have made it possible to define the basic pattern of Dacron healing and its eventual complications. Basically, Dacron heals through an early fibrinous incorporation which is gradually organized and converted into a tenacious and firmly adherent fibrous capsule (74, 77).

The initial fibrin-implant complex is relatively stable and can remain essentially unchanged for weeks or even months, depending on the species of animal utilized. Interestingly, fibrin organization by granulation tissue requires a sufficient graft porosity (19, 30, 62, 77).

From the beginning of its formation, the fibrin lining is continuously undergoing intense rearrangements. The trapped leukocytes and particularly the macrophages phagocytize fragments of fibrin and cell

debris and release proteolytic enzymes which begin to digest the protein-rich luminal coverage. Concurrently, fibroblasts and small blood vessels proliferate and spread out through the graft interstices. In time, the entire fibrinous lining is converted into a well-vascularized connective tissue. Fibrous tissue contracts in the course of weeks to months. However, the grafts presents characteristic elasticity and compliance which is not adequate enough to balance the tensile strength generated by the normal scar contraction. Particularly at the level of the graft interstices, verification of a discrepancy between diverse tensile forces with consequent breakage and thrombosis of capillary channels may occur. This event may cause either a simple delay in the healing process or may even induce a band of necrosis, in areas where the healing phenomenon proves to be particularly intense, followed by calcification directly behind the prosthetic mesh. Complications of this nature, concerning the healing graft process designated by Wesolowsky (77, and also quoted by Wesolow in 74) as inner capsule degeneration, is believed to be responsible for early graft failure, including perigraft seroma and hematoma and graft occlusion. The rate of healing complications is inversely related to graft porosity as described by the classic calcification index curve from Wesolowski (77).

Various authors have described endothelium on the inner side of the prostheses with both fibroblasts and smooth muscle cells forming the bulk of the underlying intima. There is general agreement that graft endothelialization may originate from the host artery next to the suture lines (7). In contrast, other authors claim that endothelium may derive from capillary ingrowth into the graft interstices (12, 13) as well as from a blood source (22, 46, 52, 59). In particular, Harker suggested that the endothelial lining might be generated by endothelial microemboli which may be present in the blood flow (26). Moreover, evidence of the transformation of mononuclear cells into myofibroblasts and endothelial cells was provided by Feigl et al. (18).

Most probably there is a variable contribution of the different sources to the fully endothelialized grafts. In general, the host artery represents the most common source even though a kinetic study suggests that it is not very effective (57). In the low porosity graft, a hyperplastic response develops on the part of the artery to which the graft is sutured. This reaction is called pannus (1) and slowly extends along the inner aspect of the prosthesis. By transmission electron microscopy (TEM), the pannus consists of undifferentiated mesenchymal cells, activated smooth muscle cells, fibroblasts and blood cells embedded in a loose extracellular matrix (Fig. 1). At surface level, the advancing edge of endothelial cells while migrating towards the fibrinous lining can be appreciated (48, 52). In the porous materials, the granulation tissue allows the formation of numerous blood vessels in the inner fibrinous aspect of the graft. The capillaries migrating through the interstices of the prostheses can therefore provide multiple sources of endothelium at the luminal surface (Fig. 2).

ePTFE (expanded polytetrafluoroethylene) grafts

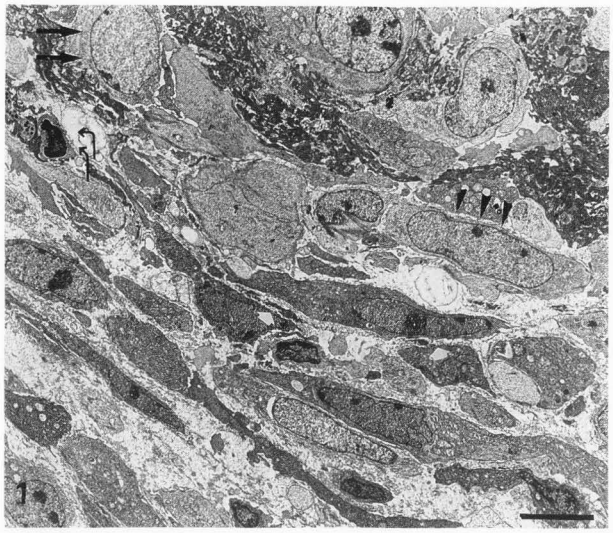
ePTFE is a non-textile material which is hydrophobic at the time of insertion, gradually becomes wet, and within 48 to 72 hours becomes filled with fibrin and blood cells. The sequence of healing is very similar to that of Dacron prostheses (8). However, the ingrowth of fibroblast and collagen tissue through the ePTFE pores proceeds more slowly than in Dacron because of the low porosity of the initially used materials. For the same reason, the endothelialization of conventional ePTFE grafts (30 micrometers internodal distance) also proceeds very slowly. According to Clowes et al. (12), by 12 months only 60% of the grafts 6-9 cm in length were fully healed. Smooth muscle cells and endothelial cells were provided from anastomotic arterial sources. In a further study, Clowes et al. (13) provided the evidence that an increase in graft porosity (60 micrometers internodal distance) can radically alter the mechanisms of ePTFE healing. By scanning electron microscopy (SEM), a confluent layer of endothelial cells associated with multiple small capillary orifices was observed within 2 weeks of ePTFE implantation, whereas a complete endothelial lining was obtained after 4 weeks in baboons. In this experimental design, endothelium was provided by rapid transmural capillary ingrowth through the graft pores whereas smooth muscle cells most probably originated from pericytes surrounding endothelial cells.

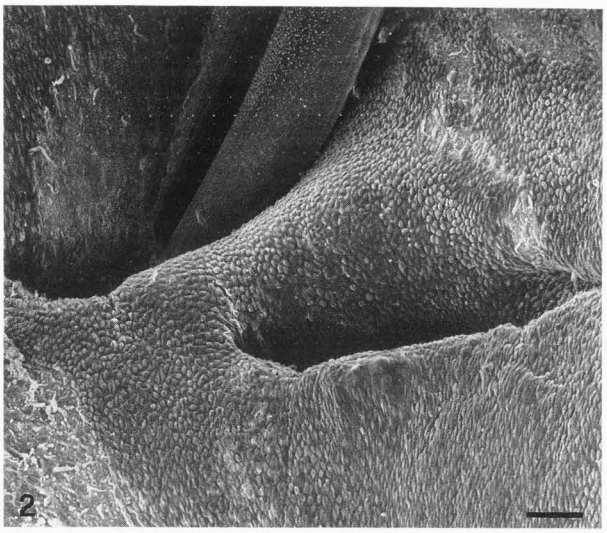
Compound prostheses

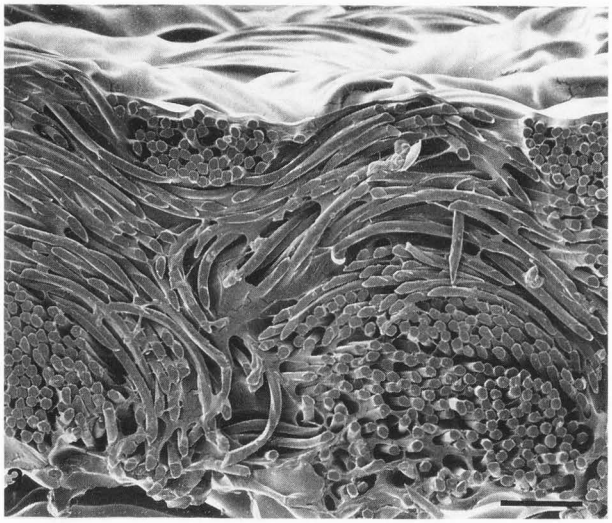
Compound arterial prostheses represent the latter advance in the field of graft manufacturing. As originally suggested by Wesolowski et al. (75) these prostheses are composed of two or more substances, where one of the components is readily resorbable by the body and the other one remains permanent. The rationale for this design is to produce a material that has a low or zero porosity, a consequent minimal controlled blood loss at implantation and a significantly higher porosity, due to resorption of the biodegradable component at the time of the required healing. Different combinations have been tested with varying results.

Glutalardehyde-fixed albumin-coated Dacron grafts have a very low porosity (100 ml/min/cm^2) at implantation, but may be somewhat thrombogenic (25). Heat-denatured albumin-coated Dacron grafts produce better results. These materials have a smooth, homogeneous, well-fixed flow surface that is apparently thromboresistant. The heat-denatured albumin is resorbed within 4 weeks after implantation, and long-term healing is not affected (61). However, a delayed early tissue incorporation is reported and no immediate advantages besides the initial low porosity are evident.

Wesolowski and associates screened numerous grafts in which the biodegradable component was collagen (75). Most of these materials developed aneurysms and subsequent hemorrhage in animals. The same authors warned that these materials can interfere with graft healing and even accelerate graft occlusion (75). In contrast, Humphries et al. (36) obtained encouraging results in dogs. Prostheses presealed with insoluble collagen were completely organized by fibrous tissue within eight months of surgery. The pseudointima had replaced all but a few of the original bovine collagen fibrils and foci of endothelium were also observed. By comparing porous knitted grafts treated with insoluble collagen (cross-linked with glutaraldehyde) and presealed grafts in which collagen was cross-linked less strongly with formaldehyde, Jonas et al. (38, 39) concluded that the delayed resorption of the sealant, because of excessive cross-linking, results in an







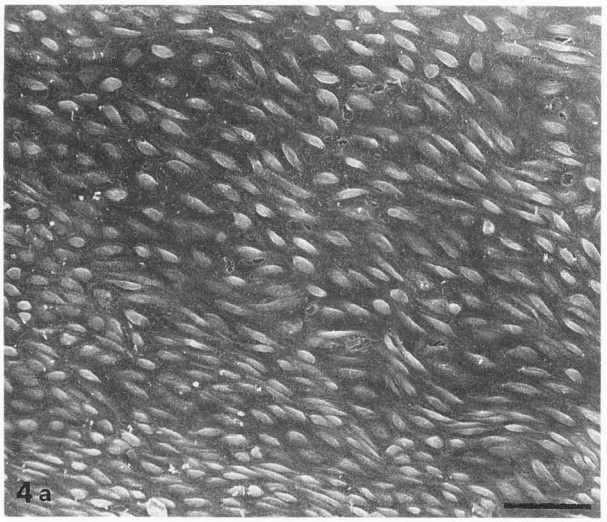
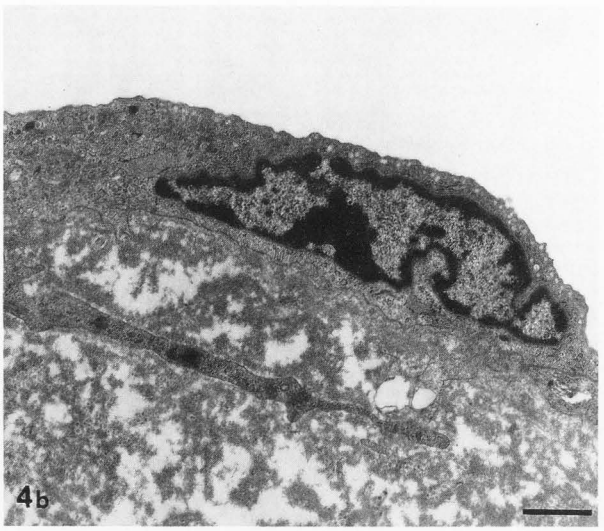


Fig.1. TEM of a knitted Dacron graft implanted in the sheep carotid seven days after implantation. At the anastomoses, the hyperplastic response is composed of undifferentiated mesenchymal cells (arrows), activated smooth muscle cells (arrowheads), fibroblasts (white arrows) and blood cells (bent arrows); bar = 5 micrometers.

Fig.2. SEM of a knitted Dacron graft implanted in the sheep carotid. After two months, the luminal opening of a neovascular channel is clearly observed. Note the continuity in the endothelial lining; bar = 100 micrometers.

Fig.3. SEM of a gelatin sealed knitted Dacron graft; bar = 100 micrometers.

Fig.4. SEM (a) and TEM (b) of a gelatin sealed Dacron graft implanted in the sheep carotid. By 4 months, the graft surface is evenly lined by endothelial cells; bars a) = 50 micrometers, b) = 1 micrometer.



undesirable lack of adhesion between the pseudointima and the luminal surface of the prosthesis. This may generate early graft failure due to large areas of hemorrhagic dissection.

Heat-denatured forms of collagen, namely gelatin, have been proposed for precoating Dacron knitted grafts (Fig. 3). Jonas et al. (40) reported that after 6 months both rat subcutaneous and canine circulatory implants reveal rapid and complete gelatin adsorption without undesirable modification of the normal healing process of knitted Dacron. In our experience gelatin sealed knitted Dacron grafts are also suitable as small caliber arterial substitutes (52). Sixteen grafts (knitted Dacron Triaxial, 8; knitted Gelseal, 8) of 6 mm diameter were inserted with end to end anastomoses into both the common carotid arteries of 8 young sheep. Multiple specimens (distal and proximal anastomoses, central portion of the graft) were retrieved at 1, 2, 8 and 16 weeks after surgery. The combined light and electron microscopic (TEM and SEM) investigation along with the immunogold labelling technique clearly demonstrated that bovine gelatin impregnation did not interfere with the neointima generation. Gelatin was reabsorbed within 2 weeks of surgery. A well-developed and thin pseudointima, composed of modified smooth muscle cells and fibroblasts embedded in a loose extracellular matrix, was observed eight weeks after implantation. By 4 months, Gelseal grafts were evenly lined by endothelial cells (Fig. 4a, b) placed on layers of mainly synthetic smooth muscle cells (Fig. 5) as well as myofibroblasts. In this experimental design, endothelial cells were mainly provided by sources at the cut edge of the host arteries. No significant local inflammatory reaction was encountered in the Gelseal Dacron grafts. In contrast, the contralateral knitted Dacron without gelatin impregnation failed to develop a complete neointimal coverage. Myofibroblasts and fibroblasts were the dominant cells covering these synthetic grafts. It was therefore concluded that gelatin impregnation was also useful in promoting the healing process.

Bowald et al. (3, 4) used Vicryl 910 as the biodegradable component. Vicryl is a copolymer consisting of 90% polyglycolic acid and 10% polylactic acid. Bowald placed a mesh of Vycril 910 into a Dacron graft. Four-cm grafts implanted in the aorta of pigs were endothelialized within 20 days and by 40 days the Vicryl mesh had virtually disappeared.

Spontaneous Graft Healing in Humans

For perhaps as long as 20 years, spontaneous endothelialization of synthetic grafts, as previously described in animals, was assumed to occur in humans (7). DeBakey and associates (15) observed the healing features of 67 patent prostheses recovered from man following implant periods of 2 weeks to almost 7 years. Most of the grafts failed to organize the inner fibrinous capsule since their surface appeared homogenously covered by fibrin. In contrast, endothelium was believed to occur in some specimens.

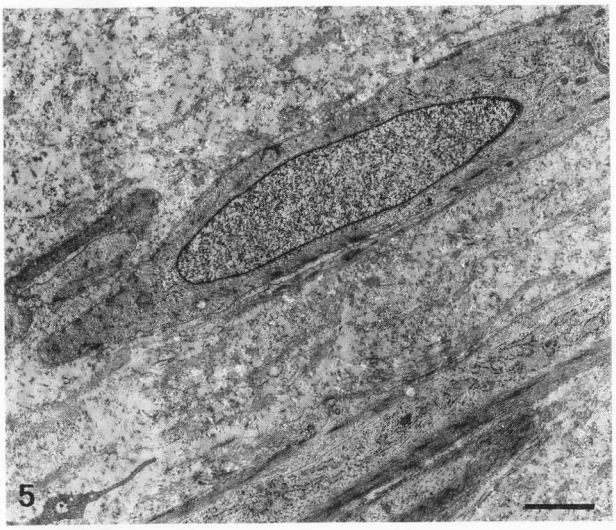
In 1964 Wesolowoski (76) stated that "unlike the pig and the dog, the human does not heal the inner capsule completely prior to the onset of intercapsular tuft degeneration". In accordance with this statement, Berger et al. clearly demonstrated graft failure to develop any endothelial coverage in man (1). Different arterial grafts of varying degrees of porosity were compared (woven, knitted, light and extra light knitted Dacron). In no instance was there evidence of endothelium on the inside of the materials, except within 10 mm of the suture lines.

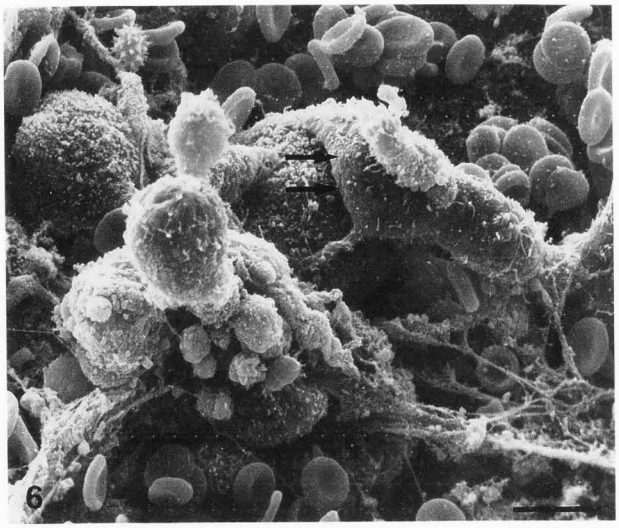
In a detailed study, Sottiurai et al. (68) reported a complete pseudointimal lining on Dacron grafts (both woven and knitted) removed en bloc during reoperation or amputation. The pseudointima consisted of fibroblastoid cells orientated parallel to the direction of the blood flow. The cells were embedded in a loose extracellular matrix composed of collagen fibres and amorphous ground substances. In contrast, ePTFE lacked any organized form of cellular coverage, being largely covered by acellular fibrinous material. Deposition of fibrin on ePTFE was also reported by Walton et al. (73). Fibrinous materials within the graft pores appeared, gradually converted into granulation tissue and later into fibrous tissue.

Our observations agree with the previous reports (1, 24, 45, 56, 62, 68, 70). Both Dacron and ePTFE grafts showed a particular form of healing in humans. This generally consists of the deposition of proteinaceous materials on the graft surface. This material organizes giving origin to a continuous sheet of fibrin in conjunction with blood cells (Fig. 6). This lining represents the major kind of lining we observed in humans. In some instances, a capillary ingrowth was evident in the inner fibrin capsule. SEM made it possible to demonstrate the surface orifices of the vascular channels in ePTFE grafts next to the anastomoses (Fig. 7). In contrast with animals, endothelial cells provided by this transmural source failed to migrate into the surrounding fibrin mesh except for a few mm. At anastomotic level, a host cellular reaction (i.e., pannus) composed of myofibroblasts, undifferentiated mesenchymal cells, blood cells and extracellular matrix was observed. The pannus growth always had a limited extension towards the graft plane. In addition, a ring-like area of intimal thickening was frequently encountered especially at the downstream anastomosis. By LM and TEM, the bulk of the lesion was composed of variously-arranged spindle smooth muscle cells with a synthetic phenotype. The cells, focally showing a whorled-liked appearance rest on a loose extracellular matrix containing proteoglycans and collagen fibers. Collagen showed great variability in width and shape and in some instances displayed a moth-eaten appearance.

Endothelial Seeding in Animals

For more than a decade, endothelial seeding has been understood as the preparation of vascular prostheses by seeding with endothelial cells taken from other vascular districts. The first experiment was carried out by Herring (28), who in 1978 described the possibility of obtaining endothelial cells by mechanical scarification of the saphena intima and then seeding these cells on Dacron prostheses. These prostheses, implanted in dogs, were compared with control, unseeded prostheses after 2, 4 and 8 weeks. The results emphasized the minor thrombogenicity and minor thickness of the neointima of the seeded prostheses as compared to the control prostheses.





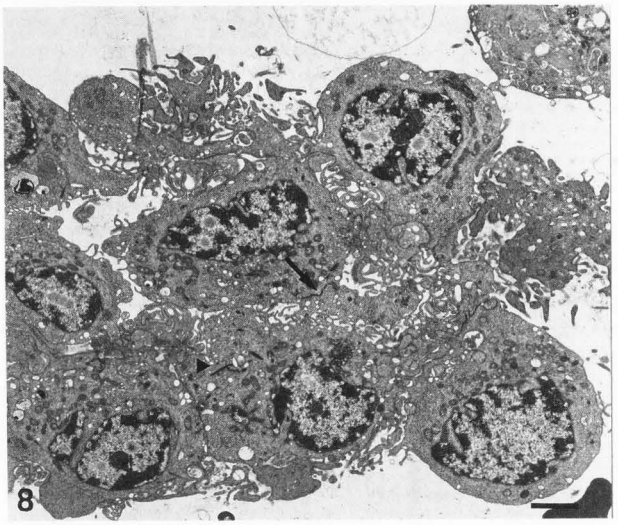


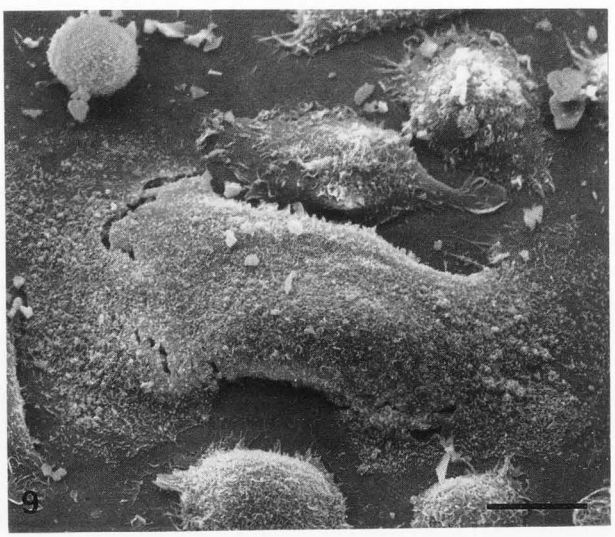
Fig.5. TEM of a gelatin sealed Dacron graft implanted in the sheep carotid. Four months after implantation, the synthetic smooth muscle cells are the major cell component of the neointima; bar = 2 micrometers.

Fig.6. SEM of a human ePTFE femoral popliteal bypass. After 21 months, the surface is still coated with fibrin, erythrocytes, and leukocytes. Note the adhesion (arrows) of endothelial or pseudoendothelial cells to the underlying macrophages; bar = 10 micrometers.

Fig.7. SEM of a human ePTFE femoral popliteal bypass. After 6 months, the transmural capillaries fails to provide endothelial cells; bar = 300 micrometers.

Fig.8. TEM of collagenase harvested human fat-derived microvessel endothelial cells. The cells are still clustered by tight junctions (arrow). Weibel-Palade bodies are clearly observed (arrowhead); bar = 2 micrometers.

Fig.9. SEM of enzymatically harvested human mesothelial cells. The cells are spreaded onto a fibronectin-coated glass surface; bar = 10 micrometers.



As an alternative to the so-called mechanically harvested endothelium, Graham, in 1980, proposed the enzymatic derived endothelium technique (20), which allows the detachment of the endothelial cells from the vessel wall by successive enzymatic washings with trypsin and/or collagenase. This method was shown to be effective both as regards quality and quantity and it established itself as the technique of choice. Subsequent modifications were made to this technique by Sharefkin (64) and Bourke (2).

As an alternative to the medium-large sized veins which were originally used as a source of endothelial cells (21, 28, 31, 35), Williams et al. (78, 79) proposed the extraction of endothelial cells from the microcirculation of the adipose tissue (Fig. 8). In addition, Clarke and Pitillo (10, 11) demonstrated that mesothelial cells (Fig. 9) extracted from dog omentum have the capacity to form a functional, non-thrombogenic lining for prosthetic grafts.

The identification of the endothelial cells was made possible by observing Weibel-Palade bodies at transmission electron microscopic level or using immunofluorescent staining for Factor VIII-related antigen (7, 28, 78, 79). The immuno-gold labelling approach for detecting the Factor VIII-related antigen, the glycoprotein IIb/IIIa, moieties for the l-fucose and cytoskeletal antigens (i.e., actin, vimentin) was also used at ultrastructural level (14).

Another important aspect is the quantity of endothelial cells which can be collected from the donor tissue for an adequate lining of the prosthesis. Herring (29) calculated that the maximum quantity of endothelial cell harvest is $25x10^4$ cells/cm^2 of vein. McCall saw that a minimum cell density of $2.5x10^4$ cells/cm^2 was necessary to develop a confluent growth of pig aorta endothelial culture on ePTFE membrane, after 5 days (47).

On the other hand, Rosenman (60) claims that endothelial cell growth in culture is essential for successful seeding. Using culture media with endothelial cell-derived growth factor and heparin, Jarrel (37) succeeded in producing 1000 cm^2 of confluent endothelial cells in 12 days from one cm^2 of vessel wall. This permitted the seeding of vascular prostheses with high density of endothelial cells.

Burkel (5) stressed the importance of morphological investigation to evaluate the success of seeding. He studied Dacron prostheses in dog, making observations between the first and twenty-eighth day after implant. There is no difference between the seeded and control prostheses immediately after the clot. The prostheses are completely infiltrated with fibrin and blood cells. After one day the prostheses are covered by a layer of activated platelets and leukocytes. A few endothelial cells can be seen between the threads of the seeded prostheses. A differentiation between the control and seeded prostheses becomes visible after 4 days. In the seeded grafts these are patches of immature endothelial cells without a basal lamina but resting on a layer of fibrin. After 7 days the control prostheses continue to be covered with fibrin, platelets and leukocytes. In contrast, the seeded prostheses show patches of endothelial cells with distinct junctions and lysis of the subendothelial fibrin mesh. Clear differences can be seen after 14 days when, in the control prostheses, 75% of the lumen is occupied by thrombotic material of varying thicknesses. On the contrary, the seeded prostheses appear to be almost completely free from thrombus and 60% of the surface is lined with endothelium, below which smooth muscle cells and fibroblasts can be seen. In some parts, the prostheses appear to be completely encorporated by a fibrous capsule. On the outside foreign body giant cells can be seen, together with dense connective tissue and numerous capillaries. After 28 days post-implantation the seeded prostheses are 80% lined with endothelium as compared to 10% in the control prostheses.

In the seeded grafts the endothelial cells have junctional complexes and basal lamina. The subendothelial connective tissue consists of smooth muscle cells, collagen and fibroblasts. Vessels can be clearly seen, penetrating through the prosthetic wall. Basically, whereas in the control prostheses there is a simple reorganization of the fibrin clot and a perianastomotic endothelialization, in the seeded prostheses, besides an almost complete endothelialization, a subendothelial layer is formed, consisting of smooth muscle cells. These observations were also confirmed in other experimental models (6, 66).

The morphological data are flanked by a whole series of functional studies on the efficacy of endothelial seeding which improves the survival of platelets and decreases the release of platelet serotonin (7, 9, 23). Moreover, the endothelial cells maintain the capacity for prostacycline synthesis (23, 65).

Following the first experiments using Dacron protheses, the possibility of treating ePTFE with endothelial seeding was also examined. Graham, in 1982, in a dog model, seeded ePTFE prostheses 6-10 mm in diameter and 20-30 mm long with endothelial cells (21). In this case also, endothelialization by seeding appeared to be more rapid and complete than unseeded prostheses. Indeed, after 4 weeks, 91% of the surface of seeded prostheses was lined with endothelial cells, whereas in the control prostheses endothelialization did not arrive at more than 7 mm from the anastomoses. In addition, Graham observed some peculiar differences between the healing of seeded ePTFE and seeded Dacron. ePTFE, indeed, appeared to be less reactive to blood components. The inner capsule appears to be thinner, and there is less infiltration and incorporation by the leukocytes. One characteristic feature observed was that in seeded ePTFE the endothelial monolayer was not regularly supported by a subendothelial tissue; in particular, unlike seeded Dacron, no smooth muscle cells were observed. These differences can be attributed to the differences in the chemical structure, in configuration and above all in the permeability of Dacron and ePTFE. In addition, Miller et al. (49) demonstrated that although both Dacron and ePTFE were efficient in the macrophage activation, Dacron surface had a greater density of activated cells corresponding to elevated levels of interleukin-1 in cultures.

Herring (32) compared the two types of prostheses with controls after 7 months and confirmed the good endothelialization capacity of seeded ePTFE, which appeared to be more rapid than in seeded Dacron. The importance was emphasized of the thinner inner capsule in ePTFE as compared to Dacron (22 ± 32 micrometers vs 169 ± 143 micrometers). This depends in particular on the low porosity of ePTFE (internodal distance 30 micrometers) which hinders the transparietal diffusion of cellular elements (myofibroblasts and smooth muscle cells) and

the formation of vasa vasorum. Herring attributed the minor tendency of seeded ePTFE to develop a myointimal hyperplasia to these characteristics.

According to other authors (41), the absence of a subendothelial structure does not favour a stable and well functioning endothelium; it is thus suggested that ePTFE prostheses with a greater porosity should be used (internodal distance 45 micrometers).

Although the results reported are encouraging, Hollier (35) raises doubts as to the origin of the endothelial lining in seeded protheses. He made a study in male pigs, seeding prostheses with endothelial cells collected from female pigs. After 4 weeks the implanted prostheses appeared to be lined with endothelial cells with male karyotype, thus excluding successful seeding.

The possibility of staining the seeded endothelial cells with indium^{111} was explored by Rosenman (60), with the intention of quantifying over time the presence of cells seeded on a vascular prosthesis once the blood flow had been re-established. The result was that after 24 hours only a small part of the seeded endothelial cells were present on the prosthesis. In the first thirty minutes after restoration of blood flow there is a rapid loss of endothelial cells. This is followed by a stage, from 30 minutes to 24 hours, in which the endothelial cells are lost at a slower but constant rate so that at 24 hours only 17% of the endothelial cells adhering at the beginning remains. However, Patterson et al. (53) demonstrated the spontaneous loss of indium^{111} from isolated canine endothelial cells. It was therefore suggested that indium^{111} oxine labeling is not a reliable technique for the study of endothelial cell kinetics in vivo. Recently, some authors (50, 80) used recombinant retroviruses to transduce beta-galactosidase genes into harvested endothelial cells. This technology appears particularly suitable in order to estimate the presence of seeded endothelial cells on vascular substrates.

A problem thus emerges of great importance for the success of seeding: how to improve the adhesion of the seeded endothelial cells to the prosthetic material.

In the first place, several methods of endothelial seeding have been proposed to improve seeding efficacy. Kesler (43) suggested sequential inoculation; Fasol (17), uses a rotation device; Shindo (67) seeds the endothelial cells on prostheses in a glassware apparatus for 48-72 hours before implanting.

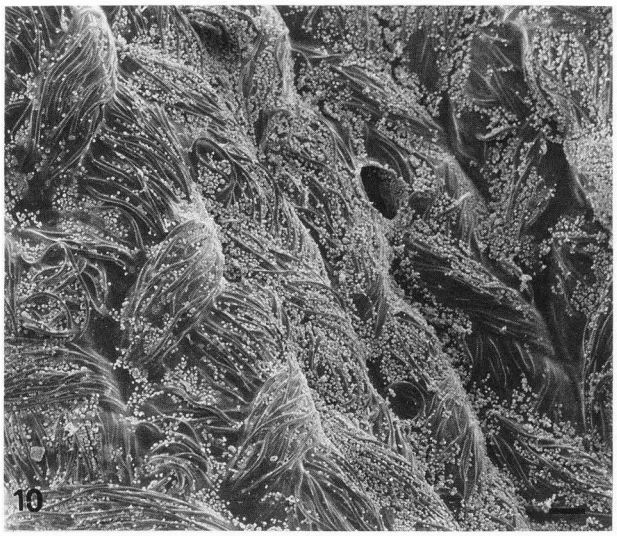
At the same time, research in the field of prosthetic substratum has been developed. The precoating of material with fibronectin has been shown to be very effective, starting with the first experiments by Kempczinski and Ramalanjaona (42, 55). This method increases the adhesion of the seeded endothelial cells, reducing their loss once blood flow has been restored.

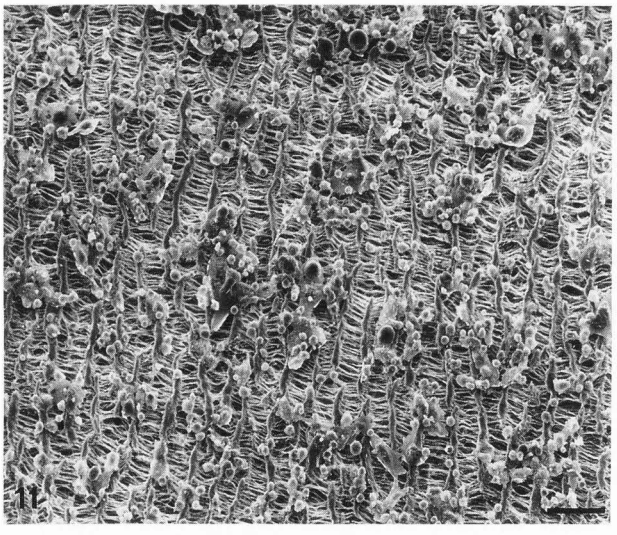
Our approach was designed to analyze the interaction between endothelial cells and various synthetic vascular grafts by means of SEM (14). Endothelial cells were harvested from adipose tissue by using collagenase treatment. Cell viability, as determined by Trypan Blue exclusion, resulted in higher than 90% of isolated cells. Endothelium was characterized by the demonstration of positivity for Factor VIII-related antigen and glycoprotein IIb/IIIa. Endothelial cells were seeded and left to adhere for one hour onto different synthetic grafts. As expected, knitted Dacron and ePTFE alone are not suitable for generating endothelial cell monolayers. In fact, SEM showed no cell adhesion on these substrates. Blood preclotting did not significantly improve the rate of cell adhesions and basically resulted in the presence of areas with cell attachments. Commercially available coated grafts (Gelseal Dacron, Albumin-coated-Dacron, Hemashield Dacron) were further investigated. Interesting results were obtained with gelatin-coated Dacron (Gelseal). In agreement with other authors (27), we found that gelatin permits the adhesion of seeded endothelium. A high density of adherent endothelial cells was seen on the Gelseal surface even though the rate of cell spreading was low (Fig. 10). Adherent cells were mainly round in shape. However, many cells showed thin filopodia while others exhibited complex surface corrugations. A few spread cells were also found. In our experiments these results were not constant; this most likely depends on variations in the physico-chemical characteristics of the gelatin contained in different industrial supplies (14). In contrast, Albumin-coated-Dacron and Hemashield Dacron (sealed with type I collagen) did not seem to favor endothelial cell adhesion. In particular, Albumin-coated grafts lacked endothelial coverage while Hemashield Dacron showed an intermediate behavior. By SEM, clusters of endothelium were observed particularly in the hollows of the material. These results do not comply with those reported in experimental models (54) and in animal models (67) utilizing collagen-coated-Dacron. However, this discrepancy could be attributed to the fact that Shindo et al. (67) impregnated their prostheses with a mixture of I/III type collagen. In our experience, the best results are provided by adsorbed ePTFE grafts with bioactive molecules. As previously described, ePTFE alone did not permit any cell adhesion. However, incubation of ePTFE with 20 mg/cm^2 of fibronectin for 2 hours at 37°C resulted in a dense lining of seeded endothelial cells (20-29 cells/micrometers^2) with a significant tendency to spread out (Fig. 11). The exposure of endothelial cells to this substrate induced rapid cell morphological changes. Endothelium lost their round appearance and extended long, slender filopodia making tenuous contacts with the substrate. As the endothelial adhesion proceeded, the interaction appeared more stable. Endothelial cells presented peripheral cytoplasmic flaps and in some areas completely spread over the substrate. Analogous results were provided by ePTFE grafts preliminary treated with fibrin-containing mixtures. SEM examination of fibrin glue (Immuno AG) coated ePTFE grafts clearly revealed a sharp transition in cell adhesion between coated and uncoated ePTFE surfaces (Fig. 12) thus confirming data provided by Zilla et al. (82).

Finally, the experiments carried out with carbon-coated ePTFE (63) should also be kept in mind.

Endothelial Seeding in Man

The first experiments of endothelial seeding in man were carried out by Herring (31), who in 1984 reported the results of a trial with implanting of Dacron prostheses seeded with endothelial cells collected with the mechanical method. The data concern bypasses made in different vascular areas: axillo-femoral, femoro-femoral and femoro-popliteal; in this last group the seeded prostheses are also





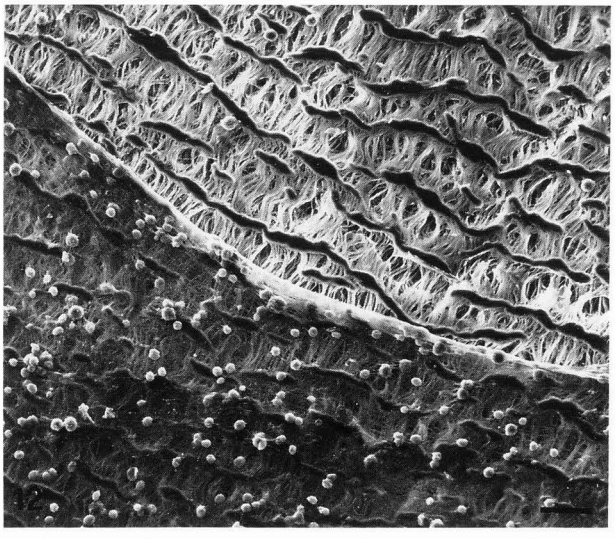


Fig. 10. SEM of collagenase harvested human fat-derived microvessel endothelial cells. After 1 h of incubation on a gelatin coated Dacron graft, the density of cell adhesion is high; bar = 100 micrometers.

Fig. 11. SEM of collagenase harvested human fat-derived microvessel endothelial cells. After one hour of incubation on a fibronectin-coated ePTFE graft, there is a good cell adhesion on the substrate. Spread endothelial cell are also present; bar = 50 micrometers.

Fig.12. SEM of collagenase harvested human fat-derived microvessel endothelial cells. After 1 h of incubation on a fibrin glue coated ePTFE graft, there is a sharp transition in cell density between coated and uncoated PTFE surface; bar = 25 micrometers.

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compared with the autologous vein. The results assessed on the basis of the patency and the platelet function, are not the same as those obtained in experiments performed in animals. Although he reports an improved patency in seeded prostheses of some sub-groups (femoro-popliteal of non-smokers), Herring did not, in fact, observe great differences between seeded and control prostheses. He did, however, confirm the validity of the autologous vein. The small number of histological observations did not reveal seeded endothelial cells on the prostheses after 4 months, but only fibroblasts and smooth muscle cells.

It was Herring again, who made an interesting morphological observation of seeded ePTFE at 90 days after implant in man (33). Unlike the experience with Dacron, areas of endothelialization could be recognized in this case, characterized by a monolayer of endothelium-like cells, resting on a thin layer of matrix with no elastin, collagen or smooth muscle cells. Given the distance from the anastomosis of the area examined (10 cm) and the absence of myofibroblasts, Herring concludes that endothelialization is probably secondary to seeding.

In 1986 in Vienna, the results were presented of 4 clinical trials carried out in different centres: Indianapolis, Vienna, Goteborg and Dundee (17, 34, 58, 72). Although there were a few differences due to the harvesting and seeding techniques employed, the prosthetic material used and the postoperative control methods, the various authors concluded that endothelial seeding in man gives less positive results than in animals. Although, on the basis of long-term patency and platelet reactivity, the comparison between seeded and control protheses appears to indicate the major healing characteristics of seeded prostheses; the comparison of data does not reveal statistically significant differences.

Discussion

Several groups of investigators have described the clinical and experimental results of graft incorporation using synthetic materials of various designs and compositions (7, 62, 71, 74, 77, 81). Some reports have also provided detailed and correlated documentation of the arterial cellular and subcellular healing responses to graft implants in animals (12, 13, 54, 56, 59, 70, 77). Most of these papers support the view that synthetic vascular grafts of both Dacron and ePTFE fabrics heal through a process of

graft arterialization which, within certain limits, seems to recapitulate the normal architecture of the replaced artery. At the final stage, a continuous luminal graft repopulation with endothelial cells and/or modified smooth muscle cells (neointima) takes place. As far as graft thrombogenicity is concerned, this neointimal coverage represents the ideal blood/graft interface (7, 44, 79).

In contrast to experimental animals, the flow surface of both Dacron and ePTFE grafts in humans remains unhealed, with a surface of more or less compacted, cross-linked fibrin. Healing in humans consists of outer wall attachment by fibrous tissue. Further healing of the prosthesis occurs very slowly and rarely involves the inner wall. This consists of a thin and relatively stable fibrin lining due to a critical equilibrium between fibrin neo-deposition and its degradation by the monocyte/macrophage system. In addition, endothelium and smooth muscle cells provided from the arterial sources (anastomoses) migrate only a few mm from the cut edge of the artery (1, 15, 76). Patches of cell coverage most likely related to the graft capillarization remain circumscribed to the luminal openings of the neointimal capillaries mainly at the anastomoses and the cells provided do not extend any further. Therefore, the usual patterns of graft endothelialization seem to be ineffective in man. In any case, the luminal fibrin coverage remains essentially unchanged for months or even years and appears as a relatively non-thrombogenic lining. In the large conduits, in fact, this lining seems to be sufficient to minimize graft complication. In fact, the patency rate of Dacron and ePTFE grafts is particularly high in the aorto-iliac position. However, with diameters of 5 mm or less the failure rate of both these materials is unacceptable. After 3 months, approximately 30% of the grafts are closed by early thrombosis. After 36 months, only 20% of artificial grafts are still patent (7). In this context one of the causes of the lack of success of artificial prostheses when compared to autologous vein implants, besides the evident difference in compliance, may be attributed to the lack of functioning endothelium at the moment of implant. Therefore, the presence of a continous endothelial layer should be considered as an essential prerequisite for the success of low-flow, small-diameter grafts.

The need to favour a rapid and, as far as possible, complete endothelialization of implanted vascular prostheses has induced some researchers to introduce and develop the endothelial seeding technique (28, 79). The experiments carried out, with various prosthetic materials in animal, are without doubt encouraging. From a clinical point of view, seeded prostheses give immediate patency results and, in the long run, better results than unseeded prostheses. From a functional point of view, there is a more effective restoration of the synthetic endothelial activity as well as an improvement in the main platelet functioning parameters in seeded prostheses. In addition, seeding permits an almost complete and rapid endothelialization of the prosthesis segment with different aspects linked to the chemico-physical and structural characteristics of the various materials studied. The results obtained with endothelial seeding in animals indicate that although the actual capacity of seeded endothelial cells to remain attached to the prostheses, subject to hemodynamic stress, has not been clearly established; this method does favour prosthetic endothelialization.

Equivalent results have not been obtained in the few experiments carried out in man. It may be postulated that there are many causes which interfere with the efficacy of endothelial seeding in man. In the first place, the difficulty of harvesting a sufficient number of endothelial cells must be considered. To this can be added the need to overcome the limitations involved in using human endothelial cultures and the need to find substratum which favour a major adhesion of seeded endothelial cells. In the second place, along with the well-known differences between species (16), it should be remembered that in a man requiring a prosthetic implant, a multidistrict steno-obstructive disease is present, inducing complex variations in the regional hemodynamics. This disease is also maintained and aggravated (70, 73) by the presence of various factors such as smoking, diabetes, hypertension and hyperlipidemia.

In conclusion, the thirty years of experiments in the field of synthetic vascular prostheses have revealed large differences in the results in animal models and those in human models. In man, while in the aorto-iliaco-femoral district a fibrin layer appears to be sufficient for long-term patency of such materials; in small caliber districts an improvement in compliance and an endothelial lining is essential. This may be favoured by endothelial seeding. Some encouraging results in man have been achieved with this method. The long-term fate of seeded grafts in man, however, can only be assessed with an increase in clinical experiences together with the use of more sophisticated morphological and functional analyses.

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Discussion with Reviewers

K.A. Robinson: What are your criteria for the characterization of syntjetic smooth muscle cells and their distinction from myofibroblasts at the TEM level? Clowes et al. (13) have shown that, at least in ePTFE grafts, the spindle-shaped cells of the neointima were all smooth muscle cells. This may have significance in terms of extracellular matrix production and any retraction of the intimal thickening.
Authors: According to F.N. Ghadially (Diagnostic Electron Microscopy of Tumours, IInd edition, Butterworths, London, 1985, pp. 456-466.), the following TEM features were used to distinguish myofibroblasts from synthetic smooth muscle cells: i) abundance of elements of the Golgi complex and rough endoplasmic reticulum; ii) paucity of micropinocytotic vesicles; and iii) absence or relative infrequency of basal-lamina-like material.

K.A. Robinson: You cite recent work regarding adipose tissue as a source of endothelial cells from graft seeding. What are the advantages of this technique, particularly with respect to cell yield and seeded cell density?
Authors: The major advantages of this technique are: i) large quantity of endothelial cells are rapidly (2-4 hr) available (average yield: 10^4-10^6 cells/g fat tissue); ii) reproducibility in yield; iii) high endothelial cell yield for immediate monolayer generation; and iv) large vessels for cell procurement are spared.

K.A. Robinson: Could the divergent healing responses of experimental animals and humans be due in part to discrepance in relative age? Perhaps a greater regenerative capacity is seen in the former because relatively young animals are used.
Authors: Yes, we agree with your comment.

K.A. Robinson: Another possible reason for graft failure may be cytokine stress from adherent leukocyte (Fig. 6). Could you comment on the role of chronic inflammation in graft failure?
Authors: Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are important cytokines produced by activated mononuclear cells. They share many biologic properties and also act synergistically. In particular, IL-1 and TNF produce local effects on endothelium which may be relevant to the process of graft failure. IL-1 and TNF induce on endothelium the synthesis of surface adhesion molecules, which stimulate increased adhesion of inflammatory cells to the endothelium. They stimulate the endothelial cell synthesis of platelet activating factor, which is a powerful platelet activator and aggregator. They produce an increase in procoagulant and a decrease in anticoagulant properties of endothelial cells, rendering the endothelial surface potentially thrombogenic.