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ENDOTHELIALIZATION OF A NEW DACRON GRAFT IN AN EXPERIMENTAL MODEL: LIGHT MICROSCOPY, ELECTRON MICROSCOPY AND IMMUNOCYTOCHEMISTRY

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Abstract

Two types of synthetic vascular grafts, Dacron Triaxial and Dacron Gelseal Triaxial, were implanted into both the common carotids of sheep. The animals were sacrificed 1, 2, 8, and 16 weeks after surgery. Multiple specimens, obtained from grafts and anastomoses, were studied by light microscopy, transmission and scanning electron microscopy. A parallel immunocytochemical analysis was performed on some specimens. Dacron Triaxial grafts failed to develop a complete neointimal coverage. Myofibroblasts and fibroblasts were the dominant cells in such synthetic graft. Moreover, focal areas of stripping, platelet deposition, and thrombosis were observed at 8 and 16 weeks.

In contrast, a stable endothelial coverage developed on the Gelseal Triaxial grafts after 16 weeks.

KEY WORDS: Dacron grafts, Dacron Gelseal Triaxial, sheep, pseudointima, neointima, endothelial cells, scanning electron microscopy, transmission electron microscopy, immunocytochemistry.

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Introduction

Endothelialization of prosthetic grafts in humans remains a controversial issue (1, 11, 19, 25). As specifically discussed by Sottiurai et al. (22), Dacron grafts, in contrast to all the biological vein grafts, lack endothelium and, after incorporation, appear to be lined only by a pseudointima consisting of myofibroblasts, fibroblasts and fibrous matrix.

The absence of a true endothelial coverage has been considered a prerequisite for graft failure in animal models (10, 12). The endothelial ingrowth, therefore, is an important factor influencing patency or occlusion of prosthetic grafts.

A new Dacron graft (Gelseal Triaxial, G.T.), impregnated with a biodegradable modified mammalian gelatin of low thrombogenicity, is the result of an extensive research program. Our preliminary experience in the clinical application of G.T., which represents a zero porosity graft requiring no preclotting or pre-treatment prior to implantation, is very encouraging.

An accurate characterization of the cell types involved in the graft incorporation seems, at present, indispensable to shed light onto the mechanisms which subtend the long-term patency of synthetic grafts. Thus, we performed a morphological analysis by light microscopy (LM), transmission and scanning electron microscopy (TEM/SEM) on Dacron Triaxial grafts implanted into the common carotids of sheep. Both G.T. and simple Dacron Triaxial (D.T.) grafts were investigated.

The aim of our study was to point out:
- the evolution of the cellular response both at the prostheses and at the anastomoses;
- the differences in healing between G.T. and more conventional D.T. prostheses and, in particular, in the production and maintenance of a stable graft coverage.

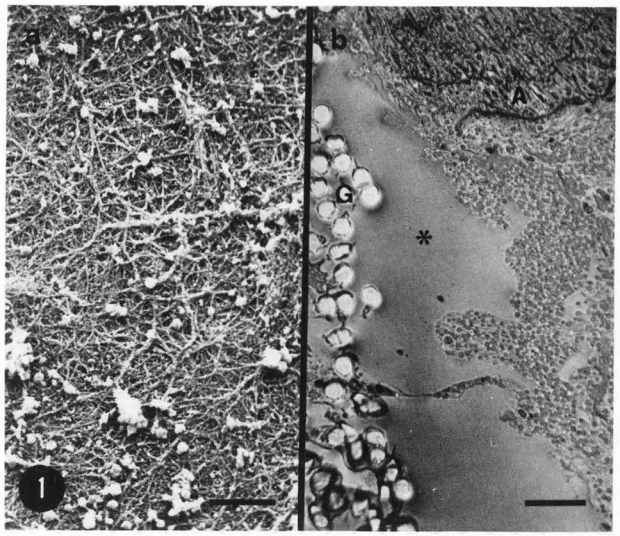
Moreover, immunocytochemical criteria have

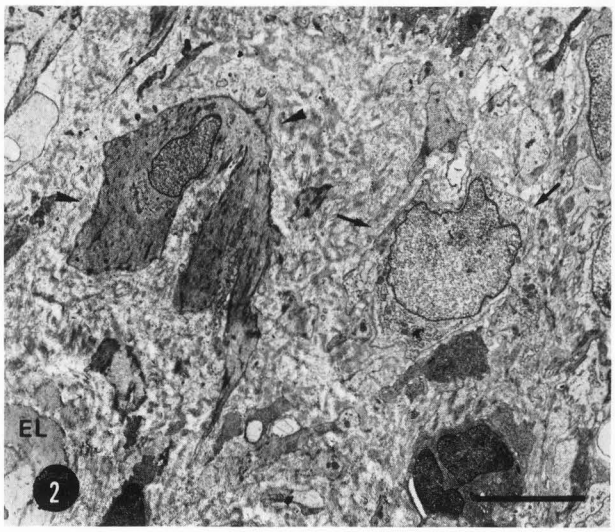
been utilized to fully differentiate neointima from pseudointima production.

Materials and Methods

Two different prosthetic grafts, G.T. and D.T. (6 cm in length, 6 mm i.d.), have been implanted with end to end anastomoses into both the common carotids of sheep. All the grafts were kindly provided by Vascutek Limited, Scotland.

A total of 8 animals, weighing approximately 90 kg, were sacrificed at 1, 2, 8, and 16 weeks. The vessels containing the grafts were carefully resected en bloc and particular care was taken to avoid trauma to the luminal lining as suggested by Durand et al. (5). They were successively processed for light and electron microscopy. Multiple specimens (artery, distal and proximal anastomoses, central portion of the graft) were immediately fixed in 2.5% cacodylate-buffered glutaraldehyde for 2 h. After washing in three cacodylate-sucrose buffer baths, the specimens were post-fixed for 1 h in a 1% solution of osmium tetroxide in 0.1 M cacodylate buffer and then rinsed in cacodylate-sucrose buffer. Tissues for SEM were critical-point dried after ethanol dehydration, coated with a semithin film of gold (20-30 nm) and observed in a SEM Philips 505. Fixed tissue for TEM was cut into small fragments, dehydrated in a series of increasingly concentrated ethanol baths and embedded in araldite. Semithin sections (1 µm) for LM observations were taken before thin sectioning with a glass knife. Thin sections (60-80 nm) collected on Formvar-coated grids were stained with uranyl acetate and lead citrate and coated with a thin layer of evaporated carbon before observation. TEM analysis was performed with a JEOL 100 B. Moreover, some tissue fragments were fixed in periodate-lysine-paraformaldehyde (15) and subjected to the protein A-gold immunocytochemical technique (21) for the detection of actin and vimentin. The immunolabelling was carried out on semithin and thin sections from araldite embedded material by using two mouse monoclonal antibodies (anti-actin and anti-vimentin, Amersham). The sections were subsequently incubated in rabbit anti-mouse immunoglobulin in order to allow protein A-gold complex binding. The gold labelling was revealed at LM level by a silver enhancement (2). Moreover, the same protein A-gold-silver technique was applied to frozen sections for the detection of desmin by means of a mouse monoclonal antibody (anti-desmin, Amersham).





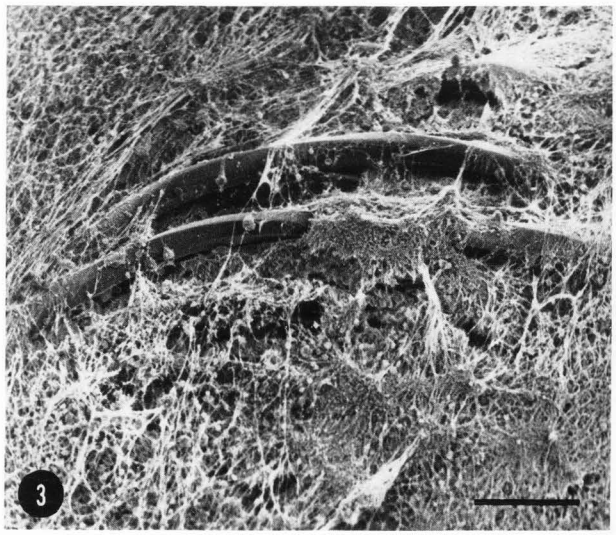


Fig. 1. Seven days. Dacron grafts are covered by a fibrinous layer. a) SEM view of fibrin and platelets overlying the G.T. graft; b) Gelatin impregnation as revealed by LM. A = artery; G = graft; * = gelatin. Bar: a) 10 μm; b) 50 μm.

Fig. 2. Seven days. At anastomoses, a mesenchymal cell (→) and a contractile smooth muscle cell (►) are disclosed just below the endothelium by TEM. EL = elastic lamina. Bar = 5 μm.

Fig. 3. Seven days. SEM shows D.T. graft with patches of uncoated Dacron fibres. Bar = 50 μm.

←

Results

Seven days after implantation

Our LM data, as for early deposition of fibrin and blood cells on Dacron surfaces, agree with others (4, 24, 25). In the initial stage of incorporation, prosthetic grafts were covered by a fibrin lining easily visualized by SEM (Fig.1a), whereas a layer of granulation tissue surrounded the tubular graft. Gelatin was still present on and in between the interstices of the G.T. fabric (Fig. 1b). Foreign body giant cells were not detected.

Worthy of attention was the occurrence, by LM, of a flow of rounded and spindle-shaped cells migrating from the perianastomotic artery media to the adjacent fibrin lining the prosthesis. By TEM, most of these cells showed features of mesenchymal cells: large rounded to oval nuclei with dispersed chromatin and prominent nucleoli (high nucleus/cytoplasm ratio). Their cytoplasm contained numerous free polyribosomes, cisternae of rough endoplasmic reticulum (RER) as well as some bundles of myofilaments. A basal lamina coated most of the cell body (Fig. 2). Others were smooth muscle cells of the contractile phenotype. A few, mainly located next to collagen fibrils, showed characteristics of both fibroblasts (well developed RER) and smooth muscle cells (myofilaments, pinocytotic vesicles, basal lamina), thus resembling the cells described by Gabbiani et al. (8) as myofibroblasts. Typical fibroblasts were focally present. The cellular submicroscopic features of D.T. and G.T. were quite similar. However, a significant difference in the rate of healing between the two types of prostheses was underlined by SEM. The D.T. graft focally showed patches of uncoated Dacron fibers (Fig. 3). On the contrary, a continuous layer of fibrin, erythrocytes, leukocytes, platelets and plasma proteins adhered to the G.T. surface, thus forming an early pseudointima.

Fifteen days after implantation

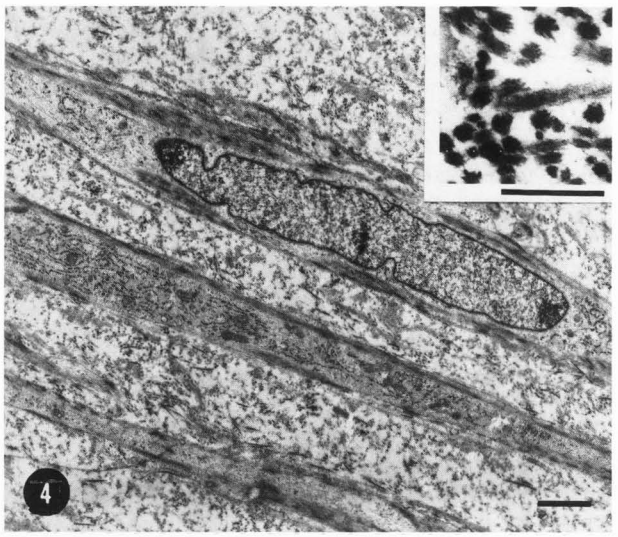
At this time, the fibrin lining, next to the anastomoses, showed an increased cellularity. LM evidenced multiple layers of spindle-shaped cells of low thrombogenicity, embedded in a loose fibrous matrix. These cells, arranged parallel to the blood flow, were characterized as myofibroblasts and fibroblasts by TEM (Fig. 4). In particular, myofibroblasts appeared to be actively involved in the synthesis of extracellular matrix components as demonstrated by the presence of dilated cisternae of RER. Collagen fibrils, some with dysplastic aspects ("flower-like appearance", Fig. 4 Inset) as well as proteoglycan particles were seen in the intercellular matrix. SEM showed that the pseudointimal surface was constituted by flat, elongated, loosely interconnected cells with very smooth surfaces; a few mononuclear cells were seen spreading over and into this lining (Fig. 5).

The central portion of both D.T. and G.T. grafts showed, by LM, a poor development of the pseudointimal coverage. Foreign body giant cells adhered to the Dacron fibrils. No trace of gelatin was detected on the G.T. graft (Fig. 6). The surface of the loose inner layer consisted of numerous round cells with dark, slightly crenated nuclei. Monocytes, activated macrophages (showing an increased number of lysosomes and endocytic vesicles), as well as resting foam cells (with intracytoplasmic lipid droplets and vacuoles containing digested fibrin) were easily recognized by TEM (Fig. 7). Clusters of elements of the mononuclear macrophage system (13) were also seen within the spindle-shaped cell area.

Eight weeks after implantation

Unlike conventional D.T., G.T. prostheses had a more complete and mature coverage, and neointimal patterns of healing were also evidenced at some distance from anastomoses.

G.T. was completely covered by a thin pseudointima (Fig. 8), whose inner layer was, in part, lined by endothelial cells (neointima constitution). At anastomoses, a continuous advancing front of endothelial cells was observed by SEM (Fig. 9a). Proliferating endothelial cells were focally grouped in characteristic, well-demarcated clusters. TEM revealed that the cluster forming cells were polygonal in shape and displayed large nuclei with dispersed chromatin and one or two prominent nucleoli. The cytoplasm contained numerous free polyribosomes (Fig. 9b). Islands of endothelium were, at times, observed in the central portion of the graft.



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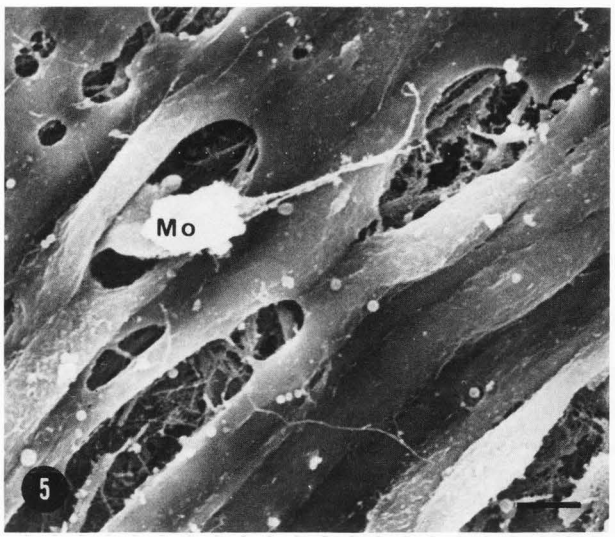

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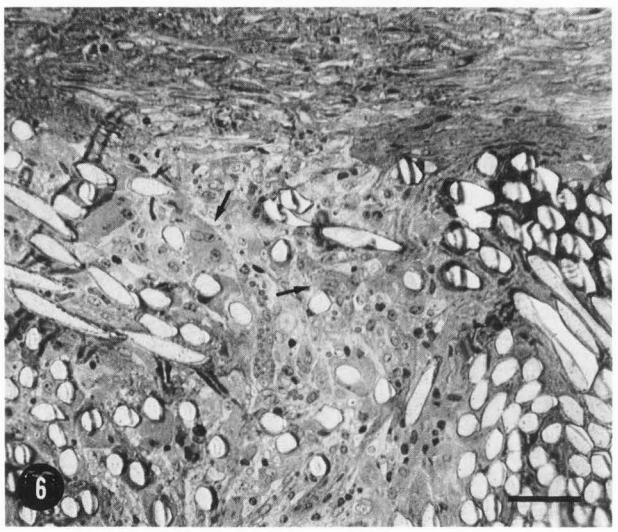

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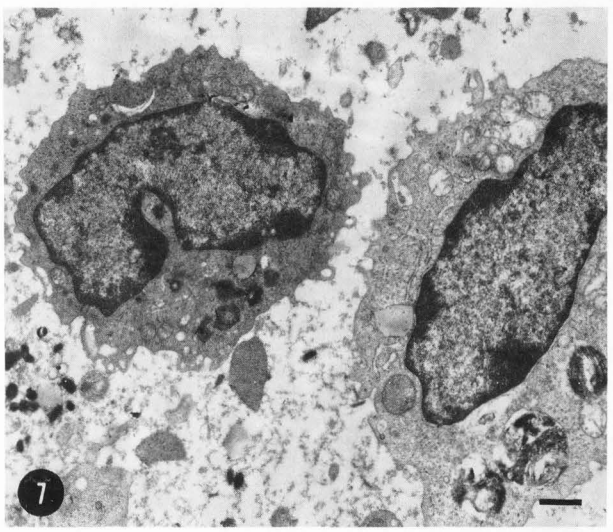

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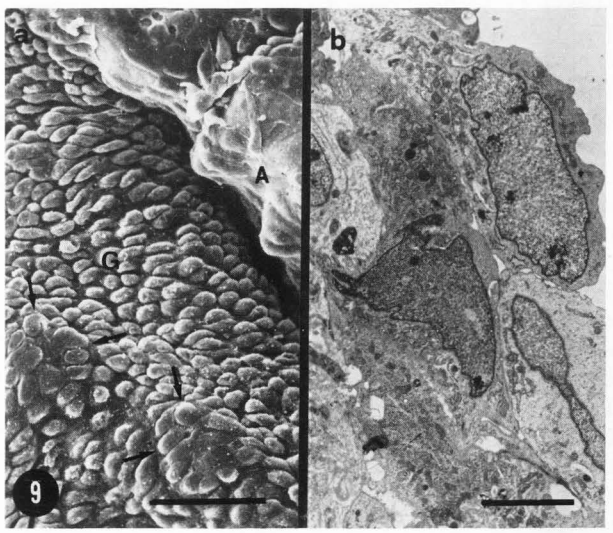

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Fig. 4. Fifteen days. TEM of developing pseudointima at anastomoses. Myofibroblasts are the prominent cell type. They are embedded in a loose extracellular matrix. Inset shows a high power view of some collagen fibres with a flower-like appearance. Bar = 5 μm; Inset, Bar = 0.5 μm.

Fig. 5. Fifteen days. SEM of pseudointima over an anastomosis. The cells are elongated, loosely interconnected with very smooth surfaces. Mo = mononuclear cell. Bar = 5 μm.

Fig. 6. Fifteen days. LM image of G.T. graft. Gelatin was completely removed at this time. Some foreign body giant cells (→) are seen close to Dacron fibres. Bar = 50 μm.

Fig. 7. Fifteen days. The central portion of the graft as viewed by TEM. At this level the graft appears unhealed and monocytes and macrophages are easily visualized. Bar = 1 μm.

Fig. 8. Eight weeks. A thin and homogeneous pseudointima covers G.T. graft. A SEM view. [= pseudointima; G = graft. Bar = 50 μm.

Fig. 9. Eight weeks. Neointima constitution at anastomoses. a) SEM view of endothelial cells migrating from the artery (A) towards the graft side (G). Clusters of proliferating endothelial cells are also observed (→); b) A cluster of endothelial cells as depicted by TEM. Bar: a) 50 μm; b) 5 μm.

←

In contradistinction to G.T., D.T. lacked endothelial coverage and pseudointima, in the central portion of prosthesis, showed focal areas of stripping as well as massive platelet deposition (Fig. 10). Mononuclear cells were also prominent.

The endothelial cells, which were beginning to cover G.T. grafts, were characterized according to ultrastructural and immunocytochemical criteria. SEM depicted slender elongated cells with slightly bulging nuclei and few surface projections. The intercellular junctions were flat (Fig. 11). TEM showed endothelial cells with large, oval nuclei as well as prominent nuclear fibrous laminae (Fig. 12). Their cytoplasm contained a fair amount of intermediate filaments and pinocytotic vesicles. Tight junctions were also observed; Weibel-Palade bodies were not found. The intermediate filaments (8-10 nm o.d.) formed loosely packed bundles running throughout the cytoplasm. They were immunocytochemically characterized as vimentin filaments (Fig. 13). Immunolabelling for desmin was negative. Microfilaments of actin (5-7 nm o.d.) were specifically localized both in the subplasmalemmal and in the junctional area of the cytoplasm.

Sixteen weeks after implantation

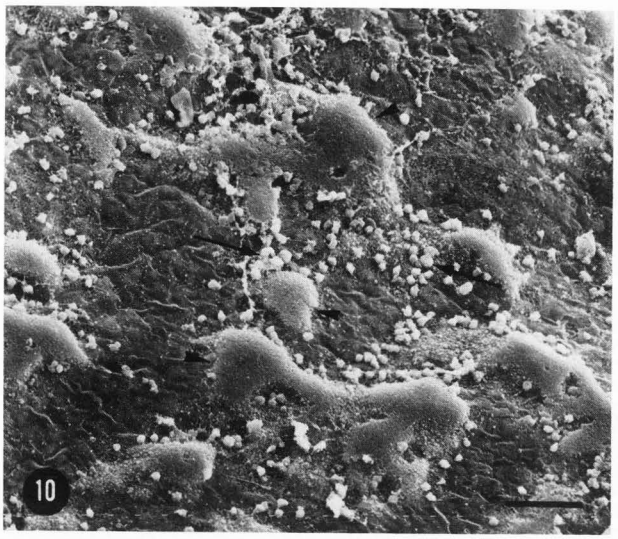
The differences in healing between G.T. and D.T. were pronounced.

G.T. graft displayed a continuous neointimal coverage. By SEM, neointima was homogeneously increased in thickness (compare Fig. 14 to Fig. 8) and endothelium appeared to be oriented in an orderly pattern (Fig. 15). TEM showed quite normal endothelial cells which were arranged parallel to the direction of blood flow. Characteristic peripheral bundles of actin filaments, comparable to the stress fibres of cultured endothelial cells, were detected within their cytoplasm (Fig. 16a, b). Underlying endothelium were myofibroblasts and smooth muscle cells embedded in an abundant fibrous extracellular matrix consisting of proteoglycans, collagen fibrils and scant elastin. The phenotype expressed by neointimal spindle-shaped cells was also investigated at LM level (protein A-gold-silver technique). The cells showed an intense labelling with anti-actin (Fig. 17a) and an intermediate one with anti-vimentin (Fig. 17b). Poor differentiated smooth muscle cells were suggested by the presence of weak staining with anti-desmin (Fig. 17c).

Conversely, D.T. graft developed an occlusive thrombus. By LM, the thrombosed graft appeared totally filled with an organized clot. Its innermost portion was traversed by many newly formed recanalized channels also detectable by SEM (Fig. 18). At the same time, a marked reaction of foreign body giant cells against the Triaxial fabric was recognized. The yarn bundle arrangement was subverted, and some isolated giant cells containing fragments of fabric were easily visualized.

Discussion

The nomenclature of the linings of prosthetic grafts is very confusing. This mainly depends on the inadequate identification of the cellular structure at LM level. An accurate ultrastructural and immunocytochemical characterization of the cellular coverage seems, therefore, indispensable. On the basis of TEM and SEM observations, we used the term "neointima" for describing a continuous lining of endothelial cells placed on a multiple layering of spindle-shaped cells (myofibroblasts, fibroblasts, smooth muscle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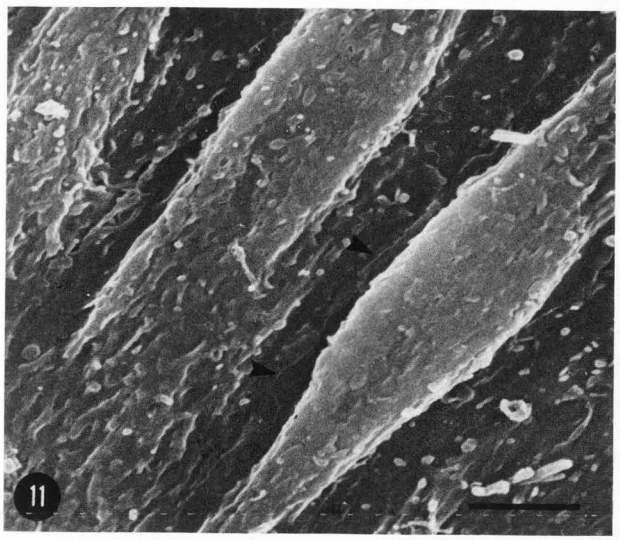
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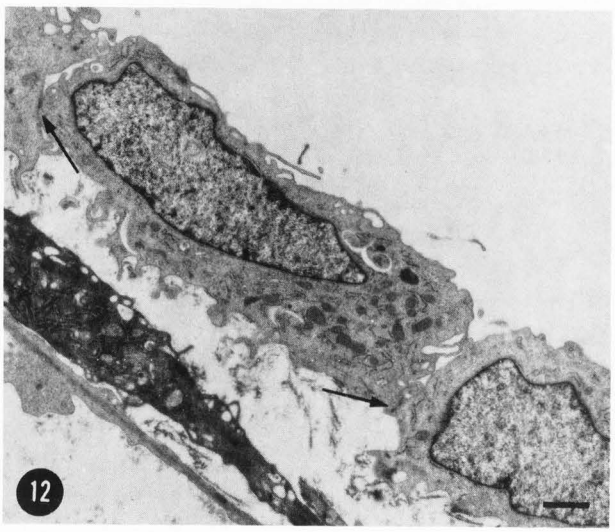
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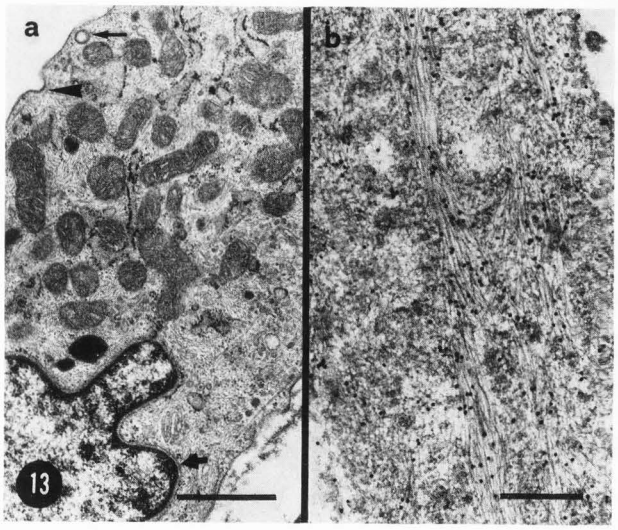
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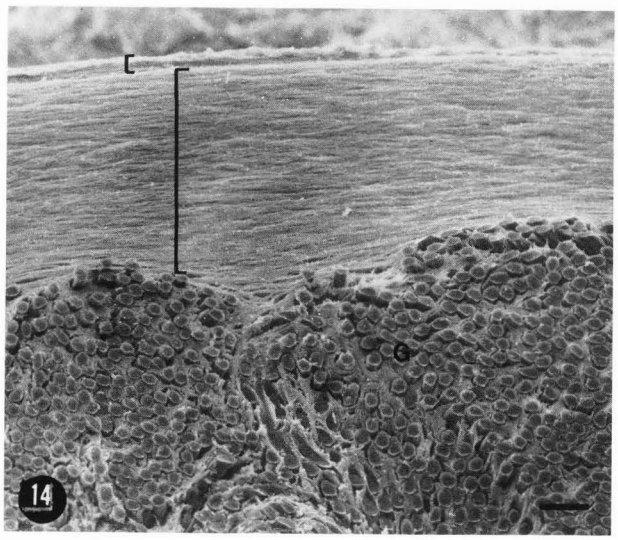
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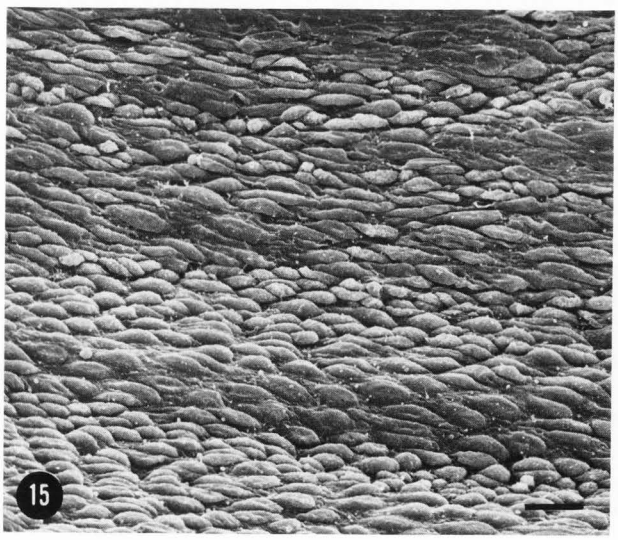
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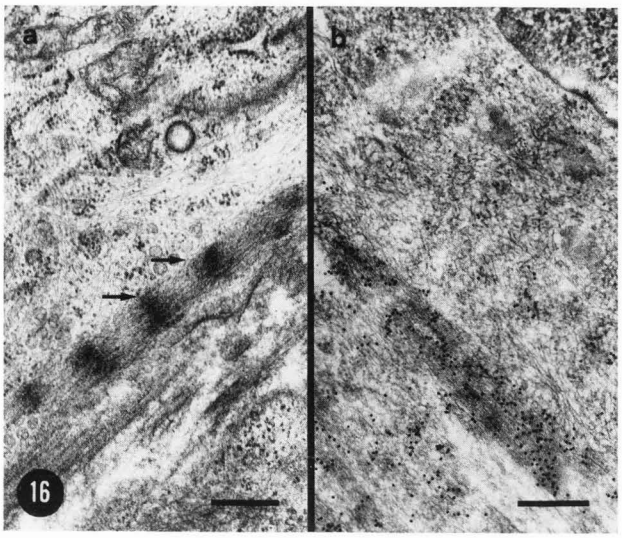

Fig. 16. Sixteen weeks. a) TEM shows peripheral bundles of microfilaments within the cytoplasm of endothelial cells (➟); b) The microfilaments are composed of actin as demonstrated by the protein A-gold technique.
Bar: a) 10 µm; b) 10 µm.

Fig. 10. Eight weeks. Massive platelet (➤) and mononuclear cell (➟) deposition over D.T. graft by SEM. Bar = 50 µm.

Fig. 11. Eight weeks. SEM of the endothelial lining. The cells are elongated, with bulging nuclei and few surface projections. The intercellular junctions appear flat (➤).
Bar = 5 µm.

Fig. 12. Eight weeks. TEM view of endothelial cells. Note the presence of tight junctions (➟). Bar = 1 µm.

Fig. 13. Eight weeks. High power view of endothelium. a) Endothelial cells show a prominent nuclear fibrous lamina (◗) as well as a fair amount of intermediate filaments; coated pits (➤) and coated vesicles (➟) are also observed by TEM; b) Characterization of intermediate filaments as vimentin filaments by means of protein A-gold immunolabelling.
Bar: a) 1 µm; b) 0.5 µm.

Fig. 14. Sixteen weeks. Cross-section of the central portion of G.T. graft. SEM reveals that neointima is evenly increased in thickness. ▮ = endothelium; [= multiple layers of spindle-shaped cells; G = graft. Bar = 50 µm.

Fig. 15. Sixteen weeks. The neointimal surface as viewed by SEM. Endothelial cells are arranged parallel to the direction of the blood flow. Bar = 20 µm.

cells) and the term "pseudointima" for designating a lining of spindle cells, mainly myofibroblasts, embedded in a loose fibrous matrix.

The healing characteristics of Dacron prostheses have been investigated both in human and animal models (1, 7, 11, 19, 20, 22, 23, 25). From these reports, Dacron grafts failed to develop an indisputable neointimal coverage. Fibroblasts and myofibroblasts were the dominant cells in such synthetic grafts (22).

In the present study, we investigated the healing properties of two types of Dacron prostheses, both implanted in the sheep carotids: a D.T. graft and a new modified D.T. graft whose fabric was embedded with a biodegradable mammalian gelatin prior to implantation.

As for the conventional D.T., we were able to confirm the previous reports from literature (16, 17, 19, 20, 23). Eight weeks after implantation, a pseudointima lined most of the lumen of this graft. Neointima occasionally occurred next to anastomoses, but never completely covered the graft. Focal areas of stripping as well as platelet deposition were also observed. After sixteen weeks, the lumen of D.T. was occluded by a well recanalized thrombus through which blood flow could, at least in part, be reestablished. Moreover, a marked reaction of giant cells against the Triaxial fabric was well recognized.

On the contrary, a true endothelial lining developed on G.T. in our experimental model. The endothelial colonization apparently required the following events: i) the activation of medial smooth muscle cells which undergoing phenotype modulation could represent the major source of the spindle-shaped myofibroblasts; ii) the recruitment of the mononuclear macrophage system, which is involved in the remodelling of the fibrinous lining both by clearing fibrin and cellular debris and by modulating the smooth muscle cell and endothelial proliferation (3, 6, 18); iii) the synthesis of a suitable extracellular matrix by myofibroblasts.

These processes, which arise early after surgery, seem to be critical for the subsequent neointimal deposition. Aggressive factors, such as turbulent flow, mechanical stress and toxic injury could modify this pattern of healing thus

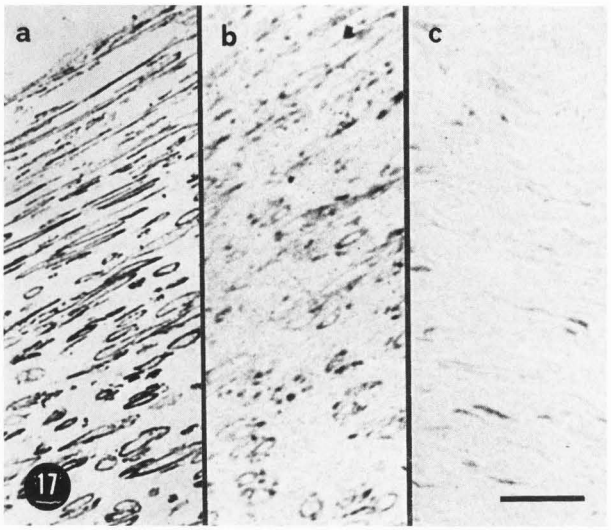

Fig. 17. Sixteen weeks. Immunocytochemical characterization of neointimal spindle-shaped cells in healing G.T. grafts. a) Protein A-gold-silver labelling for actin. LM on semithin section. b) Protein A-gold-silver labelling for vimentin. LM on semithin section. c) Protein A-gold-silver labelling for desmin. LM on cryostat section. Bar: a) 50 μm; b) 50 μm; c) 50 μm.

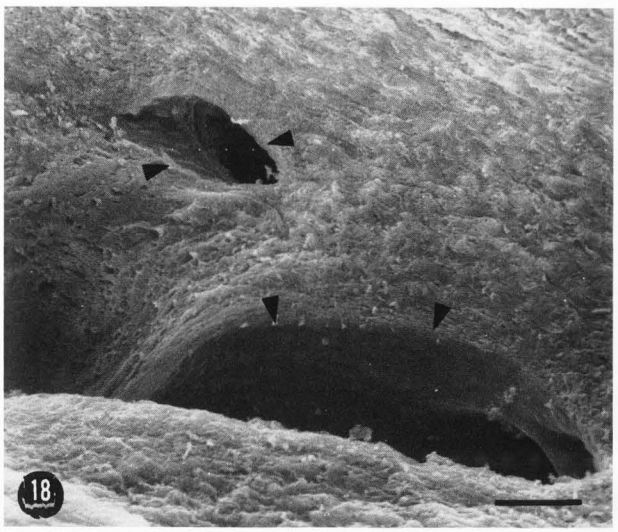

Fig. 18. Sixteen weeks. D.T. with an occlusive thrombus. SEM shows the openings of some recanalizing vascular channels (►). Bar = 50 μm.

rendering the graft prone to failure. In this framework, gelatin may promote an early graft incorporation. Fibrin lining of the luminal G.T. surface is extremely rapid and appears to be complete by 7 days. At eight weeks, a neointimal deposition was observed both on anastomoses and on the central portion of the graft. Proliferating endothelial cells, often grouped in clusters, were characterized according to ultrastructural (surface and internal morphology) and immunocytochemical (presence of actin, vimentin and absence of desmin) criteria. Sixteen weeks after surgery, a more mature neointima was formed, and G.T. graft displayed a continuous endothelium. Anastomotic narrowing due to myofibroblast proliferation (4, 14, 22) was never detected. Instead, the neointimal thickening was evenly distributed along the entire length of the graft. The endothelial cells characteristically showed a rearrangement of their actin microfilamentous network. Peripheral bundles of actin filaments, stress fibres (9, 26), were observed within the resting endothelial cells. Since focal contacts were often found at the perimeter of such cells, it is somehow possible that peripheral stress fibres are related to the sites of endothelium adhesion to its substratum, thus preventing the cells, subjected to hemodynamic stress, from translocation.

Conclusion

Our findings indicate G.T. as a suitable graft for small vessel implantation. Furthermore, it was shown that the gelatin impregnation was useful in promoting the healing process. This feature is believed to be of importance in the subsequent graft endothelialization.

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Discussion with Reviewers

K.A. Rosenbauer: Did you observe Weibel-Palade bodies in the endothelial cells of neointima?

Authors: We did not. However Feigl et al. (see reference 6) did not observe any Weibel-Palade bodies in their study which was also performed on sheep. As to the distribution of the "rod-shaped

microtubulated bodies", F. Ghadially (Ultrastructural Pathology of the Cell and Matrix, II ed., 1982, Butterworths, London, pp 623-628) stated that they were found in seven species besides man. Sheep was not included in this list. Moreover, we used a polyclonal antibody (Dako) directed to Factor-VIII related antigen both on semithin and cryostat sections. At the same time the immunolabelling was performed on human common carotid samples. The results showed that human endothelium stained whereas sheep endothelium did not. Data sheet provided by Dako did not mention sheep as cross-reactive species. In our opinion, these data suggest that endothelial cells from various sources as well as species may exhibit slightly different morpho-functional behaviors.

P. Frasca: What is the specific feature of the mammalian gel in the G.T. graft that it makes the graft respond so favorably ?
Authors: G.T. graft is a Triaxial warp knitted Dacron prosthesis which has been impregnated with an adsorbable protein. The main advantage of this impregnation is to provide a vascular prosthesis which does not require to be preclotted. The other one is to improve the healing characteristics of the graft. In fact, it is known that compound, biodegradable vascular graft materials have a low thrombogenicity (i.e. low reactivity with platelets). Gelatin can be defined as a type of natural hydrogel derived from mammalian collagen by thermal or chemical processes. It is a non-antigenic, non-toxic protein, a fact which is reflected by its extensive use as plasma expander. Crosslinked gelatin surfaces have a high blood compatibility which corresponds to the ability of the contact surface to interact with plasma proteins and yet demonstrate the capacity to be free of thrombus. It can function as a scaffold for the regeneration of arterial tissue thus leading to a rapid perigraft tissue ingrowth (27, 28). Moreover, it guarantees a blood-tight graft on implantation which prevents graft bleeding. The gel is resorbed over a period of 15 days. At this time, a porous graft results on healing. This means a good blood supply through the open interstices into the inner pseudointimal coverage thus avoiding necrosis, haemorrhage and calcification.

D.W. Gregory: What is the "nuclear fibrous lamina" ?
Authors: According to F. Ghadially (ibid, pp. 38-43), the nuclear fibrous lamina appears as a band of fine-textured material lying adjacent to the inner membrane of the nuclear envelope. Now, some authors refer to the nuclear fibrous lamina as a part of the cellular cytoskeleton. Anyway, it is a dynamic, transient structure which can be disclosed in numerous cell types (both mesenchymal and epithelial cells). It seems to be more prominent in mesenchymal cells involved in tissue repairing.

D.W. Gregory: Both Figs. 13b & 16b show some gold labelling of the background cytoplasm. Is this due to non-specific labelling or 'invisible' antigen? Please will you describe in detail, and preferably illustrate, the controls used for your immunocytochemical studies?
Authors: Control experiments were performed by using a non-immune serum followed by protein A-gold complex as well as by omitting the first incubation with the specific antibody. Under these conditions very few gold particles were present. They were randomly dispersed over the tissue sections as a non-specific background staining. An increased labelling was observed over the cytoplasm of endothelial cells only when anti-actin and anti-vimentin antibodies were applied to the sections. Anyway, the endothelial cell intermediate filaments tend to form loosely packed bundles running throughout the cytoplasm. Thus, the gold particles (see Fig. 13b) can obscure those filaments which are transversally sectioned. As to Fig. 16b, this micrograph shows actin stress fibers which develop in endothelial cells subjected to mechanical stresses. Besides this particular cytoskeletal arrangement, there are, within endothelial cells, other constant actin microfilaments which are mainly located close to the plasma membrane as webs of interwoven filaments. These filaments should also be considered in the evaluation of the specificity of the reaction (specific vs. non-specific labelling).

D.W. Gregory: In Fig. 10, what is the nature of the large patches of material with a slightly wrinkled surface, between the deposits of platelets and mononuclear cells, which are obscuring the Dacron fibrils in this D.T. graft?
Authors: It should be a thin cellular layer which was shrunk by the CPD procedure.

D.W. Gregory: How do you know the chemical composition (stated to be proteoglycans, collagen fibrils and scant elastin) of the fibrous extracellular matrix described for G.T. grafts at sixteen weeks after implantation?
Authors: In the transmission electron micrographs, the extracellular matrix components are well recognized. In particular, the two main components of the connective tissue matrix, viz: (i) the fibrous component (collagen fibres and elastic fibres), and (ii) the ground substance which contains mainly proteoglycans, can be morphologically identified.

S.L. Goodman: In your conclusions you suggest that the GT graft is suitable for small vessel implantation. Patency is only one criteria for graft suitability while the absence of thromboembolism is another. Do you have evidence, such as necroscopic evaluation, that thromboembolism does not occur with the GT graft during the healing process?
Authors: No, we do not. As you know, no single synthetic graft has yet satisfied all the mechanical and blood compatibility demands of the vascular system. However, several materials (PTFE, compound Dacron , PU/PLLA grafts) have proved to be usable. Gelatin coated surfaces have been shown to have a low reactivity with platelets. Our results show rapid and complete endothelialization as well as absence of thrombosis on G.T. grafts. The presence of endothelium is of fundamental importance due to its complex antithrombogenic function (for instance, the synthesis of prostacyclin, heparin-like molecule, thrombomodulin). Our 1 year experience concerning patients who underwent arterial substitution with G.T. grafts is also encouraging. However, the risks of thromboembolism and occlusion remain.

S.L. Goodman: Islands of endothelium were observed in the central portion of the GT graft. Do you have evidence on how these arose? Did these endothelial cells come from upstream or perhaps appear via migration through the graft as described by A. Clowes (Mechanisms of Arterial Graft Failure: The Role of Cellular Proliferation, New York Academy of Sciences, Blood in Contact with Natural and Artificial Surfaces, Nov 12-14, 1986, 47.)?
Authors: In our experimental model, the endothelial layer was provided by migration as well as proliferation of endothelial cells from sources at the cut edge of the host artery. However, the blood stream source should also be taken into account. On the contrary, the mechanism described by A. Clowes has to be excluded. In fact, the transmural capillary ingrowth is strictly related to the porosity of the graft. For instance, the PTFE graft used by A. Clowes showed a 60 μm mean internodal distance. On the contrary, the G.T. is a zero-low porosity graft (its porosity changes during the process of healing). For this reason, we never observed capillaries within the luminal coverage of this graft. They were numerous into the neoadventitia and stopped close to the interstices of the Dacron fibres.

S.L. Goodman: You state that anastomotic narrowing was not observed on the GT graft. However, comparison of Figures 8 and 14 show an increase in pseudointimal thickness from 50 μm to 230 μm over two months. If this process continued the 6 mm graft would eventually become occluded. Indeed, A. Clowes (ibid) described this type of neointimal hyperplasia in baboons implanted with porous PTFE (Gore-tex) grafts. Do you have evidence that this will not occur with the GT graft? Do the cells of the pseudoneointima, including endothelial cells, myofibroblasts and smooth muscle cells, continue to show signs of proliferation at four months?
Authors: Intimal hyperplasia appears as a myofibroblast proliferation occurring at the heel and toe of the anastomosis and the floor of the host artery. We never found this kind of narrowing. As stated in the paper, the luminal coverage was homogeneously increased in thickness along the entire length of the graft. Moreover, Fig. 19 is a LM image of the luminal coverage of a G.T. graft at eight weeks. The thickness of the pseudointima is about 250 μm. This value is close to the thickness of the neointima observed at sixteen weeks. A prominent oedema is visible (see the area between the arrowheads). Thus, a proportional shrinkage should be expected from a similar specimen processed for SEM. By contrast, the neointima is compact at sixteen weeks. This means a more dense and less oedematous extracellular matrix. So the influence of the CPD procedure will be minimized.
As to the last question, we have no information on the rate of cellular proliferation. However, it can be suggested that endothelial cells stop to proliferate when a confluent monolayer is reached. In grafts completely covered by endothelium the smooth muscle cells of the neointima can return to the contractile state, regaining an apparently normal complement of myofilaments with a concomitant decrease in the amount of synthetic organelles (see next page for Fig. 19).

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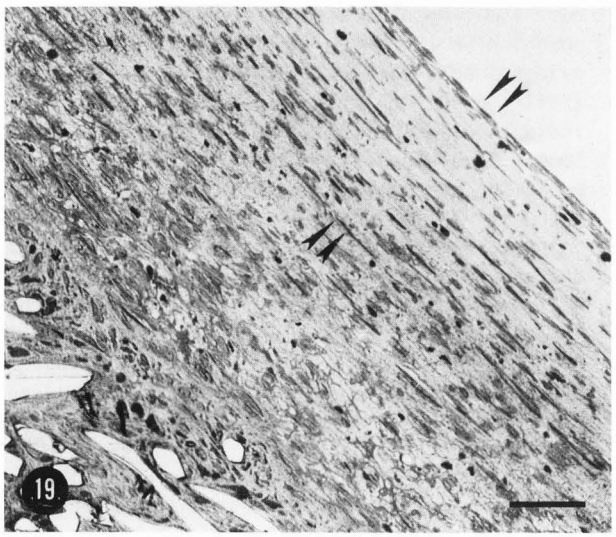

Fig. 19. Eight weeks. LM view of G.T. graft pseudointimal coverage. Multiple layers of spindle-shaped cells are embedded in a loose extracellular matrix. The area between the arrowheads shows a very prominent oedema. Bar = 50 μm.