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Angiogenic Vascular Grafts[1]

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The development of an endothelial cell lining on prosthetic vascular grafts may be achieved in three ways, as illustrated in figure 1. *Pannus ingrowth*, or endothelial cell migration from the native artery, is generally limited to a short distance from each anastomosis [1, 2]. Until the factors that restrict endothelial migration over prosthetic surfaces are better elucidated, the contribution of this mechanism to vascular graft endothelialization remains limited.

The second approach, direct *endothelial cell seeding*, has been successful in both animal models and in early clinical trials [3, 4]. For example, seeding of autologous canine endothelial cells onto Dacron or polytetrafluoroethylene (PTFE) grafts results in an endothelial monolayer within 3–4 weeks following graft implantation [5]. Although a number of challenges remain to be overcome before endothelial cell seeding will be applicable on a broad scale clinically [6], the experimental results of these techniques remain the 'gold standard' by which other approaches to graft endothelialization will be judged.

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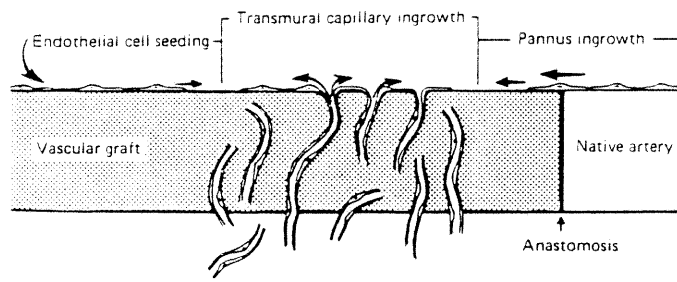

Fig. 1. Diagrammatic illustration of three ways by which prosthetic vascular grafts may acquire an endothelial cell lining.

A third approach, illustrated as *transmural capillary ingrowth*, relies upon migration of capillary endothelial cells through the graft wall to provide a source of cells for surface endothelialization. Although this process was suggested as early as 1962 [7], it has only recently been demonstrated that transmural ingrowth is a feasible means of achieving an endothelial cell-lined surface on prosthetic vascular grafts [8–12]. We have used the term 'angiogenic' to describe vascular graft materials which elicit this type of healing response after implantation.

Angiogenic vascular grafts appear to develop surface endothelium and a cellular neointima through the process of transmural capillary ingrowth. The angiogenic graft materials currently under study include textiles constructed from absorbable polymers and porous fabrications of PTFE. In addition, composite materials constructed from absorbable polymers and a permanent structure, such as Dacron or PTFE, are also under evaluation. This presentation reviews the feasibility or graft endothelialization by transmural capillary ingrowth, our work towards understanding the mechanisms of angiogenic vascular graft healing, and a potential means to modify this healing response.

Feasibility of Surface Endothelialization

The possibility that transmural cellular ingrowth could result in surface endothelialization during early vascular graft healing was first convincingly demonstrated with absorbable textile grafts [8]. Greisler et al. [11] compared the healing of Dacron grafts and polyglactin 910 (Vicryl) grafts after implan-

tation in the rabbit aorta. Dacron grafts demonstrated fibrous tissue ingrowth with a flow surface composed of protein, fibrin, and platelet thrombus. In contrast, Vicryl grafts demonstrated cellular ingrowth with capillary migration, a flow surface of endothelial cells, and a smooth muscle cell-like neointima within four weeks. Further studies have demonstrated a similar healing response to polyglycolic acid, polydioxanone, and polyurethane/poly-*L*-lactic acid graft materials [9–11].

Porous fabrications of PTFE represent a permanent graft material that elicits an angiogenic healing response. This has recently been demonstrated by Clowes et al. [12, 13, 18] using the baboon carotid artery model. Although the healing of clinically utilized PTFE grafts (30-μm internodal distance, reinforced outer wrap of PTFE film) is characterized by little tissue ingrowth and a fibrin-like flow surface, the early healing of porous PTFE grafts (60-μm internodal distance, nonreinforced) demonstrates rapid transmural capillary ingrowth, surface endothelialization, and a cellular neointima.

The histological appearance of porous PTFE grafts removed four weeks after implantation in the baboon carotid artery shows surface endothelial cells and a neointima composed of smooth muscle cells [12]. In addition to a complete endothelial cell monolayer, scanning electron microscopy of the lumenal surface reveals numerous capillary pores, at a distance of 150–400 μm apart. Injection of the graft lumen with low viscosity latex demonstrates communication of these pores with the tissues outside the graft, through small transmural vascular channels. These observations suggest that migrating capillary sprouts, upon reaching the lumenal surface of the graft, provide multiple sites from which endothelial cells may migrate to cover the graft surface.

Mechanisms of Angiogenic Vascular Graft Healing

Transmural capillary ingrowth may result in the development of surface endothelialization and neointimal tissue. We have focused on the mechanisms that might promote this response to prosthetic vascular grafts after implantation. It is clear that porosity is an important element, from a structural viewpoint, as woven textiles and microporous reinforced PTFE grafts prevent the ingrowth of capillaries. On the other hand, knitted and velour Dacron grafts are porous, yet these materials do not develop an angiogenic healing response. This suggests that non-mechanical factors may also be important [14, 15].

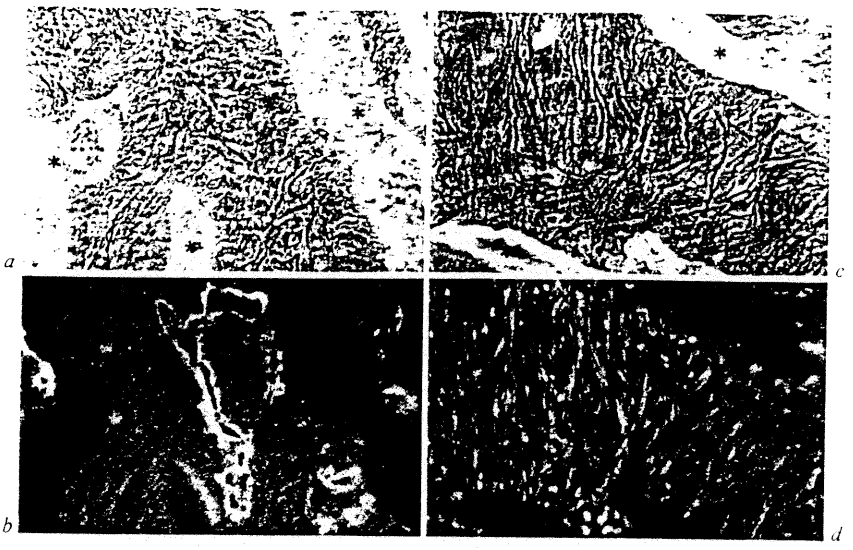

Fig. 2. Immunohistology of porous PTFE graft (60-μm internodal distance, nonreinforced) removed from canine carotid artery 2 weeks after implantation. Phase-contrast *(a)* and corresponding indirect immunofluorescent *(b)* views of graft matrix stained for laminin. Laminin localized to the basement membrane of capillaries migrating through the graft wall. A similar pattern is obtained for collagen types IV and V. Phase-contrast *(c)* and corresponding indirect immunofluorescent *(d)* views of graft matrix stained for fibronectin. Fibronectin is distributed in a fibrillar array throughout the graft wall, prior to the migration of capillaries. A similar pattern is obtained for collagen types I and III. The PTFE nodules are marked by asterisks. ×38.

Surface Endothelialization

We have studied the early healing response of porous PTFE grafts (60-μm internodal distance, nonreinforced) in the canine carotid artery. Of particular interest has been the role of specific extracellular matrix components and cellular growth factors in the process of transmural capillary ingrowth.

Figure 2 demonstrates histologic sections of a porous PTFE graft removed from the canine carotid artery 2 weeks after implantation, stained by immunological techniques for extracellular matrix components. Laminin, a basement membrane-associated glycoprotein, is distributed along the capillary sprouts migrating through the graft wall. A similar pattern was observed for types IV and V collagen. In contrast, fibronectin, a cell-adhesion

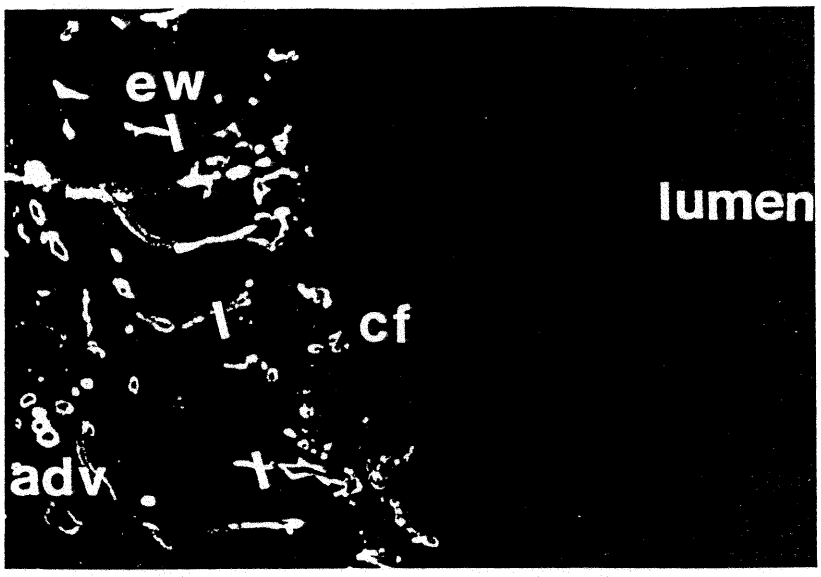

Fig. 3. Low-power indirect immunofluorescent view of porous PTFE graft (60-μm internodal distance, nonreinforced) removed from canine carotid artery 2 weeks after implantation, stained for laminin. The graft lumen is on the right, and the adventitial (adv) surface is on the left. The external wall (ew) is marked by the dashed white line. Transmural capillary ingrowth can be seen to extend as a 'capillary front' (cf), which has transgressed 35% of the graft width at this time. ×2.5.

glycoprotein, is distributed in a fibrillar pattern throughout the matrix of the graft wall, and interstitial collagens (types I and III) were localized in the same pattern. The early appearance of fibronectin and interstitial collagens within the graft wall, along with the initial cellular infiltrate, may play a role in the subsequent migration of capillaries. For example, several investigators have shown that interstitial matrix components stimulate the migration of capillary endothelial cells in vitro, while basement membrane components promote differentiation into capillary-like sprouts [23].

The use of immunohistological techniques has also allowed an assessment of the rate and extent of transmural capillary ingrowth into porous PTFE grafts. Figure 3 demonstrates the extent of capillary ingrowth during the healing of a porous graft 2 weeks after implantation into the canine

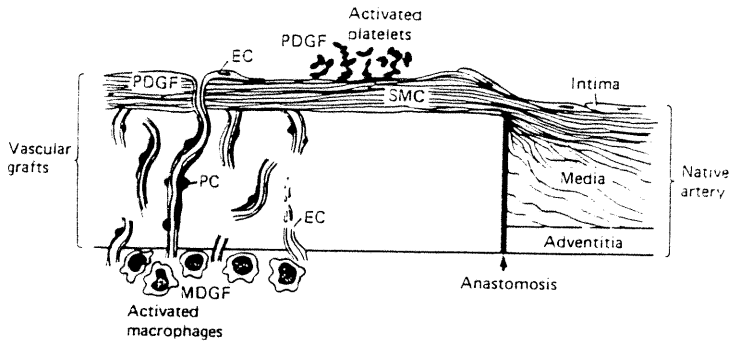

Fig. 4. Diagrammatic illustration of the development of neointimal hyperplasia in prosthetic vascular grafts. Platelet-derived growth factor (PDGF) and macrophage-derived growth factor (MDGF) may arise from a number of sources in the healing graft environment. Anastomotic compliance mismatch may also play a role. Neointimal smooth muscle cell (SMC) may be derived from microvascular pericytes (PC). EC = Endothelial cells.

carotid artery. The migration 'capillary front' has transgressed only 35% of the graft wall width at 2 weeks. By 4 weeks, the capillaries have reached the lumenal surface, and surface endothelialization can be observed in some areas [Thompson et al., manuscript in preparation].

It has been suggested that endothelial cell growth factors in the environment of healing vascular grafts may stimulate the transmural migration of capillaries. We have identified mitogenic activity in salt extracts from porous PTFE grafts removed at 2 and 4 weeks. Examination of this activity by heparin-affinity and by immunological analysis for acidic and basic forms of fibroblast growth factor (FGF) may further our understanding of the role that heparin-binding endothelial cell growth factors play in the process of capillary ingrowth. Immunolocalization techniques are currently under study to identify the distribution of these factors in the healing graft environment. Endothelial cell mitogens may arise from a variety of cell types, including activated macrophages [16, 17]. The frequent observation of activated macrophages and foreign body giant cells along the outer wall of healing PTFE grafts suggests that these cells may mediate much of the angiogenic graft healing response.

Fig. 5. High-power indirect immunofluorescent view of porous PTFE graft at 2 weeks, stained for laminin. Transmural capillary ingrowth is observed, with basement membrane outlining pericapillary cells which may be microvascular pericytes. These cells may give rise to the neointima. × 65.

Neointimal Hyperplasia

Although the healing of angiogenic vascular grafts is associated with early surface endothelialization, the development of progressive neointimal hyperplasia is a problem seen with most of these materials. This occurs not only at the anastomoses, but along the graft length. The mechanisms that may lead to this process are illustrated in figure 4.

Intimal hyperplasia is generally attributed to smooth muscle cell proliferation, which may be stimulated by mechanical factors such as compliance mismatch, or by mitogenic factors such as platelet derived growth factor (PDGF) [18]. It has recently become apparent that PDGF-like mitogens are produced by a variety of cell types, including activated macrophages and proliferating endothelial cells [19, 20].

Using tritiated-thymidine labelling, Clowes et al. [12] have examined cellular proliferation in porous PTFE grafts in the baboon. Neointimal proliferation persists long after an endothelial monolayer has been established. Despite a morphologically normal appearance, the endothelial cells lining porous PTFE grafts proliferate more rapidly than those of native arteries, even 3 months after implantation. Continued elaboration of PDGF by a non-

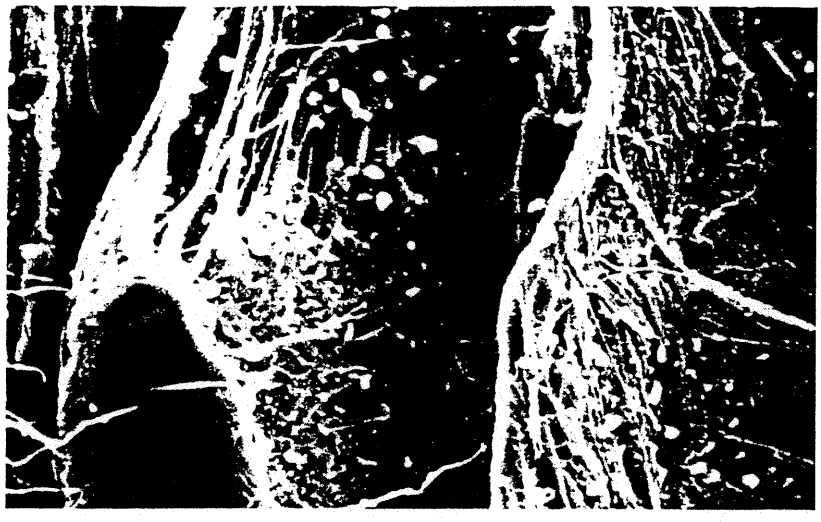

Fig. 6. Scanning electron micrograph of the luminal surface of a porous PTFE graft (60-μm internodal distance, nonreinforced) coated with polyanhydride, incorporating retina-derived endothelial cell growth factor (bovine) and heparin for sustained-delivery. The internodal spaces of the graft are well preserved, and a thin polymer coat is achieved.

quiescent endothelial monolayer, and its effect on the underlying smooth muscle cells, may explain the progressive development of neointimal hyperplasia.

As subendothelial smooth muscle-like cells appear concomitant with surface endothelium, it has been suggested that these cells are derived from microvascular pericytes that migrate with capillary endothelial cells through the graft wall [12, 18]. Examination of capillaries within the graft wall by immunofluorescent techniques for basement membrane reveals cells lining the capillary sprout, which may be pericytes (fig. 5). Immunological staining for specific actin-isotypes has been shown to distinguish pericytes from other cells [21], and these techniques may allow clarification of the role pericytes play in transmural capillary ingrowth and the development of neointimal hyperplasia.

Modification of Angiogenic Graft Healing

As our understanding of the mechanisms of graft healing become clearer, modifications of this healing response may be possible. Several inves-

tigators have explored the use of composite materials, utilizing an absorbable polymer and a permanent graft scaffold [9, 10, 14]. These alterations significantly affect the rate of transmural healing, as well as subsequent proliferation of the neointima. While Dacron appears to inhibit surface endothelialization by transmural healing, polyurethanes appear to promote this process, and may limit the development of intimal hyperplasia.

We are currently investigating the use of absorbable polymers as sustained-release drug-delivery systems, combined with porous PTFE grafts. A novel class of polyanhydrides has been developed by Leong et al. [22], which allows the incorporation of biologically active macromolecules for sustained-release over prolonged time periods. By coating porous PTFE grafts with a thin layer of polyanhydride, we have been able to deliver peptide growth factors and glycosaminoglycans into the local graft environment via sustained release.

Figure 6 shows the appearance of porous PTFE coated with polyanhydride, incorporating heparin and a heparin-binding endothelial cell growth factor. The porosity of the material is maintained, permitting rapid tissue ingrowth, and the handling characteristics of the graft are similar to the uncoated material. The effect of growth factors and highly sulfated glycosaminoglycans (to inhibit smooth muscle cell growth) on the subsequent healing response in vivo are currently under evaluation. Although these efforts are in an early stage of development, they may lead to an effective means of modifying the healing response to angiogenic graft materials.

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