1st European Workshop on Advanced Technologies in Vascular Surgery,
Vienna, November 5–6, 1986

Endothelialization of Vascular Grafts

Editors
P.P. Zilla, R.D. Fasol, M. Deutsch, Vienna

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Why Seeding?

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The Germination of Endothelial Cell Seeding

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The field of endothelial seeding has been reviewed at two previous symposia [1, 2]. Still, the issue of endothelial seeding may be reviewed from yet a different perspective; that is the perspective of a germinating idea. Innovations often require critical developments to occur in related fields. An example of this is the construction of the Wright brothers' airplane, which drew heavily from their understanding of lightweight tubular bicycle frames and previous flying attempts by others. Endothelial seeding evolved concurrently with developments in vascular grafting, knowledge about the basement membrane, and endothelial cell physiology. Developments in each of these subjects belong to three general time periods. The first period includes those times that preceeded endothelial seeding, the period of the first successful endothelial seeding and the period of the veritable explosion of new information that has occurred in all three areas since that time.

The design of endothelial cells began before man walked on earth, and in the evolution that preceded man, endothelium apparently found its first application in the linings of lymphatic channels [1]. Of course man had no idea about this extraordinary cell; indeed, his understanding of the circulation began with Harvey in the 17th century. Years passed before his intervention into the vascular system began with experiments by Eck in 1879 in which vascular anastomoses were performed. Carrel and Guthrie began to explore synthetic vascular replacements in experiments performed during the first part of this century.

Meanwhile, man's understanding of endothelium was generally limited to the fact that it was present histologically, and that it formed some type of liner for blood vessels. However, the blood vessel itself was regarded as little

more sophisticated than a garden hose, a simple conduit through which blood would flow.

In 1906, vascular grafting had its clinical debut with a vein interposition graft for a popliteal aneurysm performed by Goyannes. Vascular replacement grafting remained infrequent until the 1950s when Voorhees first used Vinyon N as a graft replacement for arteries. He intuitively recognized the need for an endothelial lining on his synthetic grafts. Vinyon N was quickly supplanted by Dacron and Teflon grafts that were promoted by DeBakey and Edwards. The idea of endothelialization of synthetic grafts lay fallow for nearly two decades. However, it soon became apparent that the Dacron and Teflon graft materials were well suited to large vessel grafting, marginally suited to medium-sized arterial grafting, and poorly suited to small artery grafting.

In the early 1970s, it became possible to tissue culture endothelium [3], but Carrel was doing tissue culture as early as the 1920s and established fibrin as a substrate on which fibroblasts could be grown [4]. Jaffe et al. popularized the collagenase harvesting of endothelium; in 1973, Mansfield et al. [5] drew upon this, culturing cells onto bovine vascular grafts, but the cell linings proved unstable during flow. However, at about this time, expanded PTFE began to emerge as a better arterial substitute [6]. Yates et al. [7] were working concurrently with Dacron grafts, attempting to inhibit thrombin activity on the graft surface with heparin.

Thus, the stage was set for our first experiments with endothelial seeding. Autogenous endothelial cells were scraped from veins with a crude steel wool pledget and mixed with whole blood. Dacron grafts were preclotted with the blood and cell mixture, thereby holding the seeded cells in a fibrin network on the Dacron material. Our choice of a fibrin substrate was based on intuition rather than knowledge of the work by Carrel [4], and our first experiments in 1976 failed to achieve endothelial healing.

Fortunately, we chose to heparinize the second set of experimental grafts after they were seeded, modifying the technique of Yates et al. [7]. These experiments were successful, and resulted in endothelial linings within a month of seeding [3]. Like our choice of a fibrin substrate, intuition surrounded our decision to heparinize the grafts; we did not begin to understand the important effects of heparin that would be described subsequently. Our naivete was not limited to the heparin and fibrin issues; endothelium itself was remarkably mysterious as exemplified by this statement in the 1976 literature [8]: "Most recently, endothelium has been shown to possess hitherto unrealized metabolic properties that may play vital roles ... in control of ...

hemostasis and thrombosis.' 1976 marked the beginning of great proliferation in cell seeding and three related fields: vascular grafting, cell substrate and endothelial cell physiology.

In 1976, heparan sulfate was already known to be on endothelial surfaces, but in 1976, Moncada [9] discovered prostacyclin. A litany of endothelial functions was assembled in the subsequent years including the description of plasminogen activator function in 1980 by Laug [10]. In 1983, thrombin binding and inhibition were described by Dryjski [11], thromboxane A2 synthesis by Eldor [12], antithrombin 3 function by Bauer [13], and the binding of clotting factor Xa by Heimark [14]. In 1984, there were the discoveries of thrombospondin by Mumby [15], protein C function [16, 17], thrombomodulin activity by Johnson [18], plasminogen activator inhibitory functions by Van Hinsbergh [19], tissue factor (thromboplastin) release by Maynard [20], platelet activating factor by Prescott [21], and nexin production by Knauer [14]. Finally, the binding of coagulation factors IX and IXa was reported by Nawroth [22] in 1985.

The complex interaction of endothelium with its substrate has progressed as well. Jaffe et al. [23] found that endothelial cells made basement membrane microfibrils, elastin and collagen. Through the late 1970s, fibronectin was described and further characterized [23]. Other basement membrane proteins were subsequently described including laminin and vitronectin in 1979 [24], and nidogen in 1983 by Timpl [25]. Other cell adhesion molecules have been discovered in other cell systems and may eventually relate to endothelium too [26].

Our understanding of what prompts the endothelial cells to proliferate was nil until Folkman [27] identified the tumor angiogenesis factor in the early 1970s. By 1978, a mitogenic effect was recognized with certain prostaglandins [28], serum [29], and macrophages [30]. Endothelial cell growth factors appeared on the market in 1979 [31]. By 1983, it was apparent that heparin augmented the effects of endothelial cell growth factors [32], and one relationship between seeding and heparinization was clarified.

Our initial seeding experiments [3] were followed by histologic confirmation of the nature of the resultant lining [33]. Dilley [34], working in our laboratories, adapted factor VIII related antigen staining to endothelial cell identification on grafts. In 1980, Graham et al. [35] used enzymatic harvesting of endothelium, thereby gaining a more pure endothelial inoculum. They confirmed [35] our work and introduced the idea of culturing the cells before seeding [36]. In 1981, they reported successful seeding of PTFE grafts [37], which were previously not thought to be suitable for seeding [38].

In 1982, Stanley et al. [39] reported improved patency of small artery Dacron grafts using endothelial seeding plus aspirin and dipyridamole therapy. Other reports in 1982 partly explained Stanley's results since seeding improved platelet survival [40] and platelet serotonin levels [41]. The same year, Sharefkin [42] labeled endothelial cells with indium-111 and provided a laboratory tool that shaped the seeding studies that followed.

In 1983, Allen et al. [43] explained the seeding effect on platelet survival by demonstrating that seeding reduced platelet uptake on grafts. Schmidt et al. [44] demonstrated improvements in seeded small artery graft patency during low flow. Also during that year, we reported improved patency in small artery prostheses that were lined with endothelium in tissue culture, all without aspirin and dipyridamole [45]. The first clinical trial of seeding was reported; mechanically derived endothelium were seeded on Dacron grafts. The reports were discouraging; among smokers, graft patency was worse than with unseeded grafts, and there was histologic evidence of atherosclerotic changes in the wall of the graft [46].

The following year saw the first successful seeding experiments in primates by Callow et al. [47], and our detailed description of the long-term cellular healing patterns [48]. Sicard et al. [49] reported that seeding diminished thromboxane production on the walls of grafts compared to controls, and Graham [50] showed that a single-step inoculation of endothelium was more efficient than the three-step inoculation method that we originally described.

1985 proved to be a very innovative year in endothelial seeding. The inefficiencies of seeding on PTFE were explained by Rosenman et al. [51]. The increased production of prostacyclin on seeded graft walls was also identified by Schmidt et al. [52]. Rosenman reported improved resistance to bacterial infection on seeded grafts. Some of the new ideas that were tried included seeding with homografted endothelium by Zamora [53], seeding with xenografted endothelium by Pennell [54], the seeding of endarterectomized artery by Bush [55], and experimental seeding of coronary artery grafts by Hunter [56]. Finally, in 1985, we found endothelium growing on a seeded clinical PTFE graft [57], giving us encouragement that endothelial seeding would eventually find clinical applicability.

In 1986, a new method of endothelial harvesting was described by Jarrell [58] and Pearce. Methods of improving the cell distribution during inoculation were described by Kesler [59], and the effects of fibronectin on the improved binding of endothelium were established by Kesler [60] and Ramalanjona [61]. However, many factors contributed to endothelial slough-

ing during in vivo perfusion [62], namely leukocyte interaction with endothelium [63], and the effects of at least two serine proteases [64]. Species differences in the cell attachment phenomenon were clarified by Lundgren [65]. A second clinical trial was also reported in 1986; seeding improved patency in femoral popliteal bypass grafting when enzymatically harvested endothelium was inoculated onto PTFE grafts [66].

The germination of endothelial cell seeding has really only just begun. Many applications and improvements on the current technology will be forthcoming in the next few years. Indeed, as Allan Callow has stated, 'vascular prostheses have entered the biologic era'.

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Perspectives on Arterial Graft Function and Failure

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Probably the greatest urgency leading to the development of arterial grafts was the arteriosclerotic aneurysm of the abdominal aorta. There were numerous and largely unsuccessful makeshift attempts to control the expanding aneurysm during the 1940s and 50s. Spontaneous thrombosis was attempted by the insertion of several hundred feet of stainless steel piano wire within the sac, reinforcement of the wall was attempted by the application of epoxy resins which hardened upon polymerization, and induction of fibrosis was attempted by application of sheets of cellophane with additives to stimulate an inflammatory reaction. Following these failures it next seemed reasonable to try to replace a diseased artery with a normal human one. Fresh allogenic grafts were extremely antigenic, rapidly underwent rejection, and therefore had a very short clinical trial. A number of methods to denature the protein within the graft and overcome the antigenicity were introduced by application of chemicals, by freeze drying, and by 2 meV radiation. Although such grafts were not antigenic, they lost their tensile strength. Reports of dilatation and aneurysm formation appeared throughout the mid to late 1950s. The prevalence of abdominal aneurysms, their progressive expansion, and a consequent high mortality rate stimulated the search for an arterial substitute. In the late 1940s and early 1950s, Voorhees noted that an endothelial-like surface, relatively free of macroscopic thrombi, coated the surface of synthetic fabrics inserted into the blood stream. Such tubes supported fibrin deposition within the interstices of the graft. From this came the assumption that ingrowth of fibroblasts from the adjacent soft tissue could occur.

Subsequent migration of endothelium was anticipated. In the dog, endothelial regeneration did take place. In 1954, Voorhees and Blakemore reported replacement of 17 abdominal and one popliteal aneurysm by the implantation of a synthetic tube. This was the beginning of present day arterial reconstructive surgery and ushered in the age of vascular synthetics. The surgical vocabulary acquired words and concepts such as flexibility, durability, warp, weft, denier, porosity, compliance, and biological inertness. It was postulated that the ideal graft was blood tight after interstitial clotting, possessed a pore size sufficient to prevent fibroblast reorganization, would be conformable to surrounding tissues and thus avoid kinking, would correspond in compliance to the host artery, and was biologically inert. The trellis concept was introduced by which was meant porosity not only permitted but enhanced interstitial fibroblastic ingrowth. Internal and external velours were added with the intent of assuring firm host incorporation of the graft and attachment of what was thought to be endothelium on the luminal surface [1–6].

Had these large diameter grafts been used only in the high flow, low resistance aortoiliac position, graft refinement would have been unnecessary. In diameters of 4 mm or below, however, the failure rate of these synthetic fabric grafts is unacceptable. Below the inguinal ligament, the patency rate of saphenous vein grafts at eight years is 65–70%, for Dacron it is 10% [7]. Dacron grafts yield a 99% patency rate at 10 years in the aortoiliac position, about 50% in the femoropopliteal position, and in the distal calf approximately 15–20%, this last less than three years [6]. The same experience has been reported with expanded polytetrafluoroethylene when inserted below the knee. At the end of 3 years, the patency rate for PTFE was approximately 25 v. 60% for the saphenous vein [8, 9]. Denatured human umbilical vein grafts gave no better results.

Probably it was Strandness who first suggested that saphenous veins work better than the best of synthetic tubes because of the vein's endothelial surface. For perhaps as long as 20 years, endothelial regeneration such as seen in the canine model, was assumed to occur in the human. Such is not the case except for the perianastomotic zone of 1–2 cm. A completely lined endothelial graft in humans has not been realized with any of the prosthetic materials in use. This human failure of endothelialization of the synthetic graft may be the major shortcoming of all small caliber grafts currently available. Material largely of a proteinaceous nature gradually accumulates within the lumen. Various cellular elements – collectively referred to as myofibroblasts, and labeled pseudointima, neointima or neointimal hyperplasia