Endothelialization of Prosthetic Vascular Grafts: Current Status and Future Directions

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Synthetic vascular grafts perform satisfactorily as large caliber (e.g., aortoiliac) arterial substitutes but commonly fail when employed in small diameter and low flow applications.[1] It is likely that prosthetic grafts in humans do not become endothelialized except for a few centimeters from each anastomosis.[2] The lack of an endothelial lining has been postulated as an important factor contributing to the poor patency rates of prosthetic bypass grafts placed in the distal arterial circulation. Increased appreciation of the diverse functions of endothelium in maintaining vascular homeostasis, coupled with improved techniques for in vitro cultivation of human endothelial cells (ECs), spawned efforts to employ endothelium to produce a less thrombogenic inner lining for vascular prostheses.

HISTORICAL DEVELOPMENT

In 1970, Mansfield introduced the concept of induced endothelialization on prosthetics by using granulation tissue as a source of cells to line Dacron patches implanted into the hearts of dogs.[3] This resulted in a thrombus-free surface at three weeks. In 1978, Herring and his associates described a technique for endothelial "seeding" of Dacron grafts in a canine model.[4] Endothelial cells were obtained from saphenous vein segments by mechanical scraping and were immediately returned in suspension in the blood used to preclot the Dacron graft. This produced a complete vascular endothelial lining of the prosthetic graft at four to eight weeks after implantation by histochemical analyses.[5] Subsequently, investigators at the University of Michigan developed enzymatic techniques (employing collagenase) for reliably isolating canine venous endothelial cells, with decreased risk of contamination from other cell types (e.g., smooth muscle cells [SMCs], fibroblasts) as compared to mechanical scraping.[6] This group also employed tissue culture for expansion of the harvested venous ECs prior to seeding, resulting in greater seeding density and improved endothelial surface coverage of grafts at implantation.

Improved patency of seeded grafts has been demonstrated in a number of animal studies and was first reported for iliofemoral Dacron grafts in dogs in 1983.[7] Subsequent reports confirmed improved patency for seeded commercial ePTFE, experimental (porous) ePTFE, and Dacron grafts in the iliac and carotid positions in dogs.[8-10] Common features in study designs during this period included

immediate harvest and return of cells, the use of antiplatelet therapy for a brief, defined period of time, the use of large numbers of cells, and definitive analysis of patency at the time of explantation. Other animal studies demonstrated decreased platelet accumulation, enhanced prostacyclin production, and increased resistance to bacterial infection for seeded prostheses.[11-13]

With encouraging early results from animal experiments, attention was turned to refining harvesting techniques, cell culture to maximize the endothelial cell pool, and improving cell adherence to the graft surface using a variety of precoating substrates and techniques. For infrainguinal applications, most efforts have concentrated on ePTFE prostheses, since they are the most common non-autogenous material employed for these bypasses. Cell attachment to ePTFE requires the use of a precoating substrate which serves as a basement membrane mimic (Fig. 1). A number of substances, alone and in combination, have been employed (e.g., preclotted blood, serum, fibronectin, fibrin glue, collagen, laminin).[14-17] While both fibronectin and preclotted blood have shown promising results for ePTFE grafts, determination of the optimum graft material-substrate combination for supporting EC adherence and growth remains an area of active investigation.

Low harvest efficiency using enzymatic techniques, combined with the limited amount of vein available in patients requiring prosthetic bypass, encouraged the investigation of alternative sources of ECs for seeding. Microvascular endothelial cells (MVECs) are an attractive option in this regard. Omentum and falciform ligament have been employed as sources of MVECs for seeding vascular grafts.[18-20] Animal experiments have shown that prosthetic grafts seeded with cells derived from the above sources result in the formation of a confluent monolayer of endothelium and result in significantly improved patency rates of Dacron grafts used for coronary artery bypass in dogs.[21] Questions regarding identity of the isolated cells, freedom from contamination with other cell types, and complexity of the isolation techniques have been issues limiting the clinical application of this approach thus far.

CLINICAL TRIALS

Early human clinical trials resulted from the enthusiasm generated by the encouraging results in animal studies (Table 1). In the late 1980s, a number of reports appeared in which an immediate seeding approach (one-stage with harvest of venous ECs at the time of graft implantation) was employed in patients requiring lower extremity prosthetic bypass. In the first of these published series (1984), Herring and associates seeded Dacron femoropopliteal, axillofemoral, and femorofemoral grafts but failed to show any statistically significant improvement in patency rates.[22] In 1987, however, the same authors seeded ePTFE grafts (femoropopliteal) and showed an improved patency of seeded grafts as compared with unseeded grafts (82% vs. 31% at 1 year).[23] Several small studies from Europe at about the same time showed no difference in patency rates between seeded and unseeded grafts using the immediate seeding technique.[24-28] In some cases, decreased thrombogenicity of seeded grafts was documented by platelet-labeling studies.

In 1992 Magometschrigg and his associates reported early results in a series of reoperative distal ePTFE bypass grafts in 26 patients, half of which (nonrandomized) were seeded with previously harvested, cultured venous ECs.[29] Secondary patency rates were 92% for seeded and 53% for unseeded grafts at 30 days, and amputation rate was reduced by 50% in the seeded group at 18 months. In the most recent series reported from Indiana University, a multicenter randomized trial compared seeded PTFE femoropopliteal grafts (immediate seeding approach) with vein grafts.[30] Cumulative patency at 30 months was 92% for vein versus 38% for seeded grafts; unseeded ePTFE grafts were not included in the study. Failed seeded grafts were associated with anastomotic hyperplasia.

In a recently reported, well-performed study, Zilla and associates at the University of Vienna employed a delayed seeding approach with follow-up out to 32 months.[31] Forty-nine patients who required a lower extremity bypass and had no saphenous vein available were randomized to receive a seeded or unseeded ePTFE graft. Follow-up was based on angiography, platelet-labeling studies, ankle-brachial index measurements and duplex ultrasound. The patency rates at 32 months were 84.7% for seeded and 55.4% for unseeded grafts.

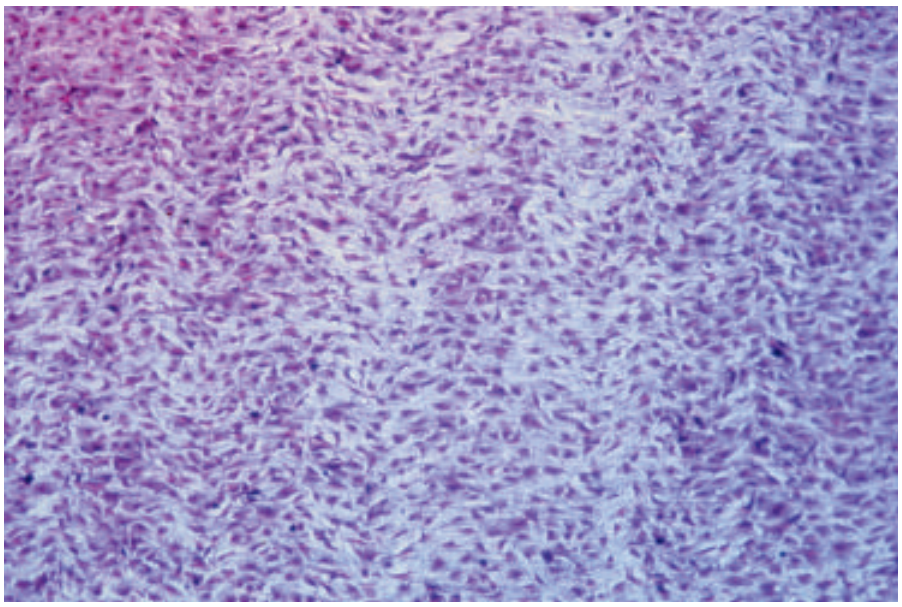


Figure 1. Typical appearance of human saphenous vein endothelial cells on fibronectin-coated ePTFE grafts. Cells were seeded and maintained in culture for 24 hours prior to fixation and staining with crystal violet. (x32, Reprinted with permission from Miyata T, Conte MS, Trudell LA, et al. J Surg Res 1991;50:485-93.)

CRITICAL TECHNICAL ISSUES

The optimal method for seeding prosthetic grafts to achieve a durable endothelial lining remains undefined. One of the most fundamental questions is whether a "delayed" seeding approach, which incorporates a tissue culture interval, offers any advantage

Table 1. Summary of clinical trials of endothelial cell seeded prosthetic grafts

Study	Prosthesis (type)	Number of Patients	I/D	P/UP	Pt. Selection	Patency	Comments
Herring et al.[22] 1984	Dacron Ax-fem Fem-fem Fem-pop	 11 28 147	I	 UP UP UP	 Alternate Alternate Randomized	 No difference S vs. US No difference S vs. US V better than S, US	Seeded grafts performed better in non-smokers.
Herring et al.[23] 1987	ePTFE (fem-pop)	28	I	UP	Alternate	S (82%) vs. US (31%) @ 1 year	ePTFE used-Enzymatic harvesting of ECs.
Örtenwall et al.[24] 1987	Dacron (aorto-bifem)	9	I	P	Consecutive	No difference S vs. US @ 4 months	⇓ thrombogenicity of S grafts @ 4 months.
Zilla et al.[26] 1987	PTFE (dist. fem-pop)	18	I	UP	Randomized	No difference S vs. US @ 14 weeks	No difference in thrombogenicity @ 14 weeks.
Örtenwall et al.[27] 1989	ePTFE (fem-dist)	23	I	P (1/2 graft seeded)	Not specified	Not compared	Thrombogenicity of seeded portion @ 6 months.
Fasol et al.[25] 1989	PTFE (dist. fem-pop)	26	I	UP	Not specified	S (100%) vs. V (100%) vs. US (77%) @ 1 year	No difference in thrombogenicity between S & US. ⇓ thrombogenicity of V grafts @ 1 year.
Örtenwall et al.[28] 1990	Dacron (aorto-bifem)	22	I	P	Randomized	No difference S vs. US @ 1 year	⇓ Thrombogenicity of S grafts @ 1 year.
Magometschrigg et al. [29] 1992	ePTFE (fem-dist)	26	D	UP	Non-randomized	S (92%) vs. US (53%) @ 1 month	In vitro seeding used. All redo grafts.
Zilla et al.[31] 1994	PTFE (fem-dist)	49	D	UP	Randomized	S (85%) vs. US (55%) @ 32 months	In vitro seeding. ⇓ thrombogenicity of S grafts.
Herring et al.[30] 1994	PTFE (fem-pop)	66	I	UP	Randomized	S (38%) vs. V (92%) @ 30 months	Multicenter trial. Failed grafts developed anastomotic hyperplasia.

I = Immediate seeding
D = Delayed seeding

P = Paired
UP = Unpaired

S = Seeded prosthetic grafts
US = Unseeded prosthetic grafts
V = Vein graft

over "immediate" seeding with freshly harvested ECs at the time of graft implantation. Tissue culture adds cost, complexity, risk of microbial contamination, and the possibility of phenotypic alterations in vitro. Potential advantages include increased number of cells available for seeding and improved retention due to maturation of cell–cell, cell–matrix, and graft–matrix bonds (see below). The question of cell number is an important one, given the limited vein available for EC harvest in patients requiring prosthetic bypass, low harvest efficiencies using the enzymatic techniques commonly employed, and the documented loss of substantial numbers of cells from seeded grafts on initial exposure to flow (Fig. 2).[32] The reproductive capacity of adult human venous ECs may be insufficient to achieve complete endothelialization in vivo once the density of cells remaining falls below some minimum value. It seems likely that unless alternative sources of ECs (e.g., microvessels) are employed, tissue culture will be required to obtain adequate cell numbers for seeding infrainguinal prosthetic grafts.

Fat-derived MVECs can theoretically be isolated in large numbers from easily obtainable, disposable adipose tissue. Several reports have appeared describing the isolation of large numbers of MVECs for seeding prosthetic grafts at supraconfluent densities.[33,34] As previously mentioned, contamination of MVEC isolates with other cell types and complexity of the isolation protocol remain important practical limitations. The function of MVECs in a large vessel arterial environment is speculative and is at most a theoretical concern. Further investigation is needed to determine the ultimate usefulness of this approach.

Despite a great deal of investigative

effort, the goal of durable, flow-resistant EC attachment to prosthetic graft surfaces remains elusive. Durable attachment requires firm bonding at three levels: substrate–prosthetic, cell–substrate, and cell–cell. Uniform binding of matrix protein to the hydrophobic ePTFE surface has been difficult and has led many investigators to employ combinations of substrates. A theoretical concern after implantation is that areas of cell loss will leave exposed these highly thrombogenic proteins to circulating blood. Previous studies have documented improved retention of seeded cells if the graft segments are maintained in tissue culture for some period of time (days) prior to exposure to flow, providing another rationale to favor delayed over immediate seeding protocols.[35] It has been hypothesized that maturation of cell–substrate and particularly cell–cell bonds requires the formation of junctional complexes and secretion of extracellular matrix, both of which require de novo protein synthesis. Even under the best of circumstances, it appears likely that a significant percentage of cells (20% to 80%) will be lost from the graft surface in the early postimplantation period. The function of these grafts may ultimately depend upon the retention of a critical minimal density of cells with sufficient reproductive capacity to grow to confluence in a short time interval.

The ability to achieve durable endothelialization of a prosthetic surface is strongly dependent on the nature of the prosthetic material employed. Commonly employed conventional prostheses such as commercial ePTFE have major drawbacks in this regard, and alternative materials or construction methods may be needed if endothelialization is considered an important therapeutic goal. A review of the numerous design constraints and experimental materials under investigation is beyond the scope of this monograph; it suffices to say that prosthetic materials development may facilitate EC seeding and allow for a better appraisal of its potential benefits.

The rationale for EC seeding rests on the belief that the beneficial functions exhibited by endothelium on native vascular surfaces in vivo will be maintained on the prosthetic graft. It is also well known that under certain circumstances, "activated" endothelial cells can exhibit a number of deleterious (e.g., procoagulant, immunologic, proliferative) functions, and thus characterization of the physiologic state of ECs on prosthetic grafts is of critical importance.[36-38] There has been some evidence from both animal and clinical trials that graft surface thrombogenicity, assessed by platelet deposition, may be decreased by EC seeding. Virtually nothing is known about any of the other of the myriad EC functions on seeded grafts, and this remains an important area for technical development. Ultimately, prosthetic bypass grafts in humans fail most often because of downstream anastamotic intimal hyperplasia, not "thrombogenicity" per se, and the impact of EC seeding on this process is unclear.[39,40]

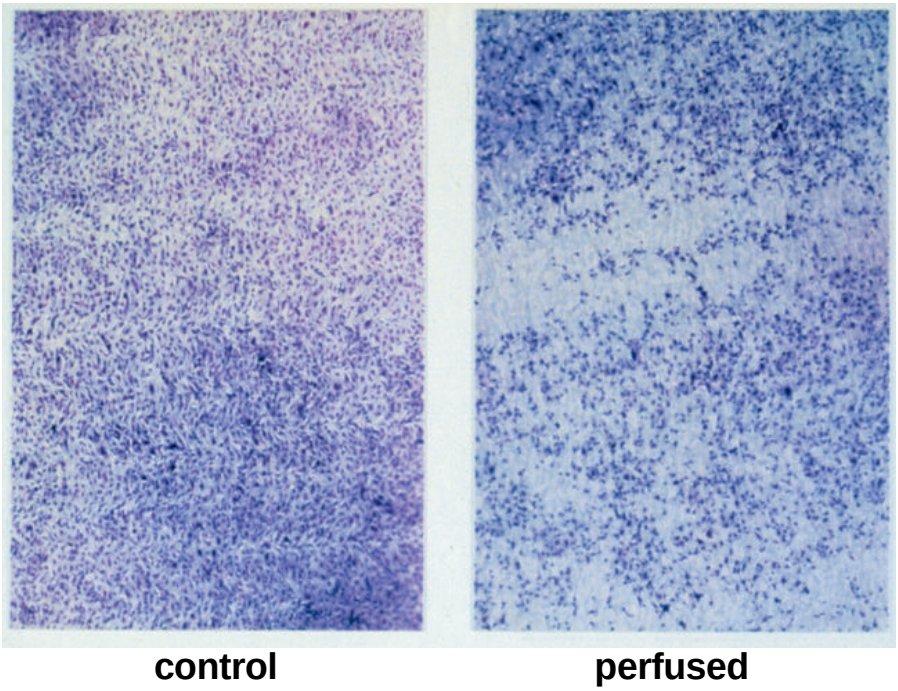


Figure 2. Appearance of EC-seeded fibronectin coated ePTFE graft before and after perfusion in an in vitro pulsatile flow circuit. Graft was seeded at confluent density, incubated for 90 minutes, then exposed to flow for 90 minutes prior to fixation and staining. (x5, courtesy of T. Miyata, LK Birinyi, Harvard Medical School, Boston, Mass.)

OTHER POTENTIAL APPLICATIONS OF SEEDING TECHNOLOGY

In animal models of arterial injury and healing, endothelial denudation results in a characteristic response of smooth muscle cell (SMC) migration to and proliferation within the intima. There is evidence to suggest that the magnitude of this response (and the resulting neointimal lesion) is related to the extent of endothelial removal, the degree of underlying injury to the media, and the rate and completeness of re-endothelialization. The pathogenesis of restenosis after therapeutic interventions such as endarterectomy, balloon angioplasty, or atherectomy is likely to involve similar events within the vessel wall. In addition, removal of atherosclerotic plaque by endovascular techniques results in a highly thrombogenic luminal surface which attracts platelets and leukoctyes, predisposing the vessel to early thrombosis in low-flow situations. Rapid re-endothelialization with seeded endothelial cells may provide a way to accelerate luminal healing and return the vessel wall to a quiescent state after injury, and has been an area of recent interest.

The damaged arterial surface following endarterectomy or angioplasty provides an excellent bed for rapid attachment of seeded endothelial cells. In 1987, Bush and his colleagues reported their observations on the effects of endothelial seeding on healing of canine carotid arteries subjected to endarterectomy.[41] In this study, which employed a large inoculum of previously harvested and cultured venous ECs, seeded vessels demonstrated a significant reduction in subsequent neointimal thickening. Other investigators have demonstrated the feasibility of seeding arteries after

angioplasty, resulting in reduced platelet deposition in a rabbit model.[42]

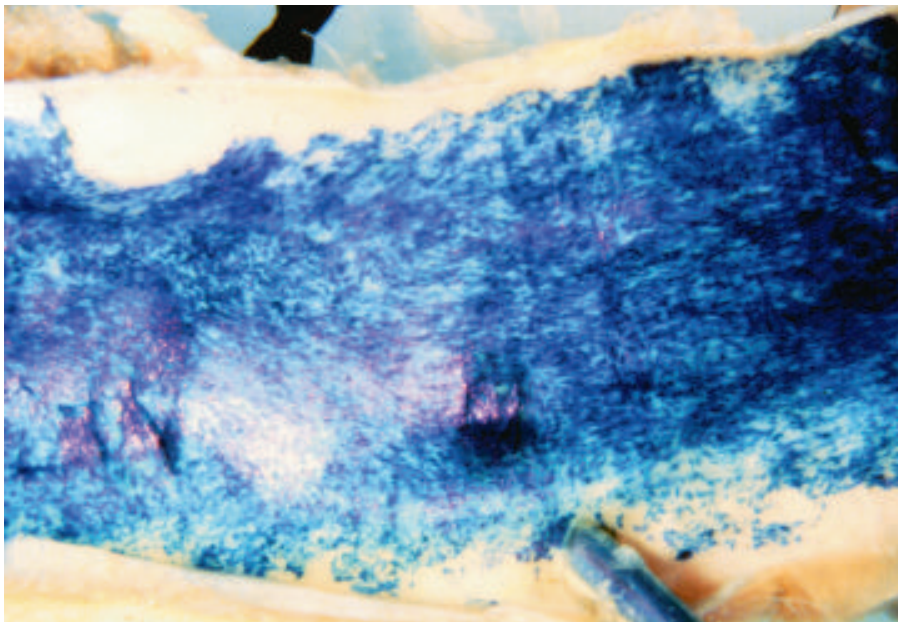
We have recently reported observations on the healing events in balloon-injured rabbit arteries seeded with autologous venous ECs. Employing retroviral mediated gene transfer to identify seeded cells unambiguously, we found that these vessels may be completely resurfaced within seven days by cultured ECs seeded at subconfluent densities. The seeded cells are capable of rapid proliferation on the arterial surface, and persist in vivo where they continue to express the inserted marker gene for up to 14 days after seeding (Fig. 3).[43] Subsequent experiments revealed that although EC seeding accelerated endothelial monolayer formation, it failed to attenuate neointimal thickening in the injured arteries.[44] Further investigation is needed to assess the functional state of the seeded cells in the reforming monolayer, and more stringent models are required to assess the impact of seeding on vascular remodeling and restenosis.

Within the last decade, profound advances in gene transfer methodology have stimulated immense interest in the application of this technology to cardiovascular disease. By virtue of their location at the blood-tissue barrier, endothelial cells are an attractive target for the therapeutic delivery of secreted proteins. Endothelial cell seeding (of both prosthetic grafts and native vessels) has been employed as a method of implanting genetically modified cells into the body.[45,46] Genetically modified cells seeded to the surface of a prosthetic graft or to an acutely denuded artery could deliver secreted gene products locally to the underlying vessel wall or neointima or downstream to a specific vascular bed. Numerous proteins and peptides have been examined as potential target molecules, including those having thrombolytic, antithrombotic, vasodilatory, and antiproliferative functions.[47-50] As currently applied, most vector systems for effecting gene transfer to ECs can be performed in a basic tissue culture facility with reproducible efficiency and could be readily incorporated into a clinical seeding protocol.

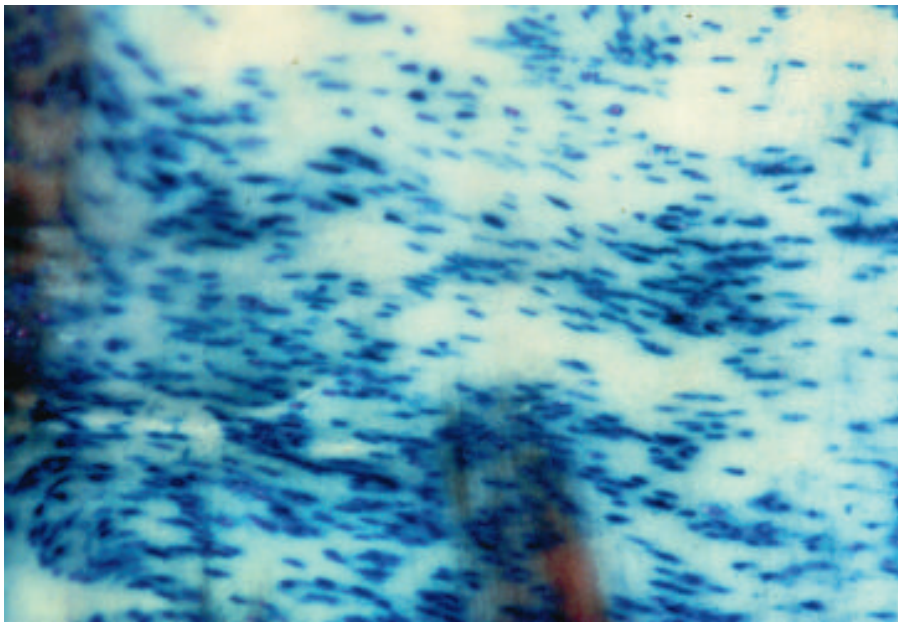
CONCLUSIONS

In summary, despite nearly two decades of active investigation, the potential role of endothelial cell seeding of prosthetic grafts remains uncertain. The patency data from animal studies, while promising, cannot be extrapolated to humans in whom the mechanism of graft failure may be quite different. Conventional prosthetic grafts in humans most commonly fail at later time points in association with progressive, perianastomotic thickening of the native artery. To date, the experimental evidence that seeding will alter the progression to mid- to late-term failure of prosthetic grafts is circumstantial. The ultimate success of this technology depends on the ability of seeded endothelial cells to persist on the surface of implanted prosthetics, to exhibit the beneficial properties they normally express on vessel surfaces in vivo, and to suppress smooth muscle proliferation which leads to perianastomotic hyperplasia. Alternatively, gene transfer may allow for selective augmentation of one or more of these desirable functions, if the appropriate target molecules are identified.

Figure 3. Appearance of rabbit arteries seeded with genetically modified endothelial cells at the time of balloon injury. Retroviral vectors were used to transduce ECs with the marker gene b-D-galactosidase prior to implantation. Explanted vessels are stained for the presence of b-galactosidase activity (blue staining positive).



(a) Vessel explanted seven days after seeding (x20).



(b) Higher magnification of surface of seven-day seeded vessel (x60).

In the long run, the clinical utility of EC seeding will depend to a great extent on its complexity and cost-effectiveness. Recent clinical studies employing the delayed seeding approach suggest some benefit in patency, but clearly more data is required to justify the effort and expense of such an endeavor. The development of alternative sources of ECs and the simplification of precoating and seeding protocols are critical technical aspects. Genetic manipulation of endothelial cells, in addition to potentially augmenting desirable functions, may also provide a future solution to the problem of cell availability for seeding. It may be possible, using the gene "knockout" approach, to design an immunologically inert, "universal donor" cell line which could be maintained in centralized tissue culture banking facilities and obtained when needed. Such a concept, while clearly years or perhaps a decade away, is no longer merely a science fiction fantasy. STI

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