Journal of the American College of Cardiology
© 2004 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 44, No. 4, 2004
ISSN 0735-1097/04/$30.00
doi:10.1016/j.jacc.2004.04.048

STATE-OF-THE-ART PAPER

Role of the Endothelium in Modulating Neointimal Formation

Vasculoprotective Approaches to Attenuate Restenosis After Percutaneous Coronary Interventions

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Restenosis at the site of an endoluminal procedure remains a significant problem in the practice of interventional cardiology. We present current data on intimal hyperplasia, which identify the major role of endothelial cells (ECs) in the development of restenosis. Considering endothelial denudation as one of the most important mechanisms contributing to restenosis, we focus more attention on methods of accelerating restoration of endothelial continuity. Prevention of restenosis may be achieved by promoting endothelial regeneration through the use of growth factors, EC seeding, vessel reconstruction with autologous EC/fibrin matrix, and the use of estrogen-loaded stents and stents designed to capture progenitor ECs. (J Am Coll Cardiol 2004;44:733–9) © 2004 by the American College of Cardiology Foundation

In recent decades, the number of coronary artery bypass graft surgery procedures has slowly declined as a result of continued conversion to percutaneous transluminal coronary angioplasty (PTCA) and stent implantation (1). The introduction of stents has resulted in a significant decrease of vessel remodeling and elastic recoil at the site of intervention and clearly has demonstrated the superiority of stent implantation to PTCA alone, with respect to restenosis in de novo coronary lesions (2–4). However, it also is evident that neointimal proliferation is not affected by stenting techniques (5). Unfortunately, this complication remains difficult to prevent and, regardless of the treatment strategy, the rate of in-stent restenosis is still unacceptably high (20% to 80% after bare-metal stent implantation) (2–4). The aim of this review is to analyze the role of endothelium in the restenotic process and to provide an update on experimental and clinical studies that use interventions designed to promote vascular healing.

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Manuscript received February 20, 2004; revised manuscript received April 10, 2004, accepted April 27, 2004.

ENDOTHELIUM AND RESTENOSIS

Endothelial denudation is considered to be a primary injury event after balloon angioplasty and/or stent implantation (Fig. 1). Besides modulating local hemostasis and thrombolysis, producing vasoactive compounds, and providing a nonpermeable barrier protecting smooth muscle cells (SMCs) against circulating growth-promoting factors, endothelial cells (ECs) produce a significant number of basement membrane components and synthesize several growth factors, including fibroblast growth factor, platelet-derived growth factor, and transforming growth factor-beta as well as heparin and other growth-inhibitory factors that are important in SMC proliferation. Endothelial cells may themselves maintain the mitogenic quiescence of SMCs by the growth-inhibitory effect of nitric oxide (NO) (6). When in a confluent monolayer, ECs cease replication. The disruption of cell contact inhibition results in rapid EC replication from the proximal and distal untraumatized segments. The SMCs proliferate and migrate to the de-endothelialized vessel surface, where they continue to proliferate and secrete extracellular matrix proteins, leading to neointimal tissue formation.

With denudation of a small area of the endothelial surface, little to no intimal hyperplasia is observed (7,8). When larger areas are denuded, there is a greater degree of intimal thickening (9). Focal fibrin deposition with thrombus formation is universally observed within the first three days of stent implantation and is proportional to the depth and extent of injury to the arterial wall. Inflammation accompanies vessel injury and attracts

Abbreviations and Acronyms

EC = endothelial cell
LPLI = low-power laser irradiation
NO = nitric oxide
PTCA = percutaneous transluminal coronary angioplasty
SMC = smooth muscle cell
VEGF = vascular endothelial growth factor

platelets and leucocytes that release growth factors and cytokines (Fig. 2), which may initiate the activation of SMCs. It also was demonstrated that SMCs appear in the intima only in areas that are not re-endothelialized seven days after injury (10). We also showed that more severe and deeper injury to the vessel wall results in delayed re-endothelization (Fig. 3).

Grewe et al. (11) described three phases of stent integration. In the acute phase (<6 weeks), the border between the vascular lumen and arterial wall is constituted by a thin, multilayered thrombus, and no ECs are found in the implantation zone. During the time course of integration, increasing numbers of SMCs and amounts of extracellular matrix can be detected. In the intermediate phase (6 to 12 weeks), the neointima consists of extracellular matrix and increasing numbers of SMCs. Increasing numbers of ECs are found on the luminal surface of the stent neointima. In the chronic phase (three months), complete re-endothelization is first noted. Matrix structures increase, whereas the number of SMCs decreases. During all phases of stent incorporation, the stent material is covered by a thin proteinaceous layer.

A time-course analysis of stent endothelization in a rabbit iliac artery model disclosed <20% stent re-endothelization at 4 days, <40% at 7 days, and near-complete endothelization at 28 days after stent implantation (12). The time course for re-endotheliization of the injured vessel has been studied in several other animal models with different types of injuries with controversial results (Fig. 4), suggesting a multifactorial process.

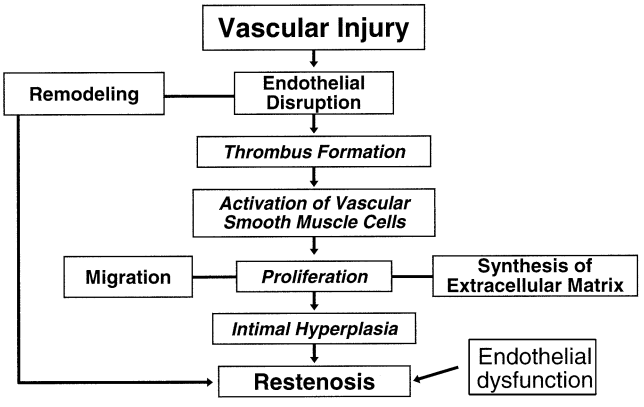

Figure 1. Proposed mechanism of restenosis.

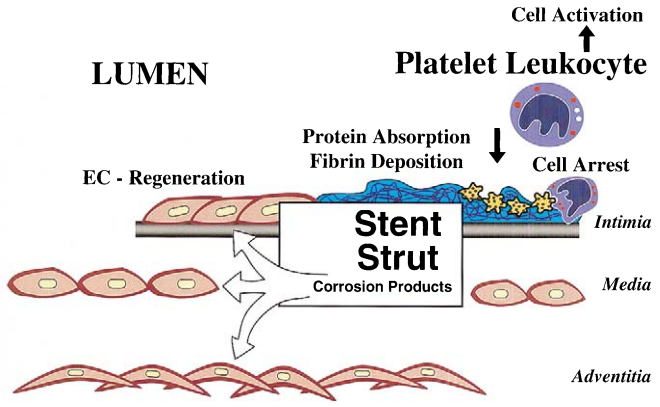

Figure 2. Impact of the stent implantation on the vessel wall. EC = endothelial cell.

The ECs can have heterotopic origins and supply sources. In 1963, Stump et al. (13) provided evidence that ECs can also be derived from the peripheral blood. Further studies confirmed their origin from the hematopoietic stem cells and from hemangioblast precursor cells (14,15). Endothelial progenitor cells isolated from peripheral blood also can be derived directly from monocytes/macrophages (16). There is strong evidence that mature ECs that have a constant supply of blood-borne multipotent endothelial-like cells can undergo transdifferentiation and serve as a potential source of at least certain mesenchymal cells, including SMC (17).

The regenerating endothelium experiences long-term (>3 months) dysfunction, characterized by decreased vascular integrity and increased permeability. Dysfunction is more pronounced after stenting than after PTCA (18) and also is characterized by impaired endothelium-dependent vasodilatation. Influences that lead to increased intracellular cyclic adenosine monophosphate may promote re-endothelization, reduce endothelial permeability, and attenuate fibromuscular proliferation (19). In addition, intima that is affected by atherosclerosis contains ECs with lowered cyclic adenosine monophosphate content, which points to similarities in the development of both pathologic conditions: restenosis and atherosclerosis. Thus, the re-endothelization of an injured vessel requires a long period of time; this delay could substantially contribute to restenosis.

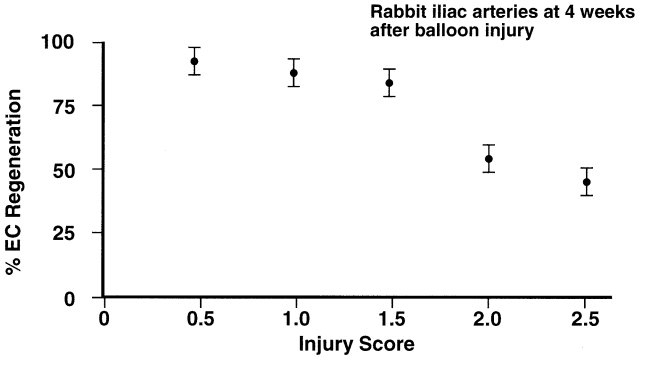

Figure 3. Relationship between severity of the vessel wall injury (by injury score) and endothelial cell (EC) regeneration (%).

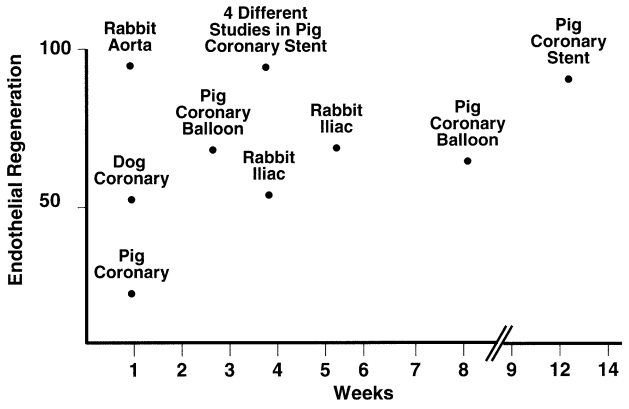

Figure 4. Time course of endothelial cell regeneration after percutaneous coronary interventions in different animal models.

In theory, early confluent re-endothelialization by EC seeding may reduce SMC proliferation, migration, or both.

The disruption of deeper vessel wall structures may lead to the activation of other mechanisms involved in neointima formation (Fig. 5), pointing to its importance along with de-endothelization in the development of vessel stenosis.

Besides playing a primary role in neointimal hyperplasia, continuous inflammation may be an important factor in the restenosis of stenting. Despite the many impacts of the implanted stent on the vessel wall, triggered inflammation response is believed to be the factor responsible for more severe intimal hyperplasia after stenting compared with balloon angioplasty (5). Corrosion products of metallic stents found in the subendothelial space at the site of stent implantation may be of great importance in this process. Furthermore, in some individuals, a contact allergy to certain metallic ions that are present in stainless-steel stents (e.g., nickel, chrome, molybdenum) may potentially trigger excessive intimal growth (20). There also is experimental evidence that the degree of inflammation and subsequent neointima formation is proportional to the degree of penetration of the vessel wall by the stent struts (21). Endothelial regeneration seems to be significantly diminished in the presence of metallic wires, causing delayed re-endothelization of bare metallic stents (22). These problems are successfully being resolved by coating the stents with different polymers, which also provide a biologically inert barrier between the stent surface, vascular tissues, and circulating blood and can be a feasible platform for local drug delivery, gene therapy, and recolonization of the stent with ECs.

INTERVENTIONS TO PROMOTE ENDOTHELIALIZATION

Mechanical approach. Numerous reports suggest that low-power laser irradiation (LPLI) is capable of affecting cellular processes in the absence of significant thermal effect (23–25). We observed in vitro (26) that LPLI of vascular and cardiac cells results in a statistically significant increase of vascular endothelial growth factor (VEGF) secretion in culture (1.6-fold for SMCs and fibroblasts; 7-fold for cardiomyocytes). This effect was dose-dependent (maximal with LPLI of 0.5 J/cm^2 for SMC, 2.1 J/cm^2 for fibroblasts, and 1.05 J/cm^2 for cardiomyocytes) and stimulated EC growth in culture. Further studies (27) showed that those effects were produced by inducible nitric oxide synthase induction and elevation of cyclic guanosine monophosphate in cells treated by LPLI. These data may have significant importance in the development of new methods for endoluminal postangioplasty vascular repair and myocardial photoangiogenesis.

Intravascular sonotherapy has demonstrated its efficacy in the prevention of neointimal hyperplasia after stent implantation (28). In addition to its capability in decreasing cellular proliferation (29), there is new evidence of a positive effect of intravascular sonotherapy on cell recovery through the

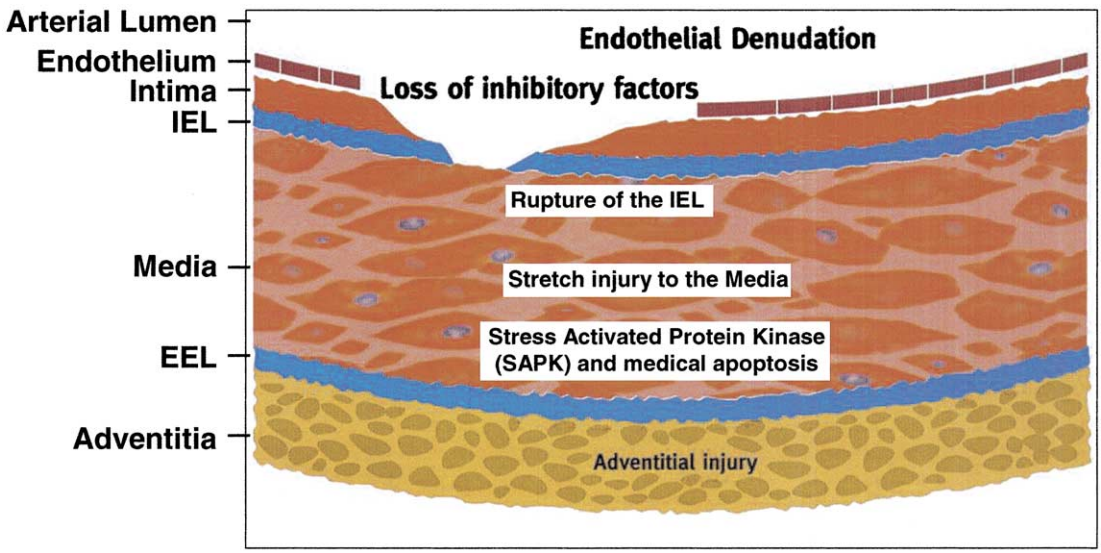

Figure 5. Severity of the vessel wall injury and activated mechanisms in the development of restenosis. EEL = external elastic lamina; IEL = internal elastic lamina.

acceleration of microtubule and microfilament reassembly, which needs further investigation (P. Fitzgerald, personal communication, 2002).

Local delivery of growth factors. Studies of the use of growth factors to accelerate endothelialization have brought very interesting and controversial results. Callow et al. (30) found VEGF stimulated rabbit EC proliferation in vitro at concentrations of 100 ng/ml. However, it had no effect on SMC cell proliferation at concentrations up to 500 ng/ml. Eight weeks of administration resulted in 88.1 ± 3.1% re-endothelialization compared with controls (44.7 ± 3.8%). Hence, VEGF appears to be a specific mitogen-inducing endothelial regeneration. Recently, Van Belle et al. (31,32) demonstrated that local delivery of VEGF accelerates stent endothelialization and reduces stent thrombosis in a rabbit model. Vascular endothelial growth factor also augments NO release from the endothelium (33). However, while growth factors increase the rate of endothelial regeneration, many of them are also potent mitogens for vascular smooth muscle cells. Indeed, Moulton et al. (34) observed that prolonged treatment with the angiogenesis inhibitor endostatin or TNP-470 reduced plaque growth in mice.

Endothelial cell seeding. Previous attempts to seed ECs using a variety of delivery devices to the vascular wall have been hampered by rapid loss of the seeded cells and also by the difficulty of maintaining cell adherence to the vessel wall when blood flow is restored (35–39). Although the seeding of ECs during or after coronary intervention is an attractive concept, major limitations include: 1) prolonged seeding time, 2) suboptimal delivery device, and 3) marginal adhesion of a functional EC to the area of vascular injury. In studies on swine femoral arteries, Nabel et al. (36) achieved 2% to 11% adherence of cells to the denuded arterial wall after 30 min of reseeding. Thompson et al. (37) have achieved 36% EC attachment to damaged human saphenous veins in vitro. The same investigators demonstrated 17% cell retention after 100 min of blood flow in previously angioplastied external iliac arteries of rabbits (38).

Nugent and Edelman (40) used EC implants grown in polymer matrices for the control of vascular repair in a porcine model of arterial injury. Porcine and bovine EC implants significantly reduced experimental restenosis three months after angioplasty compared with controls by 56% and 31%, respectively. No implanted cells or focal inflammatory reactions were detected histologically at any of the implant sites at 90 days. Allogeneic implants provide a greater benefit than xenogeneic implants.

Conte et al. (39,41) have shown that autologous venous cells can be genetically modified and returned to the surface of a balloon-injured rabbit femoral artery. Vessels examined at four to seven days after seeding displayed 40% to 90% coverage with transduced cells, even when seeded at subconfluent density, and an intact EC monolayer, as evidenced by scanning electron microscopy studies. However, their method required surgical exposure of the vessels and complete interruption of blood flow for 30 min.

It has been shown that ECs grow faster on surfaces pretreated with plasma proteins, especially those involved in coagulation, because the fibronectin, fibrinogen, and vitronectin contained in autologous cryoprecipitate preparations have specific binding sites for ECs (35,42). Recently, modified autologous cryoprecipitate has become an important reagent in the ongoing attempts to line the luminal surface of vascular prosthesis with autologous ECs (43–45). Our preliminary studies have confirmed that ECs remain viable when attached to fibrin meshwork in a three-dimensional glue matrix. Furthermore, the non-cytotoxic fibrin matrix meshwork is flexible and compliant and may be easily adapted to a circumferential contour wall and timely resorbed to leave a completely healed tissue. Areas occupied by adherent ECs and cell-free areas that may serve as depots for drug delivery characterize the structure.

We also applied an alternative technique for EC seeding using fibrin matrix and assessed the impact of this technique on restenosis at eight weeks after balloon angioplasty in iliac arteries of atherosclerotic rabbits (46). Histologic examination demonstrated the ability of this method to reattach the EC/fibrin matrix circumferentially to the denuded arterial wall segment and to significantly reduce restenosis. Moreover, after reconstruction all vessels remained patent and appeared free of thrombosis on gross examination. This result was consistent with previous studies that showed EC seeding also reduced platelet deposition in a canine endarterectomy model (32). Near-complete re-endothelialization of treated arteries was explained by the observation that the ECs from the border of denuded area then preferentially migrate into this three-dimensional matrix structure (42). Morphometric analysis showed that the lumen area was significantly greater in the EC/matrix group than in EC seeding alone or in fibrin matrix alone.

In clinical settings, this process would require the patient to be the autologous donor for the ECs and reagents for the biologic matrix. Thus, the method has the advantage of avoiding potential immunologic/rejection problems. These data indicate that related plasma proteins are able to perform some of the functions of the extracellular matrix involved in anchoring ECs to the vessel wall. Therefore, the concept of reconstruction of the arterial wall with EC/fibrin matrix to prevent restenosis remains appealing.

HEALING-ENHANCING STENTS

Vascular endothelial growth factor-eluting stents. Despite promising results with local delivery of the VEGF to the site of vascular injury, a recently conducted experimental study on VEGF-eluting stents failed to demonstrate a beneficial effect on endothelization or on intimal hyperplasia. These VEGF-eluting stents did not accelerate endothelization or inhibit restenosis, but they did reduce the stent thrombosis rate, which may make these stents less thrombogenic (47). Accelerated endothelialization by local deliv-

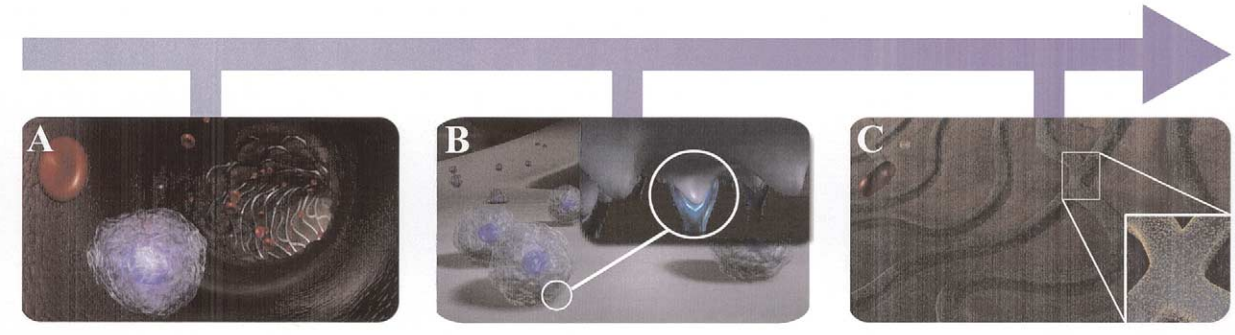

Figure 6. Time course of endothelial progenitor cell (EPC) capture. **(A)** Endothelial progenitor cells originate in the bone marrow and circulate postnatally in peripheral blood at concentrations of 3 to 10 cells/mm^3. **(B)** Anti-CD34 antibody-coated stents, once deployed, have the ability to bind EPCs to their surface. **(C)** Over time, EPCs bound to the endoluminal surface of the stent proliferate and migrate to fill the intra strut spaces establishing a confluent, functional endothelial cell monolayer on the stented arterial segment.

ery of endothelial-specific growth factors could constitute an attractive alternative to direct antiproliferative strategies.

Estradiol-eluting stents. Estradiol may improve vascular healing, reduce SMC migration and proliferation, and promote local angiogenesis. Several animal and human studies have demonstrated a protective effect of estrogen on coronary circulation (48–50). Estrogen appears not only to have a beneficial effect on lipids but also stimulates NO production by ECs, as well as inhibiting the expression of the proto-oncogene *c-myc*, which is implicated in the development of intimal hyperplasia (48,49). Estradiol may also contribute to vascular healing and to the prevention of restenosis by improving re-endothelization (estrogen receptor alpha-activation) and by decreasing SMC migration and proliferation (estrogen receptor beta-activation) (51). This explains why 17-beta-estradiol–eluting stents are associated with reduced neointimal formation without affecting endothelial regeneration with potential benefit in the prevention and treatment of in-stent restenosis (50). Recent data have shown that estradiol-eluting phosphorylcholine-coated stents that are implanted in porcine coronary arteries reduced neointimal hyperplasia by 40% compared with control stents.

The Estrogen And Stent To Eliminate Restenosis (EASTER) study was a single-center feasibility study testing 17-beta-estradiol–eluting BiodivYsio (Abbott, Abbott Park, Illinois) stents in 30 patients with de novo coronary lesions. Stents were loaded on-site by immersion in a solution of estradiol. Late loss was 0.32 mm in lesion and 0.57 mm in stent. Neointimal hyperplasia, detected using

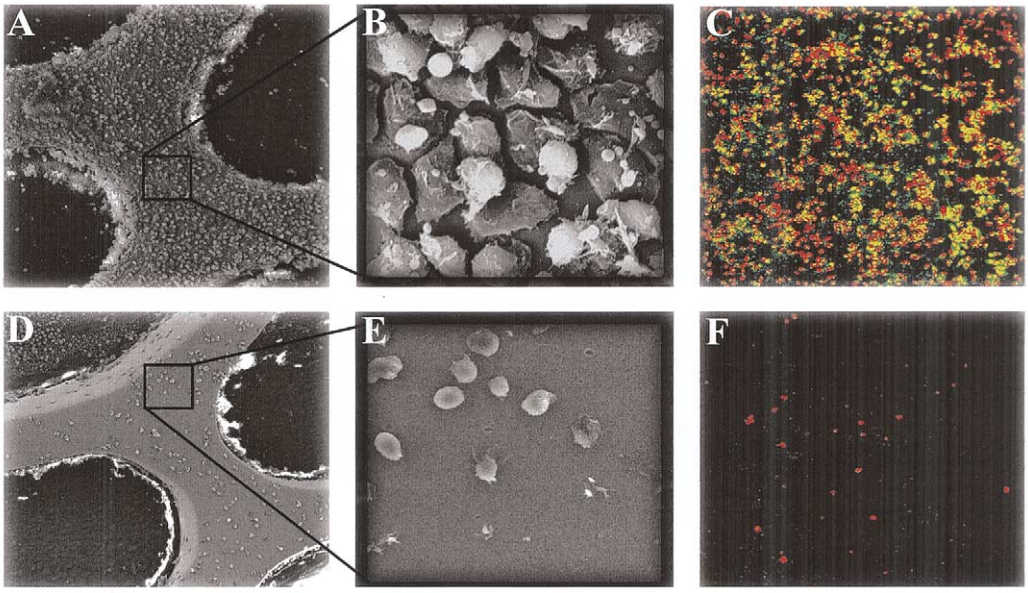

Figure 7. In vivo endothelial progenitor cell (EPC) capture at 1 h after deployment. All stents were deployed into the coronary arteries of Yorkshire Swine. **(A to C)** anti-CD34-coated stents. **(D to F)** bare stainless-steel stents. **(A)** and **(D)**, low-magnification scanning electron micrographs (×220) of stented arterial segments. **(B)** and **(E)**, high-magnification scanning electron micrographs (×2,500). **(C)** and **(F)**, Confocol microscopy images (×200) of immunohistochemical staining for the endothelial cell (EC) marker (ulex europaeus agglutinin 1). Cells were counted after staining with propidium iodine, a nuclear marker that stains all cells red. All EC marker-positive cells appear green/yellow. Anti-CD34 antibody-coated stents showed >70% cell surface coverage with EC marker-positive cells 1 h after stent deployment. Stainless-steel stents showed little to no cell coverage after 1 h.

intravascular ultrasonography, was 23.5%. At six months, there were no deaths or stent thromboses, and only one patient underwent repeat revascularization. A second phase of the EASTER study has recently concluded, and results are pending.

Stents attracting ECs. Stents may be used to attract circulating ECs. R stents (Orbus Medical Technologies, Fort Lauderdale, Florida) coated with antibodies to CD34 receptors on progenitor circulating ECs have been implanted in pig coronary arteries. Preliminary results suggest the feasibility of capturing ECs in situ (M. Kutryk, unpublished data, 2002) (Figs. 6 and 7). These nondrug-eluting stents would ultimately promote the elution of biologically active substances through a functioning endothelium monolayer. The effects of these novel stents on restenosis remain to be demonstrated.

Kutryk et al. (52) proposed the seeding of intravascular stents by the xenotransplantation of genetically modified ECs, which were capable of modifying the pathophysiologic response to vessel wall damage and provide controllable levels of active compounds. The feasibility and potential of this method has been demonstrated in animal studies. There are also preliminary data (53) suggesting that endoluminal seeding of syngeneic SMCs can be effective in reducing intimal hyperplasia in a restenosis animal model and in arterial allografts.

Conclusions. Endothelial denudation and dysfunction are common at the site of endovascular interventions and have been associated with vessel thrombosis and restenosis. In addition, delayed re-endothelialization has been associated with late side effects of potent antiproliferative therapies, such as with radiation therapy. The promotion of healing in the vascular endothelium may be a more natural and consequently safer approach in the prevention of restenosis.

One of the future approaches to overcoming the restenosis problem is promoting and accelerating re-endothelialization in the injured vessel. Along with using growth factors to enhance endothelial regeneration, EC seeding, vascular reconstruction using autologous EC/fibrin matrix, vasculoprotective compound-eluting stents, and EC-capture stents may have substantial potential in preventing restenosis. Despite the obvious benefit of interventions that promote the endothelization of stents, the clinical reduction of restenosis still remains to be proved. Further exploration into the control mechanism of the endothelium function and interaction with surrounding tissues, as well as clinical trials, would provide additional insight and support of this novel approach in the treatment of restenosis.

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