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STATE-OF-THE-ART PAPER

Vascular Inflammation and Repair

CME

Implications for Re-Endothelialization, Restenosis, and Stent Thrombosis

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CME Objective for This article: After reading this paper, the reader should be able to: recognize the various contributors to early and late DES thrombosis; assess the favorable and adverse effects of DES on vascular inflammation, neointimal proliferation, re-endothelialization, and endothelial function; discuss the role of bone marrow-derived stem cells in restenosis and vascular repair as well as the role of local vascular inflammation on stem cell recruitment; and describe novel strategies to reduce smooth muscle proliferation and enhance re-endothelialization in next-generation DES.

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Vascular Inflammation and Repair

Implications for Re-Endothelialization, Restenosis, and Stent Thrombosis

The cellular and molecular processes that control vascular injury responses after percutaneous coronary intervention involve a complex interplay among vascular cells and progenitor cells that control arterial remodeling, neointimal proliferation, and re-endothelialization. Drug-eluting stents (DES) improve the efficacy of percutaneous coronary intervention by modulating vascular inflammation and preventing neointimal proliferation and restenosis. Although positive effects of DES reduce inflammation and restenosis, negative effects delay re-endothelialization and impair endothelial function. Delayed re-endothelialization and impaired endothelial function are linked to stent thrombosis and adverse clinical outcomes after DES use. Compared with bare-metal stents, DES also differentially modulate mobilization, homing, and differentiation of vascular progenitor cells involved in re-endothelialization and neointimal proliferation. The effects of DES on vascular inflammation and repair directly impact clinical outcomes with these devices and dictate requirements for extended-duration dual antiplatelet therapy. (J Am Coll Cardiol Intv 2011;4:1057–66) © 2011 by the American College of Cardiology Foundation

Drug-eluting stents (DES) substantially reduce angiographic and clinical restenosis by 70% across broad patient and lesion subsets and decrease repeat target lesion interventions. The prototypical antiproliferative DES agents sirolimus (CYPHER stent, Cordis, Miami Lakes, Florida), paclitaxel (Taxus stent, Boston Scientific, Natick, Massachusetts), zotarolimus (Endeavor stent, Medtronic, Minneapolis, Minnesota), and everolimus (Xience stent, Abbott and Boston Scientific) have potent antimitotic actions that strongly inhibit smooth muscle proliferation and matrix production (1–3) and thus reduce neointimal formation and restenosis. Despite efficacy in reducing neointimal proliferation and restenosis, DES failure and restenosis still occurs and is more frequent in the settings of diabetes mellitus and during treatment of restenotic lesions, bypass grafts, and bifurcations (4–6). In addition to restenosis, concern has arisen about the potential for late thromboses or very late thromboses after DES implantation, and this concern has led to extended-duration dual antiplatelet therapy (7–9). Mechanisms of stent thrombosis might vary, depending on the timing of the event (10). Acute stent thrombosis (within 24 h of implantation) and early stent thrombosis (within 30 days) are likely related to mechanical issues with the stent, inadequate platelet inhibition, or pro-thrombotic patient risk factors. In contrast, late stent thrombosis (up to 1 year) and very late stent thrombosis (after 1 year) have been attributed to delayed re-endothelialization and inhibition of vascular repair. The potential for delayed re-endothelialization and inhibition of vascular repair is particularly important after implantation of DES, because the antiproliferative agents used to prevent smooth muscle cell proliferation also delay re-endothelialization in the stented segment (11,12). Angioscopic (13) and pathological (11,12,14,15) evidence suggests that there is delayed arterial healing with DES, compared with bare-metal stents (BMS), because DES-treated arteries have more histological evidence of incomplete re-endothelialization, chronic inflammatory cell infiltration, fibrin deposition, and platelet activation. It is important to recognize that inflammatory and thrombotic pathways share common signaling pathways and that inflammatory responses promote activation of the clotting cascade and stimulate platelet activation (reviewed in Croce and Libby [16]). Experimental studies also suggest that delayed arterial healing and DES-associated inflammation is greatest at sites of overlapping DES with placement of multiple stents (17). The finding of increased inflammation in areas of stent overlap suggests a possible molecular mechanism to explain higher stent thrombosis rates that are associated with overlapping stents.

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In addition to antiproliferative drug-associated delayed healing with DES, stent-induced or polymer-induced inflammation has also been identified as a possible contributor to stent thrombosis, especially because late and very late stent thrombosis occurs long after antiproliferative dugs have been eluted from the polymer (18–20). Inflammatory responses to drug, stent, or polymer might result from nonspecific innate immune responses, which have a predominance of monocyte/macrophage infiltrates, or might be related to antigen-specific adaptive immune hypersensitivity responses typified by infiltration of eosinophils, B-cells, and T-cells (reviewed in Byrne et al. [21]). Several studies have also implicated DES-polymer-induced inflammation in the pathobiology of restenosis and stent thrombosis (18,19). Currently, the 4 stent platforms approved for use by the U.S. Food and Drug Administration use different nonerodible polymeric coatings for drug delivery, and experimental animal studies suggest that biological compatibility, immunogenicity, and thrombogenicity might vary among specific polymeric compounds (22). The next generations of DES represent an attempt to reduce the possibility of polymer-induced inflammation, delayed arterial healing, restenosis, and stent thrombosis through use of polymers that have better biocompatibility and/or are biodegradable.

Emerging evidence indicates that compared to BMS, DES impair endothelial function in arterial segments distal to the stented site (23,24). Even 6 months after implantation of DES, artery segments distal to the DES show abnormal vasoreactivity (25–27). DES-associated abnormalities in endothelial function could be related to delayed vascular repair and not the DES drug itself, because the kinetics of DES are such that the drugs are completely eluted within months after implantation (28–31). It is possible, however, that in certain circumstances drug accumulation in the arterial wall (32) and the lipophilic core of stented atheroma results in prolonged drug retention/release and ongoing vascular dysfunction. The mechanism of DES-associated endothelial dysfunction is not established, and recent studies have demonstrated that there is variability in the severity of DES-associated endothelial dysfunction among specific DES agents (33–35). It is unclear whether DES-associated vascular dysfunction influences clinical outcomes after DES implantation. One small study demonstrated impaired endothelial function in patients presenting with in-stent restenosis, compared with matched control subjects (36); however, this association will require validation in larger prospective investigations.

Because of the potential for delayed re-endothelialization and repair with DES, concern was raised about possible increased mortality and late stent thrombosis following DES implantation (reviewed in Garg and Mauri [7]). Because of the insufficient power of individual trials to assess the low-incidence events of late and very late stent thrombosis, multiple meta-analyses were performed to evaluate the risk of stent thrombosis in patients treated with DES versus BMS (37–41). These meta-analyses and subsequent analyses of stent registry data (42–45) demonstrated nearly equivalent risk of stent thrombosis (approximately 0.5%) in patients treated with DES or BMS. A small increase in the risk of late and very late stent thrombosis on the order of 1% to 2% cannot be excluded, however, because available data have insufficient power to evaluate this very rare event.

Analyses of stent thrombosis and outcomes with DES are further complicated by significant differences in stent structure, drug delivery polymers, and antiproliferative drugs among the rapidly expanding panel of DES. In addition, complex biology controls vascular repair after percutaneous coronary intervention (PCI). Understanding the common and differential molecular pathways that regulate re-endothelialization versus restenosis will provide a biological context for rational use of DES and will enable development of new DES technologies that can inhibit neointimal proliferation and preserve or even promote endothelial repair. In the following sections, we will highlight key cellular and molecular pathways that regulate vascular injury and repair in the setting of percutaneous coronary revascularization, and we will discuss the role of DES in modulating vascular repair processes.

Abbreviations and Acronyms

BMS = bare-metal stent(s)
DES = drug-eluting stent(s)
EPC = endothelial progenitor cell
G-CSF = granulocyte colony-stimulating factor
PCI = percutaneous coronary intervention
SDF = stromal cell-derived factor
SMPC = smooth muscle progenitor cell

Role of Inflammation in Restenosis and Vascular Repair

Stent placement leads to mechanical injury that induces substantial local inflammation, which stimulates vascular smooth muscle cell proliferation and extracellular matrix deposition, resulting in neointimal thickening and restenosis (46,47). Vascular inflammation after PCI involves complex interactions between multiple vascular cell types, and under normal circumstances, the cellular and molecular processes that control vascular injury responses direct repair and vascular healing. In pathological conditions, dysregulation of vascular repair results in persistent vascular inflammation, neointimal proliferation, and restenotic obstruction of the stent lumen.

Immediately after PCI, platelets, neutrophils, and monocytes play a central role in the initial inflammatory response (47,48). Platelets and fibrin deposit on the de-endothelialized vessel wall and recruit leukocytes to the injured vessel segment through a cascade of cell adhesion molecules that direct leukocyte attachment and transmigration across surface-adherent platelets (49). The initial tethering and

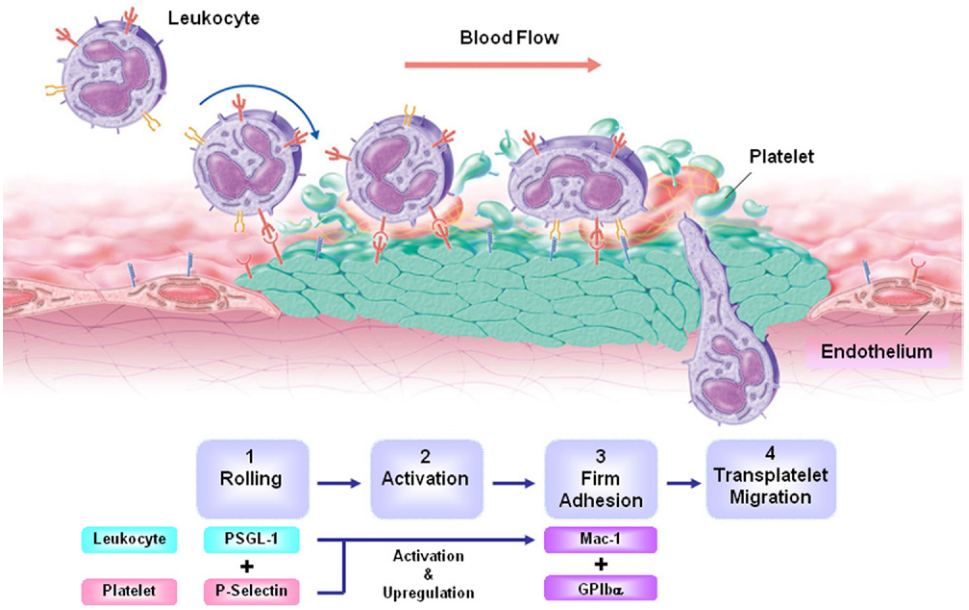

Figure 1. Transplatelet Leukocyte Migration

At the site of stent implantation after percutaneous coronary intervention, endothelial cells are denuded, and the subendothelial matrix is exposed to flowing blood. Platelets and fibrinogen immediately adhere to the surface of the injured vessel. A multistep cascade of platelet and leukocyte adhesion molecules direct leukocyte adhesion to the adherent platelets in a process termed "secondary capture." Leukocyte capture and rolling are mediated by interaction between platelet P-selectin and leukocyte P-selectin glycoprotein ligand (PSGL)-1. Arrest and firm adhesion are mediated by platelet glycoprotein (GP) Ib-alpha and leukocyte Mac-1. Chemokines stimulate transmigration into the extraluminal tissue.

rolling of leukocytes on platelets is mediated through binding of the leukocyte receptor P-selectin glycoprotein ligand-1 to platelet P-selectin (50–52). Rolling leukocytes stop and firmly attach to adherent platelets when the leukocyte integrin Mac-1 (CD11b/CD18) binds to platelet glycoprotein Ib-alpha (53) or to fibrinogen bound to the platelet glycoprotein IIb/IIIa (Fig. 1) (54). A direct role for Mac-1 in leukocyte adhesion after mechanical injury has been demonstrated in several experimental studies where Mac-1 targeting reduces neointimal thickening after experimental angioplasty (55,56). Clinical studies of patients undergoing PCI further support the premise that Mac-1 and platelet-mediated leukocyte adhesion (also termed "secondary capture") plays an important role in vascular inflammation and restenosis after coronary stenting. We have previously shown that, compared with circulating neutrophils, Mac-1 surface expression is significantly increased in the neutrophils obtained from the coronary sinus of patients who underwent PCI within the preceding 48 h and that high levels of Mac-1 expression are associated with angiographic late lumen loss and increased risk of restenosis (57–60). Increased Mac-1 expression also correlates with increased expression of P-selectin on the surface of platelets obtained from the coronary sinus after PCI (57–60).

Role of Bone Marrow-Derived Stem Cells in Restenosis and Vascular Repair

Emerging research is demonstrating that bone marrow-derived progenitor cells play an important role in vascular inflammation responses and in vascular repair. Endothelial progenitor cells (EPCs) mobilized from bone marrow into peripheral blood promote endothelial regeneration and postnatal neovascularization (61,62). In contrast to the potential protective effects of EPCs, it has been hypothesized that smooth muscle progenitor cells (SMPCs), which are also mobilized from bone marrow, migrate to the sites of vascular injury where they contribute to smooth muscle cell expansion and neointimal proliferation (63–65).

The precise function of EPCs and SMPCs once they home to sites of vascular inflammation is controversial. Previously, CD34-positive cells were believed to be committed to develop into EPCs; however, further study demonstrated that the CD34 surface antigen actually identifies undifferentiated bone marrow-derived stem cells that have the ability to differentiate into EPC and SMPCs. Transdifferentiation of CD34-positive cells into EPC or SMPC lineages depends on the local environment; ischemic conditions signal differentiation toward EPC phenotypes to promote re-endothelialization (61,66), and inflammatory

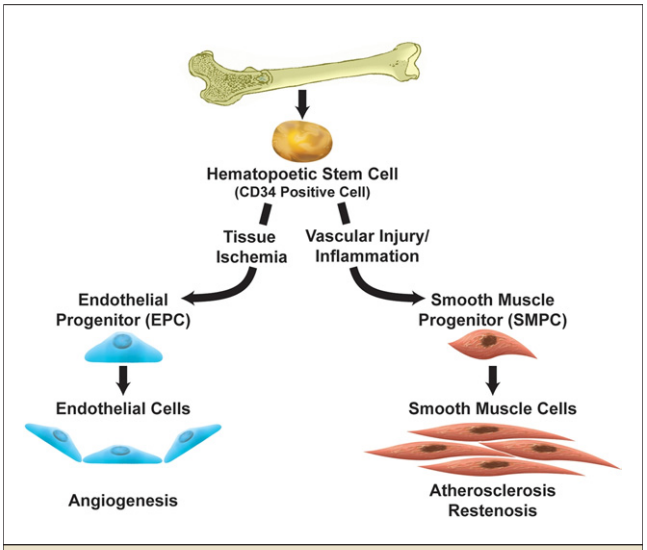

Figure 2. Differentiation of Bone Marrow-Derived Stem Cells

Previously, CD34-positive cells were believed to be committed to develop into EPCs; however, further study demonstrated that the CD34 surface antigen actually identifies undifferentiated bone marrow-derived stem cells that have the ability to differentiate into EPC and SMPCs. Ischemic conditions signal differentiation toward EPC phenotypes to promote re-endothelialization. Inflammatory conditions signal differentiation toward SMPC phenotypes that promote neointimal proliferation.

conditions signal differentiation toward SMPC phenotypes that promote neointimal proliferation (63) (Fig. 2).

Several studies have implicated CD34-positive progenitor cells in vascular injury responses after PCI. Circulating CD34-positive cells are increased in the days after acute myocardial infarction, and characterization of these circulating cells suggests that they have an EPC-like phenotype, raising the possibility that CD34-positive EPC-like cells are mobilized to promote angiogenesis in the ischemic myocardium. In contrast to ischemia-mediated mobilization, SMPC-like CD34-positive cells increase after PCI in patients with chronic coronary artery disease, presumably in response to inflammatory mediators produced at sites of stent implantation (67). In this setting, elevated levels of circulating CD34-positive cells are associated with increased rates of restenosis, suggesting possible involvement in neointimal formation (68).

We have also demonstrated that molecular signals generated at sites of local arterial inflammation promote the mobilization of CD34-positive stem cells (69). In our study, the number of CD34-positive cells in the peripheral blood increased Day 7 to 14 after PCI, and patients who received BMS had significantly more CD34-positive cells than those who received DES (Fig. 3A) (69,70). Granulocyte colony-stimulating factor (G-CSF) and Mac-1 levels were significantly reduced in patients who underwent implantation of DES, compared with those who received BMS, suggesting that the antiproliferative stent drug attenuated inflammatory cell activation (Fig. 3B) (69). This observation is consistent with our hypothesis that inflammatory signals generated at sites of coronary injury mobilize bone marrow-derived progenitor cells involved in vascular repair. To further elucidate the role of CD34-positive cells in vascular injury and repair after PCI, we isolated circulating CD34-positive progenitor cells from patients who received DES and BMS and performed in vitro differentiation assays (Fig. 4) (69). In most patients, a proportion of the cultured CD34-positive cells differentiated into both CD31-positive endothelial-like cells and into alpha-actin-positive cells with features suggestive of smooth muscle cell lineage. Several other observations were made. First, the number of differentiated colonies that formed from the CD34-positive cells correlated with the extent of restenosis during angiographic follow-up. Second, patients with more angiographic restenosis had more CD34-positive cells that differentiated into alpha-actin containing SMPC-like cells. Third, implantation of sirolimus-eluting stents resulted in reduced differentiation of CD34-positive cells into CD31-positive cells and reduced differentiation into alpha-actin-positive cells with smooth muscle cell features. This finding is consistent with in vitro data demonstrating that sirolimus inhibits differentiation of human bone marrow-derived stem cells into endothelial or smooth muscle cells (71,72).

Several lines of evidence support the premise that PCI induces local inflammatory signals that mobilize bone marrow-derived CD34-positive stem cells and that these cells have the ability to differentiate along endothelial or smooth muscle cell lines. In the setting of vascular injury, there seems to be a balance between endothelial-like stem cell responses that favor re-endothelialization and smooth muscle-like stem cell responses that promote restenosis (Fig. 2). Furthermore, it seems that, compared with BMS, sirolimus-eluting stent implantation attenuates production of local inflammatory signals that promote stem cell mobilization and differentiation into smooth muscle-like cells that contribute to neointimal proliferation. In the future, targeted pharmacological therapies might be able to promote reparative progenitor cell responses and/or inhibit responses that result in excess neointimal proliferation.

Local Vascular Inflammation Signals Stem Cell Recruitment

As described in the preceding text, inflammatory and hematopoietic cytokines produced locally at sites of vascular inflammation direct mobilization of stem cells from the bone marrow. Vascular-derived molecules involved in stem cell mobilization include G-CSF, matrix metalloproteinase-9, and stromal cell-derived factor (SDF)-1.

G-CSF, a potent hematopoietic cytokine produced by endothelium and immune cells, is expressed at sites of

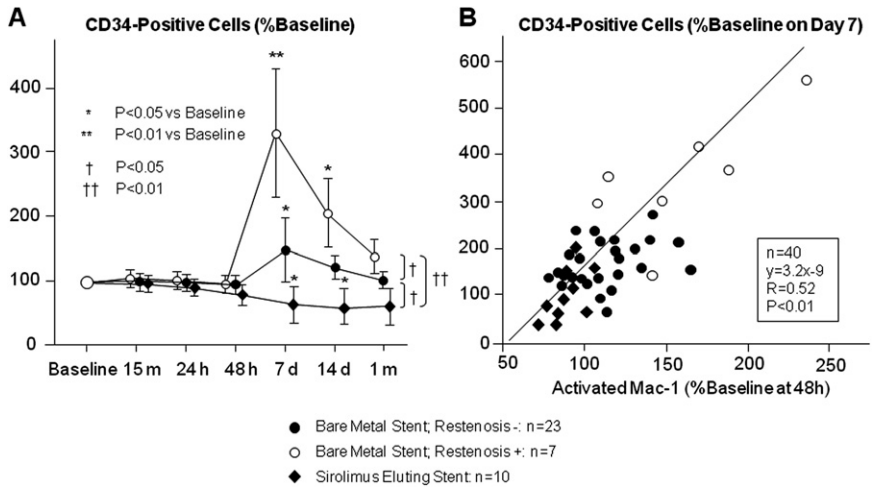

Figure 3. CD34-Positive Cell Counts and CD34-Positive Cell Mac-1 Expression After PCI

(A) Circulating CD34-positive cells increase after percutaneous coronary intervention (PCI). The highest levels of CD34-positive cells were seen in the peripheral blood of patients who received bare-metal stents that went on to have restenosis at 6-month (m) angiographic follow-up (Bare-Metal Stent Restenosis +). Implantation of drug-eluting stent was associated with a significant reduction in the number of circulating CD34-positive cells. (**B**) Neutrophil Mac-1 expression correlates with mobilization of CD34-positive cells. Forty-eight hours after PCI, neutrophils were harvested from the coronary sinus of patients who had coronary stents implanted. Neutrophil Mac-1 expression was quantified by flow cytometry. Neutrophil Mac-1 expression at 48 h correlated with circulating levels of CD34-positive cells 7 days (d) after PCI, demonstrating that higher levels of local vascular inflammation are associated with increased systemic CD34-positive progenitor cell mobilization. Data are expressed as percentage change of the baseline values. Adapted, with permission, from Inoue et al. (69).

vascular injury (73). G-CSF promotes stem cell proliferation and mobilization, and it has been hypothesized that, after PCI and/or myocardial infarction, G-CSF signals production and homing of reparative stem cells that promote angiogenesis and myocardial repair. Clinical evaluation of systemic G-CSF therapy after myocardial infarction failed to show benefit in limiting infarct size or in improving left ventricular function, despite its experimental effects on stem mobilization (74,75). It is possible that the nonselective mobilization of both EPCs and SMPCs by G-CSF might limit its therapeutic value for treating restenosis and promoting vascular repair.

Neutrophil-derived matrix metalloproteinase-9 is another inflammatory mediator that has a role in stem cell mobilization (76). Matrix metalloproteinase-9 is secreted locally in response to inflammatory inputs, including ligand binding to the leukocyte integrin Mac-1 (77). Matrix metalloproteinase-9 is required for G-CSF and chemokine-induced mobilization of hematopoietic stem cells from the bone marrow (78,79) and provides a mechanism through which inflamed vascular beds generate systemic signals that promote bone marrow-derived stem cell mobilization.

SDF-1 is a member of the CXC group of chemokines that plays a role in stem cell plasticity and engraftment (80). SDF-1 is expressed by smooth muscle cells at sites of atherosclerosis and vascular inflammation. SDF-1 signals the bone marrow to mobilize Sca-1^+ lineage progenitor cells that home to sites of vascular injury where the progenitor cells adopt smooth muscle cell phenotypes. In experimental models, SDF-1 directly regulates neointimal smooth muscle cell content, and inhibition of SDF-1 function decreases neointimal formation (80). Therapies targeting SDF-1 function could potentially inhibit restenosis after PCI.

Modulating Vascular Injury and Repair: New Frontiers in DES Technology

Current-generation DES agents prevent restenosis by inhibiting smooth muscle cell proliferation. In developing the next generation of DES agents it might be possible to harness differential drug effects on smooth muscle cell proliferation versus re-endothelialization in a manner that could accelerate repair. Vascular endothelial growth factor has attracted attention as a DES agent that could promote endothelial regeneration and angiogenesis (81). Proof-of-concept investigations have demonstrated that vascular endothelial growth factor gene-eluting stents accelerate re-endothelialization and reduce in-stent neointimal area in animal models (82). Another new strategy to promote vascular repair after PCI involves the use of antibodies (83) or peptides (84) that bind membrane receptors on circulating endothelial progenitor cells. This strategy promotes capture of these cells to accelerate healing (83). CD34 antibody-coated stents have been implanted in human

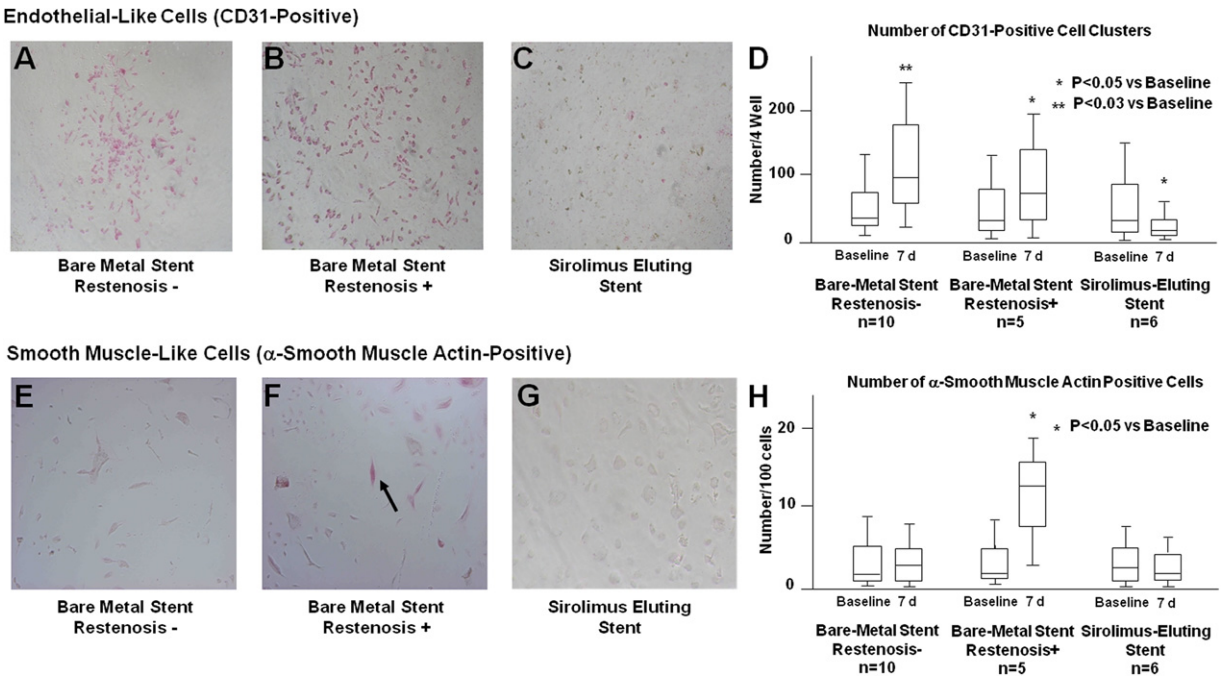

Figure 4. Differentiation of Patient-Derived CD34-Positive Stem Cells Into Endothelial-Like and Smooth Muscle-Like Cells After PCI

Circulating CD34-positive stem cells were isolated from peripheral blood of patients 7 days (d) after implantation of bare-metal stent (BMS) or sirolimus-eluting stent (SES). Immunohistochemical staining for CD31 **(A to D)**. **(A)** BMS without restenosis, **(B)** BMS with restenosis, **(C)** SES, **(D)** quantification of CD31-positive cell clusters. Patients who received BMS had similar differentiation of CD34-positive stem cells into CD31-positive endothelial-like cells, regardless of whether they went on to have restenosis at 6-month angiographic follow-up. Patients who received SES had a significant reduction in the differentiation of CD34-positive stem cells into CD31-positive endothelial-like cells, compared with patients that received BMS. Actin staining **(E to H)**. **(E)** BMS without restenosis, **(F)** BMS with restenosis, **(G)** SES, **(H)** quantification of actin positive cells. Patients who received BMS and went on to have restenosis at 6-month angiographic follow-up had increased numbers of CD34-positive stem cells that differentiated into actin-positive smooth muscle-like cells. Patients who received SES had a significant reduction in the differentiation of CD34-positive stem cells into actin-positive smooth muscle-like cells, compared with patients that received BMS. **Arrow** denotes representative actin-positive cell. Adapted, with permission, from Inoue et al. (69).

coronary arteries in the multicenter HEALING (Healthy Endothelial Accelerated Lining Inhibits neointimal Growth) II pilot trial and in later follow-up studies (85,86). The long-term safety and efficacy of this pro-healing stent technology awaits further evaluation in randomized trials.

In addition to DES technology itself, adjunctive systemic medications might also influence stem cell homing and the balance between re-endothelialization and neointimal proliferation. Interestingly, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) were recently shown to promote EPC proliferation in vitro (87) and increase the number of circulating EPCs in patients with coronary artery disease (88). Despite initial optimism that statins might favorably influence arterial healing after DES implantation, enthusiasm has tempered after release of data showing that high doses of statins started before PCI and continued thereafter increased EPC mobilization but did not increase circulating $CD34^+$ cells and did not improve the angiographic outcome after implantation of a bioengineered EPC-capture stent (89).

Thiazolidinediones, which are used to treat diabetes, function by activating peroxisome proliferator activating receptor transcription factors. Several thiazolidinedione agents increase the number of EPCs in both circulating blood and bone marrow and reduce EPC apoptosis in a phosphatidylinositol 3-kinase-dependent manner (90). Although there are several potential vasculoprotective actions of statins and thiazilidinediones, further clinical investigation will be required to determine whether these medications will positively influence vascular repair, resulting in reduced rates of restenosis and enhanced re-endothelialization after PCI.

Conclusions

Percutaneous coronary intervention results in mechanical injury that induces vascular inflammation. Vascular inflammation involves complex interactions between endothelial cells, smooth muscle cells, platelets, and inflammatory cells, including neutrophils, monocytes, and lymphocytes. Signaling molecules produced by cells at the site of vascular injury

stimulate mobilization of bone marrow-derived EPCs and SMPCs, which are recruited to the sites of vascular inflammation. The cellular and molecular processes that control vascular injury responses direct repair and vascular healing; however, dysregulation of these responses can result in adverse arterial remodeling, neointimal proliferation, and restenosis. Drug-eluting stents effectively reduce neointimal proliferation but they slow re-endothelialization and healing. Drug-eluting stents also seem to influence the mobilization, homing, and differentiation of reparative stem cells. Despite the potential for DES-induced delayed vascular healing, clinical trial investigations have demonstrated similar safety of DES and BMS in the setting of extended dual antiplatelet therapy. In the future, improved DES technologies have the potential to abolish restenosis and further improve stent safety by inhibiting maladaptive neointimal proliferation while promoting re-endothelialization and repair.

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Key Words: inflammation ■ re-endothelialization ■ restenosis ■ stent thrombosis.

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