Adherent Platelets Recruit and Induce Differentiation of Murine Embryonic Endothelial Progenitor Cells to Mature Endothelial Cells In Vitro

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Abstract—The homing and differentiation mechanisms of endothelial progenitor cells (EPCs) at sites of vascular lesions are unclear. To investigate whether platelets play a role in the recruitment and differentiation of EPCs, we made use of a robust mouse embryonic EPC (eEPC) line that reliably differentiates to a mature endothelial phenotype. We found that platelets stimulate chemotaxis and migration of these murine eEPCs. Further, the substantial adhesion of murine eEPCs on immobilized platelets that occurs under dynamic flow conditions is inhibited by neutralizing anti–P-selectin glycoprotein ligand-1 and anti–VLA-4 (β1-integrin) monoclonal antibodies but not by anti-CD11b (αM-integrin; macrophage antigen-1). Coincubation of murine eEPCs with platelets for 5 days induced differentiation of EPCs to mature endothelial cells as verified by positive von Willebrand factor immunofluorescence and detection of Weibel Palade bodies through electron microscopy. We conclude that platelets may play a critical part in the capture and subsequent differentiation of murine eEPCs at sites of vascular lesions, revealing a possible new role of platelets in neoendothelization after vascular injury. (Circ Res. 2006;98:e2-e10.)

Key Words: endothelial progenitor cells | platelets | endothelization | vascular lesion | adhesion

Vascular endothelium represents a barrier between circulating blood cells and the subendothelial matrix. Discontinuity of this barrier (eg, at sites of vascular injury or rupture of an atherosclerotic plaque) exposes various components of the subendothelial matrix, including collagen, fibronectin, or von Willebrand factor (vWF). Platelets rapidly adhere to vascular lesions, resulting in thrombus formation. If this process is controlled, platelets passivate vascular injury and initiate the healing process. However, uncontrolled platelet-mediated thrombus formation leads to acute thrombotic occlusion or plaque progression. However, the underlying mechanisms of platelet-mediated vascular remodeling and healing of lesions is poorly understood so far.

Endothelial progenitor cells (EPCs) are a circulating, bone marrow–derived cell population of large nonleukocyte cells that appear to participate in vascular repair and homeostasis. In response to cytokine stimulation and ischemia, these cells are mobilized from the bone marrow, home to the ischemic tissue, and contribute to neovascularization and angiogenesis. Furthermore, EPCs are regarded to have a key role in the maintenance of vascular integrity and to act as “repair” cells in response to endothelial injury. Several groups have raised colonies in vitro from CD34+ cells, which resemble mature endothelial cells, implying that EPCs are capable of promoting endothelization. Recruitment of EPCs toward vascular lesions has been regarded as a critical initial step in atherosclerosis and a result of the actions of various cardiovascular risk factors. Current data suggest that decrease in circulating EPCs contributes not only to impaired angiogenesis but also to the progression of atherosclerosis, and patients at risk for coronary artery disease have a decreased number of circulating EPCs with impaired activity.

However, the mechanisms that regulate recruitment of EPCs toward vascular lesions and initiate neoendothelization are not understood. In general, recruitment of circulating cells is a well-controlled process that involves chemotaxis, tethering, adhesion, and migration of cells into the subendothelial tissues. Several adhesion molecules have been described to regulate adhesion and migration of EPCs, including...
P-selectin glycoprotein ligand (PSGL-1) and β1- and β2-integrins.15–17

Adherent platelets secrete potent chemokines at sites of vascular injury and display several ligands for adhesion receptors on their surface, and therefore, they represent potential mediators of progenitor cell homing.18 In this study, we investigated the capacity of platelets to induce chemotaxis, adhesion, and migration of EPCs and provide evidence that platelets may also contribute to the differentiation of EPCs into endothelial cells.

Materials and Methods

Reagents
RB40.34 (function-blocking anti-mouse P-selectin) and 4RA10 (function-blocking anti-mouse PSGL-1) were raised as described previously.19,20 A goat anti-human polyclonal antibody (monoclonal antibody [mAb]) directed against vWF (C-20), which cross-reacts with mouse vWF, and N'2,O-dibutylryladenosine3':5'-cyclic monophosphate (cAMP) were purchased from Santa Cruz Biotechnology. Phycoerythrin or fluorescein isothiocyanate (FITC)–conjugated mAb rat anti-mouse CD162 (PSGL-1; clone 2PH1), CD49d (VLA-4; α-chain; clone 9C10), CD11b (clone M1/70), and blocking mAb anti-mouse CD29 (β1-integrin; clone 18) were purchased from Pharmingen. mAb anti-CD41 (GPIIb; clone P2) was from Beckmann Coulter. For immunofluorescence microscopy, a goat polyclonal antibody to mouse vWF (clone C20; Santa Cruz Biotechnology), an Alexa Fluor rabbit anti-goat IgG (Molecular Probes), and rhodamine phalloidin (R415; Molecular Probes) were used. A rabbit antibody to Alexa Fluor rabbit anti-goat IgG (Molecular Probes), and rhodamine antibody to mouse vWF (clone C20; Santa Cruz Biotechnology), an

Cell Culture of Murine Embryonic EPCs and Human CD34+ Cells

For our studies, we used the murine embryonic EPC (eEPC) line T17b. The cells were isolated from the egg cylinders with adjacent yolk sacs of mice embryos at embryonic days 7.5 to 8.5 as described in detail previously.21 A major advantage of this cell line is the robust growth properties in culture, the stable morphological phenotype, and the absence of difficulties to isolate and maintain a purified lineage of endothelial precursor cells. The characterization of the eEPCs has been described previously.15,21 The cells are cultured in flasks precoated with 0.1% gelatin. The culture medium consists of DMEM supplemented with 20% heat-inactivated FCS, 0.1 mmol/L β-mercaptoethanol, 1 mmol/L nonessential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L l-glutamine (Invitrogen). For immunofluorescence microscopy, the mature mouse endothelial cell line mouse heart endothelial cell clone 5-T (MHECS-T) served as positive control and was cultured as described previously.22 Human CD34+ cells were isolated from human cord blood and cultured as described previously.23

Isolation of Platelets and Preparation of Platelet Clots

Human platelets were isolated as described previously.24 Briefly, venous blood was drawn from the antecubital vein of healthy volunteers and collected in acid-citrate-dextrose buffer. After centrifugation at 430 g for 20 minutes, platelet-rich plasma was removed, added to Tyrodes–HEPES buffer (2.5 mmol/L HEPES, 150 mmol/L NaCl, 1 mmol/L KCl, 2.5 mmol/L NaHCO3, 0.36 mmol/L NaH2PO4, 5.5 mmol/L glucose, and 1 mg/mL BSA, pH 6.5), and centrifuged at 900 g for 10 minutes. After removal of the supernatant, the resulting platelet pellet was resuspended in Tyrodes–HEPES buffer (pH 7.4 supplemented with 1 mmol/L CaCl2 and 1 mmol/L MgCl2). Murine platelets were isolated from pathogen-free C57 BL/6J mice (Charles River Laboratories, Wilmington, Mass) as described.25 In some experiments, a platelet clot was generated through addition of 2U/mL thrombin to platelet-rich plasma. The forming clot was harvested with sterile instruments and used for subsequent experiments.

Migration and Chemotaxis of eEPCs

To evaluate the effect of platelets on chemotaxis and migration of EPCs, a trans-well culture system was used.26 In brief, isolated platelets were allowed to adhere to the bottom of 24-well culture plates and stimulated with a thrombin 1U/mL for 60 minutes. T17b cells (100 000/mL) were added on top using trans-well culture inserts (5.0 μm pore size; Costar) that allowed physical separation of T17b cells from platelets. After 24 hours, the trans-well insert was removed, and migrated T17b cells were stained with crystal-violet and densitometrically quantified.

Platelet Adhesion to eEPCs Under Static and Dynamic Conditions

Static Adhesion

To evaluate eEPC/platelet adhesion under static conditions, isolated platelets (2 × 10^7/mL) were allowed to adhere to 96-well plates for 2 hours. Subsequently, T17b cells were added and incubated for 60 minutes. After 3 gentle washing steps with Tyrodes buffer, residual adherent eEPCs were counted by direct phase contrast microscopy. In a second adhesion assay, T17b (1 × 10^7/mL) cells were incubated for 30 minutes with isolated platelets (2 × 10^7/mL) or buffer as control under agitation (1000 rpm) using a conventional platelet aggrerometer. Thereafter, cell suspension was washed 3 times with Tyrodes buffer, and eEPCs were incubated with FITC-conjugated anti-CD41 or control anti-CD11b mAb. The percentage of CD41-positive T17b cells was analyzed by flow cytometry and was used as index of platelet/eEPC adhesion.

Dynamic Adhesion

Adhesion experiments under flow conditions were basically performed as described previously.27 In brief, glass coverslips were coated with collagen type I (5 μg/mL) as described previously28 and used in a flow chamber (Oligene). Thereafter, isolated platelets (2 × 10^7/mL) were allowed to adhere to collagen for 30 minutes. In some experiments, adherent platelets were pretreated with anti-CD62P mAb (5 μg/mL) before eEPCs were perfused over immobilized platelets. In other experiments, eEPCs (T17b) were preincubated with anti-CD29, anti-CD162, or anti-CD11b (5 μg/mL). Perfusion was performed with T17b cells resuspended in Tyrodes–HEPES buffer (pH 7.4 supplemented with 1 mmol/L CaCl2 and 1 mmol/L MgCl2) at shear rates of 2000 s⁻¹ (high shear). All experiments were recorded in real time on videotape and evaluated offline using Capimage software (Zeintl).

Scratch Assay
eEPCs were grown to confluence in a 24-well plate. Subsequently, the cell monolayer was wounded with a plastic pipette tip generating a gap of ~1-mm width, and cells were incubated with isolated platelets resuspended in medium (0.1 to 1 × 10^7/mL) or growth medium alone as control. Migrated cells were counted after 24 hours. Thereafter, wells were incubated with an anti-vWF antibody (5 μg/mL) and a secondary Alexa Fluor 488–conjugated rabbit anti-goat antibody, as described above, and analyzed by immunofluorescence microscopy, as described below. To detect specific mediators of this platelet-mediated migration, blocking mAbs to platelet-derived growth factor-AB (Promega), CD40L (Biozol), CD11b (clone M1/70; Pharmingen), and CD54 (Beckmann Coulter), using concentrations of 5 μg/mL for each substance, were used. Activation protein tumor necrosis factor-α (Tebu-bior; 20 ng/mL) served as positive control.

Scanning and Transmission Electron Microscopy

For scanning electron microscopy (SEM), T17b cells were cultivated on coverslips in the absence or presence of platelets (2 × 10^6

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cells/mL) for 24 hours. Thereafter, the cells were washed 2 times with PBS, and the coverslips were fixed and examined using a field emission scanning electron microscope (JSM-6300F; Jeol Ltd.). For transmission electron microscopy, EPCs were grown to 70% to 80% confluence and coincubated with isolated platelets (2 × 10^6/mL) for 5 days in culture medium. Subsequently, cells were washed and fixed using a buffer consisting of glutaraldehyde (2.5%) and tannin (0.02%) in a sodium cacodylate buffer, pH 7.4, before electron microscopy was performed. 27

**Reverse Transcription–Polymerase Chain Reaction**

After incubation of T17b cells with buffer alone, cAMP (0.5 mmol/L), or isolated platelets (4 × 10^6/mL) for 5 days, the 6-well plates were carefully washed with PBS and analyzed by RT-PCR as described. 29 Briefly, total RNA was extracted using the Qiagen RNAeasy Mini Kit (Qiagen). Contaminating DNA was removed by DNase using the Qiagen RNase-free DNase Set. RT-PCR was performed using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) and Taq Polymerase (GIBCO BRL). Annealing temperature was 94°C for 30 seconds, extension at 72°C for 30 seconds, with a final 7-minute extension at 72°C. The primer sequences were as follows: c-kit forward 5'-ATA AAG GGT TCA GTG TGT TAC GTG G-3', reverse 5'-TTG GAA AGG TGC AAG AGT GTA GC-3'; CD31 (platelet-endothelial cell adhesion molecule-1 [PECAM-1]) forward 5'-GTT GTC ATT GGA GTG GTC ATC G-3', reverse 5'-CCT GCC TGC TCT CGG TGA GC-3'; flk-1 (kinase insert domain-containing receptor [KDR]) forward 5'-GCA GCG CTT CAAG-3', reverse 5'-AGC TGT CTG ACA TCG CTC ACC G-3'; aldolase, forward 5'-CAG GCA TTG TGC TTA GAG-3', reverse 5'-ACG TGT CTG ACA TCG TCT ACC G-3', 5'-CAT ACA CTG GCA GGC CCT CAAG-3'. Lowercase letters indicate nucleotides added for cloning purposes.

**Immunofluorescence Microscopy**

eEPCs were coincubated with medium or platelets for 5 days on chamber slides and processed for immunofluorescence microscopy. The mouse endothelial cell line MHEC5-T was used as positive control. Between each incubation step, cells were gently washed with PBS. T17b cells were fixed with 2% formaldehyde before electron microscopy was performed. To test whether EPCs bind to platelets, isolated platelets (2 × 10^6/mL) were added for cloning purposes.

**Data Presentation and Statistics**

Comparisons between group means were performed using Student t test or ANOVA analysis. Data are presented as mean ± SD. P < 0.05 was considered statistically significant.

**Results**

**Platelets Induce Chemotaxis and Migration of eEPCs**

Adherent platelets secrete various chemotactic compounds on activation. 18 Thus, we evaluated the effect of platelets on EPC chemotaxis in a trans-well chamber culture system. Chemotaxis of eEPCs (T17b cells) toward adherent platelets was substantially increased compared with controls (P < 0.01; Figure 1). Next, we studied the effect of platelets on eEPC migration. T17b cells were grown to confluence in 24-well plates, and the cell monolayers were wounded in a linear fashion with a pipette tip. Thereafter, the wounded eEPC monolayer was incubated for an additional 12 hours in the absence or presence of platelets (0.1 to 1 × 10^6 cells/mL medium), and the number of eEPCs that migrated into the denuded area was evaluated (Figure 2). We found that eEPC migration was significantly increased, depending on platelet number (Figure 2). At a platelet concentration of 1 × 10^6/mL, eEPC migration was enhanced by ∼2.5-fold (P < 0.01) compared with control, indicating that platelets induce migration of eEPCs. To detect the specific mediators of platelet-induced migration, blocking antibodies were used, as indicated in figure legends. By addition of blocking anti–platelet-derived growth factor-AB, a substantial reduction of platelet-induced migration was found (Figure 2D and 2E).

**eEPCs Adhere to Platelets Under Static and Dynamic Flow Conditions**

Platelets play a critical role in the recruitment and adhesion of circulating leukocytes to the vascular wall.18 To test whether EPC bind to platelets, isolated platelets (2 × 10^6/mL) were allowed to adhere to 96-well plates for 2 hours. Subsequently, EPCs were added to the wells, and adhesion of EPCs on platelets was evaluated as described in Materials and Methods. Under these static conditions, EPCs showed an ∼4-fold
enhanced adhesion to platelets compared with the plastic surface control ($P<0.01$; Figure 3A and 3B). In similar fashion, a flow cytometric adhesion assay showed enhanced coadhesion of platelets with EPCs under stirring conditions (Figure 3C). Furthermore, adhesion of isolated platelets to eEPCs could be visualized by scanning electron microscopy (data not shown).

Next, we evaluated the determinants that mediate eEPC adhesion to platelets under arterial flow conditions. In a parallel plate flow chamber, eEPCs were perfused over platelets immobilized on collagen at a wall shear rate of 2000 s$^{-1}$ as described.27 eEPCs substantially adhered to immobilized platelets under flow conditions but not to immobilized collagen type I ($P<0.01$; Figure 4A). Interestingly, rolling of eEPCs on immobilized platelets was almost completely inhibited in the presence of blocking anti-CD62P (P-selectin) or anti-CD162 (PSGL-1) antibodies but not by anti-CD29 ($\beta_3$-integrin; Figure 4). In contrast, firm adhesion of eEPCs to platelets was attenuated by both anti-CD62P/anti-CD162 and anti-CD29 mAbs (Figure 4A). Virtually no inhibition of adhesion of eEPCs on immobilized platelets was found in the presence of a blocking anti-CD11b (anti-integrin $\alpha_M$, macrophage antigen-1 $\alpha$) mAb (Figure 4A). Flow cytometric analysis of eEPCs showed substantial cell surface expression of CD162 (PSGL-1) and of CD49d (VLA-4) on T17b cells (data not shown), confirming previously published results.15

Together, these data indicate that adhesion of eEPCs onto immobilized platelets occurs under dynamic flow conditions. The adhesion of eEPCs to immobilized platelets is specifically regulated by selectins (PSGL-1/P-selectin; rolling) and integrins ($\beta_3$-integrin; firm adhesion). To test whether these findings can be transferred from the murine eEPC model to adult progenitor cells, dynamic adhesion of adult human CD34$^+$ cells isolated from cord blood was evaluated. Com-

Figure 2. Effect of platelets on eEPC migration. A, A confluent monolayer of eEPCs was wounded with a plastic pipette and coincubated with increasing concentrations of isolated platelets (0.1 to 1 $\times 10^8$/mL). After 24 hours, the number of migrated EPCs was counted by using a defined frame that was projected to each photograph. *$P<0.01$ compared with control. B and C. For subsequent experiments, migration was assessed after 24 hours using a platelet concentration of 1 $\times 10^8$/mL. B shows representative photomicrographs of migration experiments. C. Mean and SD of 6 independent eEPC migration experiments. *$P<0.01$ compared with EPCs alone (without platelets). D. After wounding of the eEPC monolayer, isolated platelets (1 $\times 10^8$/mL) were added without blocking mAbs (5 $\mu$g/mL each) for 24 hours. *$P<0.05$; n=4. E shows representative photomicrographs of these migration experiments with blocking mAbs.
were cultured in the presence of platelets for 5 days and analyzed by light and fluorescence microscopy. We found that a noticeable amount of eEPCs acquired an endothelium-like shape with a 3- to 5-fold larger, spindle-shaped appearance with cellular protrusions (Figure 5B). In contrast, no change in the phenotype of eEPCs was noted in the absence of platelets.

Immunofluorescence verified that eEPCs transform into mature endothelial cells in the presence of platelets. We found that eEPCs coincubated in the presence of platelets for 7 to 10 days showed enhanced vWF expression compared with untreated cells (Figure 6A). Moreover, eEPCs cocultured with platelets revealed a typical endothelial cell-like cytoskeleton rearrangement, as verified by staining with phalloidin. As a representative control of the differentiated endothelial phenotype, we used the mouse endothelial cell line MHEC5-T (Figure 6A). Transmission electron microscopy showed the formation and presence of Weibel Palade bodies in eEPCs cocultured in the presence of platelets similar to mature endothelial cells (Figure 6B). Additionally, RT-PCR analysis of native eEPCs showed virtually no expression of the mature endothelial cell marker CD31 (PECAM-1) and a distinct signal for the stem cell marker c-kit (Figure 6). However, after incubation of EPCs with isolated platelets for 5 days, CD31 mRNA expression increased, whereas c-kit decreased substantially (cAMP-induced differentiation served as a positive control). In a second RT-PCR analysis, the endothelial cell markers flk-1 (vascular endothelial growth factor receptor), vascular endothelial-cadherin, and thrombomodulin were increased after incubation with isolated platelets or cAMP. Consistent with previous results, expression of Tie-2 was not enhanced after incubation with cAMP, nor with platelets. To test, whether these findings can be transferred from the murine eEPC model to adult human progenitor cells, differentiation of human CD34+ cells by platelets was evaluated. After 10 days of coincubation, a change in morphology of CD34+ cells could be observed with regard to increased size and spindle-shaped morphology (Figure 6D). Furthermore, these cells displayed substantial amounts of cytoskeleton, characteristic for endothelial cells, but not progenitor cells, as verified by phalloidin staining.

**Discussion**

In this study, we have shown that platelets regulate chemotaxis, adhesion, and differentiation of murine EPCs (eEPCs). In brief, the major findings of the present study are: (1) platelets induce chemotaxis and migration of a mouse eEPC line (T17b); (2) murine eEPCs specifically adhere to immobilized platelets via PSGL-1 and VLA-4 under high-shear dynamic flow conditions; and (3) platelets induce differentiation of murine eEPCs and maturation into endothelial cells as evidenced by specific immunological (vWF) and morphological (Weibel Palade bodies) endothelial markers. The findings imply that platelets are critical for recruitment of circulating murine eEPCs to areas of vascular or ischemic lesions. Interaction of these eEPCs with activated platelets may play a prominent physiological role for vascular or tissue...
remodeling. A disturbed platelet interaction with EPCs may in turn be associated with defective vascular and tissue repair mechanisms involved in pathologies such as atherosclerosis and myocardial infarction.

The maintenance of endothelial integrity is of crucial importance for preventing atherosclerotic plaque erosion and rupture, causing myocardial infarction and sudden cardiovascular death. Recently, circulating EPCs have been shown to incorporate into sites of neovascularization and to home to sites of endothelial denudation. EPCs recruited at sites of vascular lesions accelerate re-endothelization and lesion repair. However, the mechanisms that regulate EPC homing to vascular or tissue lesions are incompletely understood.

Platelets accumulate within seconds to sites of vascular injury and release a variety of potent chemotactic factors that induce recruitment of circulating blood cells toward sites of vessel injury. Adherent platelets express various receptors (e.g., P-selectin) on their surface that regulate adhesion of neutrophils and mononuclear cells. Previous studies indicated that platelet-derived microparticles could influence

![Figure 4](image-url)

**Figure 4.** Adhesion of eEPCs to immobilized platelets under arterial shear conditions. Coverslips were precoated with collagen I (5 μg/mL) and additionally preincubated with or without freshly isolated platelets (2×10⁶/mL) to achieve adherent platelet layers as described in Materials and Methods. Resuspended T17b cells (5×10⁶/mL) were perfused over these coverslips in the presence or absence of blocking mAbs (5 μg/mL) as indicated. After 5 minutes, adherent eEPC were quantified by offline counting. The mean and SD of 4 independent experiments is shown. *P<0.05 compared with control (collagen without platelets). B, Coverslips were precoated with collagen I (5 μg/mL) and additionally preincubated with or without freshly isolated platelets (2×10⁶/mL) to achieve adherent platelet layers as described in Materials and Methods. Resuspended human CD34⁺ cells (5×10⁶/mL) were perfused over these coverslips. After 5 minutes, adherent CD34⁺ cells were quantified by offline counting. The mean and SD of 4 independent experiments is shown. *P<0.05 compared with control (collagen without platelets).
Weibel Palade bodies served as a control. Analysis revealed Weibel Palade bodies or platelets for 5 days. The mouse endothelial cell line MHEC5-T served as a positive control. B, Electron microscopic analysis of T17b cells after incubation with medium washed and analyzed using fluorescence microscopy at 568 to 61 nm; magnification ×80 000). T17b cells incubated with medium alone showed no Weibel-Palade-bodies. C, RT-PCR analysis of the stem cell marker c-kit and of the marker for mature endothelial cells CD31 (PECAM-1) in T17b cells treated with medium, cAMP (0.5 mmol/L), or isolated platelets (4 × 10^6/mL), as indicated for 5 days. The image shown is representative for 2 independent experiments. Aldolase mRNA was analyzed as internal control. D, RT-PCR analysis of the markers for mature endothelial cells thrombomodulin, flk-1 (vascular endothelial growth factor receptor), vascular endothelial-cadherin, and Tie-2 in T17b cells treated with medium, cAMP (0.5 mmol/L), or isolated platelets (4 × 10^6/mL) as indicated for 5 days. The image shown is representative for 2 independent experiments. Aldolase mRNA was analyzed as internal control. E, CD34^+ cells (5 × 10^4) were coincubated with isolated platelets (2 × 10^6/mL) in 96-well plates for 10 days. Compared with untreated CD34^+ cells (arrows, top panel), cells coincubated with platelets developed an endothelial spindle-shaped phenotype (arrows, top panel). vWF immunostaining and DAPI staining of CD34^+ cells (bottom panel) and progenitor cells after 10 days coculture with platelets (bottom panel).
dition, the homing of eEPCs in hypoxic tumors, their participation in tumor vessel formation, as well as their stimulation of angiogenesis in chronic and acute ischemia render eEPCs a relevant model system to study the biology of EPCs. To evaluate, if the central findings of this study derived from the murine progenitor cell model can be transferred to human progenitor cells, key experiments were performed using adult human CD34+ cells. We show that adult CD34+ cells adhere to platelets under high shear rates and that platelets induce differentiation of CD34+ cells into endothelial cells. Nevertheless, further studies using human bone marrow–derived stem cells have to be performed to confirm the exact mechanisms of this interaction within the human system.

In conclusion, we provide first evidence that platelets mediate recruitment of murine eEPCs and stimulate differentiation of these eEPCs into endothelial cells. If these findings can be transferred to the human system, the mechanism described in the present study may play a central physiological role for maintenance of vessel integrity and neoendothelialization of vascular lesions. Further, an altered interaction of platelets and EPCs may play a critical role in atheroprogession.

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