Shear stress regulates inflammatory and thrombogenic gene transcripts in cultured human endothelial progenitor cells

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Summary
Shear stress has an established effect on mature endothelial cells, but less is known about how shear stress regulates endothelial progenitor cells (EPCs). In vitro expanded EPCs isolated from adult human blood represent a novel tool in regenerative vessel therapy. However, in vitro culturing may generate cells with unfavourable properties. The aim of the present study was therefore to assess whether shear stress may influence the inflammatory and thrombotic phenotype of in vitro expanded EPCs. In late outgrowth EPCs, 6 hours of shear stress (6.0 dynes/cm²) significantly reduced the mRNA levels of IL-8, COX2, and tissue factor (TF) compared to static controls. This was associated with a reduced TF activity. In contrast, mRNA expression of NOS3 was significantly increased following 6 and 24 hours of shear stress. In accordance with this, NOS3 protein expression was increased following 24 hours of shear stress. Overall stimulation with the proinflammatory mediator, TNFα, for the final 2 hours increased the mRNA expression of IL-6, IL-8, MCP-1, ICAM1, and TF. However exposure to 6 hours of shear stress significantly suppressed the inductive potential of TNFα to increase the mRNA levels of IL-6, IL-8, COX2, and TF. Additionally, TNFα increased TF activity approximately 10 times, an effect that was also significantly reduced by exposure to 6 and 24 hours of shear stress. The effect of shear on the gene levels of TF and NOS3 were not blocked by the NOS inhibitor L-NAME. These observations suggest that EPCs are capable of functionally responding to shear stress.

Keywords
Late outgrowth endothelial progenitor cells, shear stress, inflammation, tissue factor

Introduction
Cardiovascular cell therapy using endothelial progenitor cells (EPCs) has recently emerged as a novel approach to repair and replace damaged endothelium (1). Accumulating evidence suggests that EPCs circulating in peripheral blood can home at sites of neovascularisation (2–4). Their clinical relevance for regeneration and repair of vascular injury has been investigated intensely (5–7). However, the efficiency of EPC transplantation may be impaired by low survival rates, low number of cells in circulating blood and impaired function in aging and during illness. Thus, the clinical success of EPC therapy for cardiovascular regeneration may depend on the development of efficient methods to increase the number and the potency of EPCs. This may be approached by in vitro manipulation of the cells.

There are a number of challenges to the concept of a therapeutic application of isolated and ex vivo expanded EPCs. First, the transplanted cells have to maintain their physiological ability to respond to stimuli. In this regard, shear stress is a critical component in the regulation of endothelial function (8). It is well known that high laminar shear stress induce an atheroprotective endothelial phenotype, while low shear stress or turbulent blood flow is linked to endothelial dysfunction (9, 10). Furthermore, it has been shown that EPCs exposed to the proinflammatory cytokine TNF (tumour necrosis factor) α behave like mature endothelial cells by inducing similar degree of procoagulant factors and surface expression of adhesion molecules (11). It is likely that patients who potentially will benefit from EPC transplantation, also have elevated levels of proinflammatory cytokines, like TNFα, in their circulation (12). Finally, recent reports have questioned the use of EPC therapy due to their proinflammatory potential, and it has been shown that transplantation of EPC can promote atherogenesis (13, 14).

Although intensively studied during the last decade, there are still an ongoing debate concerning EPC characterisation and whether or not these cells are true endothelial cells or peripheral blood monocytes expressing endothelial markers (5–7, 15). Accordingly, a number of different methods have been used to quantify and study EPCs. The two most prominent methods are flow cytometry of fresh blood samples and cell cultures of peripheral blood mononuclear cells. Since a specific marker to separate EPCs from mature endothelial cells is still missing, and the number of these cells in the circulation is very low, quantification with flow
cytometry is difficult and not fully reliable (6, 7). Importantly, in vitro culturing of peripheral mononuclear cells (MNC) has been reported to result in two different populations of EPCs. The so-called “early outgrowth” EPCs, appear 3–7 days after plating MNC in culture wells (2, 15). These cells express endothelial markers, but also the monocyte/leukocyte markers, CD14 and CD45 (15). A recent study has reported that at least a fraction of the cells defined as early EPCs are mononuclear cells that have incorporated platelet-derived microparticles giving them endothelial characteristics (von Willebrand factor, lectin binding) and angiogenic properties (16). On the other hand, the late outgrowth EPCs emerge after 9–21 days and selectively express endothelial markers (15). It seems that both populations of cells may be involved in a number of healing processes in cardiovascular diseases. The main role of early EPCs appear to be related to paracrine effects, involving release of pro-angiogenic factors resulting in enhanced neovascularisation and increased thrombus resolution (17, 18). In addition, late outgrowth EPCs can directly replace and repair damaged endothelial cells in vivo (19, 20).

In this study we have addressed the effect of shear stress on in vitro expanded late outgrowth EPCs, and have characterised the ability of such a stimulus to promote healthy endothelial cells with anti-thrombotic and anti-inflammatory properties. Also, we assessed whether shear stress make these cells resistant to the pro-inflammatory mediator TNFα.

Materials and methods

The study, and the collection of blood from healthy volunteers, were approved by the institutional ethics committee (REK-Nord 51/2004) and comply with the principles outlined in the Declaration of Helsinki.

Isolation and culturing of late outgrowth EPCs

EPCs were obtained from human peripheral blood mononuclear cells as described previously (21, 22). Human MNCs were isolated from 36 mL heparinised blood. Blood was diluted 1:1 with sterile phosphate-buffered saline (PBS), pH=7.4 and was transferred to Histopaque 1077 (Sigma-Aldrich, Munich, Germany). After 30 minutes (min) centrifugation at 740 g MNCs were washed twice with PBS, pH=7.4. MNCs were resuspended in EGM2 (Endothelial growth media 2, Lonza, Brussels, Belgium) supplemented with 10% FBS (Fetal bovine serum, Biochrom AG, Berlin, Germany). The cells were seeded at 5x10^6 cells per well onto six-wells tissue culture plates precoated with rat tail collagen type I (BD Bioscience, Bedford, MA, US) and cultured at 37°C, 5% CO2. After 24 hours (h), non-adherent cells were washed away, and medium was replaced with fresh EGM2 supplemented with 10% FBS. Medium was changed daily in six days, and then every other day until the first passage. Late outgrowth EPCs appeared in the cultures after approximately 9–21 days. Cells from passage 2–7 were used in the experiments.

Shear stress studies

A total of 2x10^5 or 3x10^5 EPCs were seeded on collagen precoated six-well culture plates and grown to approximately 90–100% confluence in EGM2 supplemented with 10% FBS. After throughout washing in PBS and 2 h incubation in EBM (Endothelial basal media 2), media was replenished with 3 ml EBM2 with 2% FBS and the cells were exposed to either 0 or 6.0 dynes/cm2 of shear stress for 6 or 24 h with or without 1 ng/ml TNFα (Sigma, St. Louis, MO, USA). TNFα was added for the final 2 h of each incubation period, in order to study the potential of the cells to acutely respond to an inflammatory environment. In some experiments, the cells were treated with the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, Sigma-Aldrich) 1 h before and during shear stress or static incubation period (6 h). Shear stress was induced by placing the culture plates on an orbital rotator (Inforas, Bottmingen, Switzerland) as described previously (23, 24). This technique applies a variable degree of shear stress across the monolayer of cells with a maximal level determined by the following equation:

$$\tau_{max} = \alpha \sqrt{\eta f(2\pi f)^3}$$  \hspace{1cm} (Eq. 1)

α is the radius of gyration of the shaker (cm), ρ is the density of the medium, η = 7.5x10^-3 dynes/cm2 at 37°C, and f is frequency of the rotation (rotations/s). Based on this equation we calculated maximal shear stress to be 6.0 dynes/cm2 at a frequency of 220 rpm. The cells were maintained at 37°C and continuously exposed to a humidified mixture of 5% CO2 in air.

RNA isolation, cDNA synthesis and real-time PCR

Following the various experiments, total RNA was extracted according to the protocol from the PerfectPure RNA cultured cell kit (5Prime, Hamburg, Germany). cDNA was prepared from 400 ng RNA using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). cDNA was diluted 1:8 before used in qPCR.

The qPCR reaction for selected genes were carried out using 2 μl of cDNA in a 10 μl final volume of TaqMan/SybrGreen Fast Universal PCR master mix (Applied Biosystems) primers and probes. The primer/probe sequences for the genes studied are given in Supplemental Table 1 (available online at www.thrombosis-onsite.com). Primers and taqman probes were obtained from Eurogentec (Seraing, Belgium) and from Roche Universal Probe Library (Roche Diagnostics, Mannheim, Germany). Samples were heated for 20 seconds (s) at 95°C and then subjected to a 40 cycle of denaturation at 95°C for 1 s and annealing and elongation at 60°C.
Figure 1: The effect of shear stress on the expression of selected genes involved in inflammation and thrombosis. EPCs were cultured under static conditions or exposed to 6.0 dynes/cm² shear stress for 6 (grey bars) and 24 h (open bars). The relative amount of each gene was obtained by normalising to the transcript level of the stably expressed reference gene, PPIA, and to the 6 h static control sample. Mean values are shown with SEM, n=4. * P<0.05 compared to the 6 h static control sample; #p<0.05 and ##p<0.01 compared to the 24 h static control sample.
for 20 s. A negative control without cDNA template was included in every assay. The PCR efficiency for all genes was determined by performing a dilution series of a pool of all samples. The efficiency was then calculated from the slope of the plot Ct vs. log dilution series (“number of amplicon”) according to the established equation (25):

\[ E = 10^{-\frac{1}{\text{slope}} - 1} \]  

(Eq. 2)

The relative expression ratio of the target genes were calculated based on its real-time efficiency and the Ct differences (Δ) of a treated sample versus a control sample. The expression of the target genes mRNA were normalised to a stably expressed reference gene.

### Tissue factor (TF) activity

A total of 3x10^5 EPCs were seeded in six-well culture plates. After stimulation with 6.0 dynes/cm^2 shear stress and 1 ng/ml TNFα as described before, the wells were washed in 0.15 M NaCl. Thereafter, the EPCs were resuspended in 300 μl 0.15 M NaCl and frozen at −20ºC.

TF activity was measured in frozen and thawed EPC cell suspension in a two-stage amidolytic assay. The assay is based on the ability of TF to accelerate the activation of factor X by factor VIIa following the conversion of prothrombin to thrombin, using bovine factor V and barium citrate (BaCi) eluate (containing clotting factor VII/VIIa, X and prothrombin). This method has been described more precisely elsewhere (26). The TF activity of a crude rabbit brain preparation clotting human plasma in 17 s was defined as 1 U.

### Western blot of NOS3

Cell cultures were washed once in ice cold PBS and then harvested in 150 μl of a 1:1 solution of Laemmli sample buffer and RIPA buffer supplemented with Complete protease inhibitor cocktail (Roche Diagnostics). Samples were boiled for 4 min, and stored at −20ºC. The proteins were resolved by SDS-PAGE and subsequently transferred onto nitrocellulose membranes. The membranes were incubated overnight with mouse anti-human NOS3 antibody (1:600; Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by incubation with Horseradish-peroxidase (HPR) conjugated sheep anti-mouse antibody (1:1500; Amersham Bioscience, Bucks, UK). The membranes were developed using Immobilon chemiluminescent HRP substrate (Millipore, Billerica, MA, USA) and immunopositive bands were visualised using a Kodak Image Station 1000 (PerkinElmer, Waltham, MA, USA). Ponceau S staining (Sigma) confirmed equal loading.

### Assessment of NOx synthesis

The culture medium samples (40 μl) were collected at each time point. The formation of nitrite/nitrate was detected by a colorimetric assay kit from Cayman (Cayman Chemical Company, Ann Arbor, MI, USA).

### Statistical analysis

The data are expressed as mean with standard error of the mean (SEM). The data were analysed using paired t-test, except for the gene expression results which were statistically analysed by REST MCS (25). P values below 0.05 were considered to be significant.

### Results

Fluorescence microscopy and gene expression analysis of the cell culture confirmed the phenotype of late outgrowth EPCs by expression of CD31, CD144, CD146, and no expression of CD45 (see Supplemental Data available online at www.thrombosis-online.com).

### Effects of shear stress on prothrombotic and proinflammatory gene expression

Expression of the genes TF (tissue factor), IL-6 (interleukin-6), IL-8 (interleukin-8), COX2 (cyclooxygenase 2), MCP-1 (monocyte chemotactic protein-1), ICAM1 (intercellular adhesion molecule 1) and NOS3 (nitric oxide synthetase 3) was compared in cells exposed to shear stress vs. static control cultures (Fig. 1A-G). After exposing EPCs to 6.0 dynes/cm^2 shear for 6 h, TF and IL-8 mRNA levels were significantly down-regulated exceeding four fold (p<0.05), while COX2 mRNA was decreased more than 3.5 fold (p<0.05). Furthermore, the mRNA level of NOS3 was significantly (p<0.05) increased with a factor of 2.8 after 6 h exposure to shear stress. No significant changes in the gene levels of IL-6 and MCP1 were detected after shear stress, but there was a trend towards reduction in both. There was also a trend towards increment of the measured gene level of ICAM-1 after 6 h of shear stress.

After 24 h exposure to shear stress (Fig. 1A-G, open bars) only TF mRNA levels were significantly (p<0.05) reduced (2.6 fold compared to the static control cells at 24 h). The expression of NOS3 mRNA after 24 h with 6.0 dynes/cm^2 increased 3.0 fold (p<0.01) compared to static control cells. The other genes measured were not significantly affected by 24 h exposure to shear stress.
Figure 2: The effect of shear stress on TNFα-induced gene expression. EPCs were cultured under static or shear stress (6.0 dynes/cm²) conditions for 6 (gray bars) or 24 h (open bars). The cells were stimulated with 1.0 ng/ml TNFα for the final 2 h of each incubation period. The amount of each gene is shown as relative expression, where the values are normalised to the stably expressed reference gene, PPIA, and to the 6 h static control sample. Mean values are shown with SEM, n=4. *p<0.05 compared to 6 h static TNF sample. #p<0.05 compared to the 24 h static TNF sample.

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To study the effect of inflammation and shear stress, 1 ng/ml TNFα was added to the perfusion medium for the final 2 h of the incubation period. Stimulation with TNFα clearly increased the mRNA levels of TF, IL-6, IL-8, ICAM1 and MCP-1 both in the 6 and 24 h treatment groups (Fig. 2). Furthermore, it was shown that cells exposed to shear stress before and during TNFα stimulation had a suppressed (p<0.05) response to TNFα on the expression of following genes; TF, IL-6, COX2 and IL-8, compared with static cells stimulated with TNFα for the final 2 h of the total 6 h culturing period (Fig. 2A-D). Shear stress did not significantly reduce the TNFα induced mRNA for MCP1 (Fig. 2E). Surprisingly, the mRNA level of ICAM1 was significantly increased 1.6 fold (p<0.05) in TNFα-stimulated EPC exposed to 6 h of shear stress, compared to static TNFα-stimulated cells (Fig. 2F). TNFα did not influence the shear stress-induced up-regulation of NOS3 mRNA level (Fig. 2G). This gene was up-regulated by a factor of 2.1 (p<0.05) following shear stress.

No significant down-regulation of the TNFα-enhanced mRNA levels was detected after 24 h exposure to 6.0 dynes/cm² shear (Fig. 2, open bars). However, also during this culturing condition, the NOS3 mRNA level was significantly increased 2.5 fold (p<0.05).
TF activity and shear stress

Shear stress per se reduced the TF activity in EPCs, from 0.36 ± 0.19 to 0.22 ± 0.12 mU/0.3 ml (p<0.05) after 6 h (Fig. 3A). After 24 h in culture, the shear stress-induced down-regulation of TF activity did not appear significant (p=0.066).

As expected, stimulation with TNFα clearly increased TF activity (Fig. 3B), compared to non-stimulated EPC. However, exposure to 6.0 dynes/cm² shear significantly reduced the ability of TNFα to enhance TF activity in EPC both at 6 and 24 h culture period [from 3.60 ± 1.16 to 0.63 ± 0.08 (p<0.005) and from 2.25 ± 1.01 to 0.89 ± 0.49 mU/0.3 ml cell suspension (p<0.01), respectively].

Table 1: The effect of L-NAME on shear stress induced regulation of TF and NOS3 mRNA levels. EPCs were treated with 0.1 mM L-NAME 1 h prior to and during the 6 h of shear (6.0 dynes/cm²) in the presence or absence of 1.0 ng/ml TNFα for the final 2 h. The level of each gene is presented as relative expression, where the values are normalised to the stably expressed reference gene, RPL13A, and to the 6 h static control sample. Mean values are shown with SEM, n = 5.

<table>
<thead>
<tr>
<th>Condition</th>
<th>TF</th>
<th>NOS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static control</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Static + L-NAME</td>
<td>1.09 ± 0.48</td>
<td>1.05 ± 0.35</td>
</tr>
<tr>
<td>Shear stress</td>
<td>0.10 ± 0.04</td>
<td>6.33 ± 2.22</td>
</tr>
<tr>
<td>Shear stress + L-NAME</td>
<td>0.12 ± 0.05</td>
<td>6.83 ± 2.39</td>
</tr>
<tr>
<td>Static + TNFα</td>
<td>29.26 ± 12.51</td>
<td>0.82 ± 0.27</td>
</tr>
<tr>
<td>Static + TNFα + L-NAME</td>
<td>31.09 ± 13.62</td>
<td>0.87 ± 0.26</td>
</tr>
<tr>
<td>Shear stress + TNFα</td>
<td>3.30 ± 1.6</td>
<td>4.30 ± 1.46</td>
</tr>
<tr>
<td>Shear stress + TNFα + L-NAME</td>
<td>3.21 ± 1.7</td>
<td>4.11 ± 1.37</td>
</tr>
</tbody>
</table>

NOS3 protein expression and NO production

Total NOS3 protein expression was determined after 24 h exposure to 6.0 dynes/cm² shear stress (Fig. 4). Shear stress induced a significant increase in protein levels of NOS3 after 24 h exposure to shear. We were, however, not able to detect any amount of the NO metabolites, NO₂⁻ and NO₃⁻ in the cell media.

The effect of NO inhibition (L-NAME) on shear stress-induced regulation of NOS3 and TF mRNA

To assess whether the NO pathway was involved in the regulation of TF and NOS3 mRNA levels, the cells were treated with the NOS inhibitor L-NAME. In the presence of 0.1 mM L-NAME, shear stress had similar effects on the gene expression of TF and NOS3 (Table 1) as observed without NO inhibition. Increased concentration of L-NAME, 0.5 and 1.0 mM (data not shown), did neither influence the shear stress induced effects on the EPCs.

Discussion

The results of the present study support the hypothesis that late outgrowth EPCs, like mature endothelial cells, are partly regulated by shear stress and can transmit the responses to an altered gene expression and a concomitantly altered protein function. This has also been observed in early and cord blood derived late outgrowth EPCs (27–29). However, to our knowledge, this is the first study...
that describes an effect of shear stress on late outgrowth EPCs isolated from the MNC fraction obtained from adult humans.

In the present study an orbital shaker was used to generate shear stress to EPCs seeded on six-well culture plates. Although this model of shear stress earlier has been described to generate laminar shear (30), a small fraction of the cells in the centre of the well is exposed to lower magnitudes of disturbed or non-laminar shear (31). However, with this in mind, our results show a clear down-regulation of genes involved in inflammation and thrombosis, like TF, IL-8, and COX2, after a short time exposure (6 h) to 6.0 dynes/cm² compared to EPCs cultured under static conditions.

TF is a membrane protein that functions as a cofactor for coagulation factor VII(α) in the initiation of the coagulation cascade, with fibrin formation as the end product (32). In order to prevent intravascular coagulation it is important that vascular cells do not express TF activity. Although cultured endothelial cells are capable of generating TF activity, endothelial cells in vivo most certainly do not express TF (for review, see [33]). It has been shown that both early (34) and late (11) outgrowth EPCs express TF activity and protein, respectively. This was also confirmed in the present study, where we could quantify TF activity and mRNA expression in late outgrowth EPCs. However, exposure of EPCs to 6 h of shear stress significantly reduced mRNA level and activity of TF compared to static EPCs. Additionally, we were able to show that shear stress significantly silenced the TNFα-induced up-regulation of TF activity and mRNA level, and also the mRNA expression of IL-6, IL-8, and COX2. This is an important observation, because patients requiring EPC therapy are likely to have inflammatory cytokines, like TNFα, in their circulation (12). Thus, pre-shearing of EPCs before therapeutic use can make them less sensitive to inflammatory exposure in the patients.

Of interest, the mRNA level of COX2 was down-regulated in response to 6 h shear stress. This observation is not in agreement with previous studies (27, 35), which have shown a significant up-regulation of COX2 mRNA after exposure of shear stress in late outgrowth EPCs obtained from cord blood (27) and in HUVEC and HMVEC (35). Brown et al. (27) speculated that up-regulation of COX2 may increase the production of the bioactive lipid, prostacyclin (PGI₂). This vasodilating prostanoïd, was assumed to be produced mainly by COX2 in endothelial cells (36). In contrary to this view, a more recent report has indicated that PGI₂ is produced predominantly by the COX1 isofom of the enzyme, and not by COX2 (37). Indeed, COX2 is not expressed by native endothelial cells from umbilical veins and only appears during cell culture (38).

Overall, the mRNA levels of IL-6, IL-8, and COX2 were suppressed after 24 h, regardless of the culture conditions (static, shear stress, TNFα stimulation). The underlying mechanism of this down-regulation of mRNA levels during incubation has not yet been elucidated. It is possible that some inhibiting factors are released from the cells, and subsequently become concentrated in the 3 ml medium exposed to the EPCs during the 24 h incubation period or due to low stability of the mRNA of some genes.

Surprisingly, but in agreement with other studies (27, 39), we found an increased mRNA expression of the adhesion molecule, ICAM1, following shear stress. Brown et al. (27) did not find that this increased gene and protein expression of ICAM1 (and E-selectin, vascular cell adhesion molecule 1 [VCAM1]) promote monocyte adhesion to the cell surface. Moreover, the enhanced expression of these adhesion genes in sheared, were small compared to the response in the presence of TNFα (27), an observation similar to our results.

Finally, we observed an up-regulation of the mRNA levels of NOS3 in EPCs sheared for 6 and 24 h. This was also verified by an increase in the protein levels of NOS3 following shear stress for 24 h. In endothelial cells NOS3 is the main producer of NO (40). A large part of the antiatherogenic and antithrombotic properties of the vascular endothelium are mediated their ability to produce and release NO, a platelet inhibitor with a strong vasodilatory activity and with an important anti-inflammatory capacity (41, 42). Note-worthy, we did not measure any concentrations of the stable NO metabolites, nitrite and nitrate in the culture media, neither in sheared nor in control EPCs. Although NO production by NOS3 is tightly regulated at various levels other than transcription and translation i.e. phosphorylation, cofactors/substrate availability and localisation of NOS3 (42, 43), previous studies (27) have found an increase in the NO production following shear stress in late outgrowth EPCs obtained from cord blood and in early outgrowth EPCs (28). Furthermore, Gulati et al. have reported that late outgrowth EPCs isolated from adult human blood were able to produce NO (44). Thus, the lack of detectable differences in NO metabolites in our study could possibly be explained by levels below the detection limit of the assay as a result of low cell number relative to the volume of media. However, the presence of the NOS inhibitor, L-NAME, did not counteract the effects of shear stress on the mRNA levels of TF and NOS3, indicating that NO was not the main responsible regulator behind this finding. This observation contradicts the knowledge of NO as a key regulator in the context of shear stress on endothelial cells by inhibiting the pro-inflammatory transcription factor nuclear factor kappa B (NFκB) (45, 46). However, the shear stress-induced upregulation of NOS3 at the mRNA and protein level may potentiate the cells for responses to
agents like bradykinin and acetylcholine, that is known to stimulate NOS3 activity, but this would not be detected in the present study.

The underlying mechanism behind the effect of shear stress on endothelial cells and how shear stress renders the cells more resistant to inflammatory stress, are not fully understood. However, previous studies on endothelial cells have revealed that shear stress has effects on gene expression by modulating MAPK- and the NFκB pathway (39, 47). Kruppel-like factor (KLF2) has also been implicated as a transcription factor involved in the antithrombotic and antiinflammatory responses to shear stress. KLF2 is known to induce eNOS expression and reduce cytokine-mediated induction of TF in HUVECs (48), and may therefore be a relevant candidate for the observed effects of shear stress in the present study. Further studies will be performed to investigate these downstream pathways of shear stress in EPCs.

In conclusion, the present results suggest that EPCs behave like mature endothelial cells in response to shear stress. We have shown that late outgrowth EPCs obtained from adult human blood are phenotypically altered by shear stress and that these cells can transmit the responses to an altered gene expression and a concomitantly altered protein function. Caution should, however, be considered when using in vitro expanded EPCs as a therapeutic tool in critical ill patients, since these cultured cells may develop phenotypes that are not in correspondence with the phenotype of endothelial cells in vivo (e.g. expressing TF and COX2). Nevertheless, it seems that shear stress is an effective approach for improvement of EPC functions towards a healthy endothelium.

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References


