African Journal of Biotechnology Vol. 10(83), pp. 19346-19355, 21 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2512
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Shear stress and interleukin-8 (IL-8) on the proliferation, differentiation and tube formation of endothelial progenitor cells (EPCs)

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Accepted 24 October, 2011

Endothelial progenitor cells (EPCs) derived from bone marrow, are also found in circulation and involved in both tumor vasculogenesis and wound healing. During the process of EPCs incorporation into tissues and neovascularization, the cells are exposed to fluid shear stress. Interleukin-8 (IL-8) has been shown to play an important role in tumor growth, angiogenesis and metastasis. In our previous study, IL-8 gene expression of cultured human umbilical vein endothelial cells was significantly increased after stimulation by low shear stress for 1 to 2 h. Hence, there may be some connection between the IL-8 and shear stress in the EPCs incorporation into tissues and neovascularization. This study aimed to investigate whether the shear stress and IL-8 played important role in this process. After the cells were pretreated by shear stress, the cells were stimulated by IL-8, and then the expressions of CD133, VWF, VE-cadherins and VEGFR-2 in EPCs were measured. The proliferation, apoptosis, cell cycle, migration and tube formation of EPCs were detected after the cells expose to shear stress stimulated by IL-8. Results show that IL-8 or shear stress alone can induce EPCs proliferation decreased. 5 and 10 dyne/cm^2 shear stress can both induce EPCs differentiation into the mature ECs. The shear stress of 10 dyne/cm^2 on the EPCs developed an extensive tubular network than that of cells stimulated by IL-8 alone, but the shear stress of 5 dyne/cm^2 depressed the tube formation ability. From the result of transwell assay, shear stress of 10 dyne/cm^2 enhanced the EPCs cell migration stimulated by IL-8, while shear stress of 5 dyne/cm^2 decreased it. In addition, the shear stress of 10 dyne/cm^2 induced an increase in wound healing. These findings suggest that EPCs are sensitive to shear stress. Both shear stress and IL-8 can influence the process of EPCs repair in wound. Also, high shear stress (10 dyne/cm^2) combined with IL-8 can induce the differentiation of EPCs to more matured endothelial cells.

Key words: Shear stress, interleukin-8 (IL-8), endothelial progenitor cells (EPCs).

INTRODUCTION

Endothelial progenitor cells (EPCs) mainly generate and exist in the bone marrow. EPCs are a class of orientation stem cells that can transfer between stem cell and endothelial cells, with the characteristics of self-renewal, proliferation and differentiation (Werner et al., 2005; Rafii et al., 2002).

And it can promote blood vessel regeneration by repair endothelial cells. Recent studies showed that EPCs contribute to postnatal vasculogenesis, namely the *de*

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novo vessel formation, during wound healing, limb ischemia, or postmyocardial infarction (Miraglia et al., 1997; Quirici et al., 2001).

During the process of EPC incorporation into tissues and neovascularization, the cells are exposed to fluid shear stress, the frictional force acting on the endothelial cell surface as a result of blood flow. Hemodynamic shear stress is an important endothelial function and phenotype determinant. Arterial-level shear stress (>15 dyne/cm^2) induces endothelial quiescence and an atheroprotective gene expression profile, while low shear stress (<4 dyne/cm^2), which is prevalent at atherosclerosis-prone sites, stimulates an atherogenic phenotype (Malek et al., 1999). Shear stress provokes embryonic mesenchymal progenitor cell (Wang et al., 2005) and EPC differentiation, but the mechanisms regulating the differentiation of EPCs have not been understood completely. *In vivo*, these factors influence the ECs function not only including shear stress, but also containing the inflammatory or chemotaxis factor. EPCs possibly promote the ECs angiogenic process through autocrine and paracrine, by which the cytokines are secreted, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (Pearson, 2010). However, pathophysiological factors recruitment of the EPCs to damage zone is not clear.

Interleukin-8 (IL-8) is a number of CXC chemokine subfamily, which can promote monocyte rolling on endothelial cells into a strong adhesion and play a role in the atherosclerotic inflammatory process (Yoshimura et al., 1987). Preliminary work in our laboratory showed that (Chen, 2003): after stimulation by low shear stress for 1 to 2 h, IL-8 gene expression of cultured human umbilical vein endothelial cells was significantly increased. Further research found that the gene expression and protein formation of IL-8 related to shear stress stimulated time and both of them anti-correlated with shear stress strength. However, whether the shear stress and IL-8 were involve in the process of EPC incorporation into tissues and neovascularization or not was not clear.

Therefore, the present study focused on the influence of the shear stress and IL-8 on the EPCs in the injury area recruitment process. The results demonstrated that both shear stress and IL-8 can influence the process of EPCs repair of wound. These findings suggest that EPCs are sensitive to shear stress. High shear stress (10 dyne/cm^2) combined with IL-8 can induce the differentiation of EPCs to more matured endothelial cells.

MATERIALS AND METHODS

All animal studies were performed according to the National Institutes of Health Guidelines for the care and use of laboratory Animals of China. Canine (Beagle's dog, female, Level I, 12 months old, 8 kg) was supplied by animal center of Sichuan University, and it was fed in a single cage. The treatment of the animal was in accordance with the regulation of ethics.

Main reagents

Endothelial basal medium-2 (EBM-2) (Lonza, USA), SingleQuots and lymphocyte separation medium were purchased from Sigma, USA. The antibody; rabbit anti-human CD34, VEGFR2, CD133, VE cadherin and peroxidase conjugated goat anti-mouse secondary mAb were purchased from Santa Cruz (Santa Cruz Inc, USA). The Matrigel was from BD (BD Biosciences, USA).

Isolation and identification of EPCs

Sterile bone marrow (5 ml) was transferred to centrifuge tube pretreatment with heparin for anticoagulation. EPCs were obtained by isolating mononuclear cells using Ficoll density-gradient centrifugation.

After resuspension in endothelial basal medium (EBM-2, Clonetics) supplemented with EGM-2-MV-SingleQuots (Clonetics) containing vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor-1, epidermal growth factor, and 5% FBS, 10^6 mononuclear cells/cm^2 were plated on fibronectin-coated tissue culture flasks. After 4 days of culture, non-adherent cells were discarded by washing with PBS. To confirm the EPC phenotype, adherent cells were incubated with DiI-labeled acLDL (Molecular Probes) for 1 h, and after fixation were incubated with FITC-labeled Ulex europaeus agglutinin I (ulexlectin, Sigma) for 1 h. Cells were visualized with an inverted fluorescent microscope, and adherent cells staining positive for both FITC–ulex-lectin and DiI-acLDL were judged to be EPCs (in another paper). The first solution change was executed at the 4th day, then every 3 days medium was changed until the cells were up to 90% confluence. In this study we selected the third to the fifth cells generation as target cells.

Immunocytochemical identification of molecular markers

Cells were plated on collagen-coated coverslip for 24 h before the experiment. After PBS washing, the coverslip was fixed with 4% paraformaldehyde for 15 min, and then blocked by normal serum 30 min at 37°C. The coverslip was then separately Incubated with rabbit anti-human CD34, VEGFR2, CD133, and VE cadherin (1:100 dilution) overnight at 4°C, after which the cells were incubated with biotinylated goat anti-rabbit IgG for 30 min at 37°C.

Cell proliferation assays and detection of cell cycle and apoptosis

After stimulation by shear stress, EPCs were harvested from the cultures and replaced into 96-well plate (2×10^5 cells/ml) in triplicates. Cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays according to the protocol of the manufacturer. Prior to the optical density (490 nm) reading, 15 µL MTT solution and 200 µL DMSO were sequentially added to each well. All groups of experiments were performed in triplicate. The OD value was continuous measure for 8 days.

Furthermore, EPCs cells to 5×10^6 / hole were seeded in a 6 well plate, and 48 h later the cells were collected. The cells were fixed by 70% cold ethanol and stained propanol iodide (PI), to enable flow cytometry analysis of cell cycle and apoptosis. Proliferation index (PI) which reflects the cell proliferation activity was given as: PI = (S + G2M) / (G0 / 1 + S + G2M) × 100%. Apoptosis index (Apoptosis index, AI, or APO): the percentage of the G0 / 1 peak of cells in the former sub-diploid peak analysis in DNA diploid cells were used for analysis of apoptosis ratio.

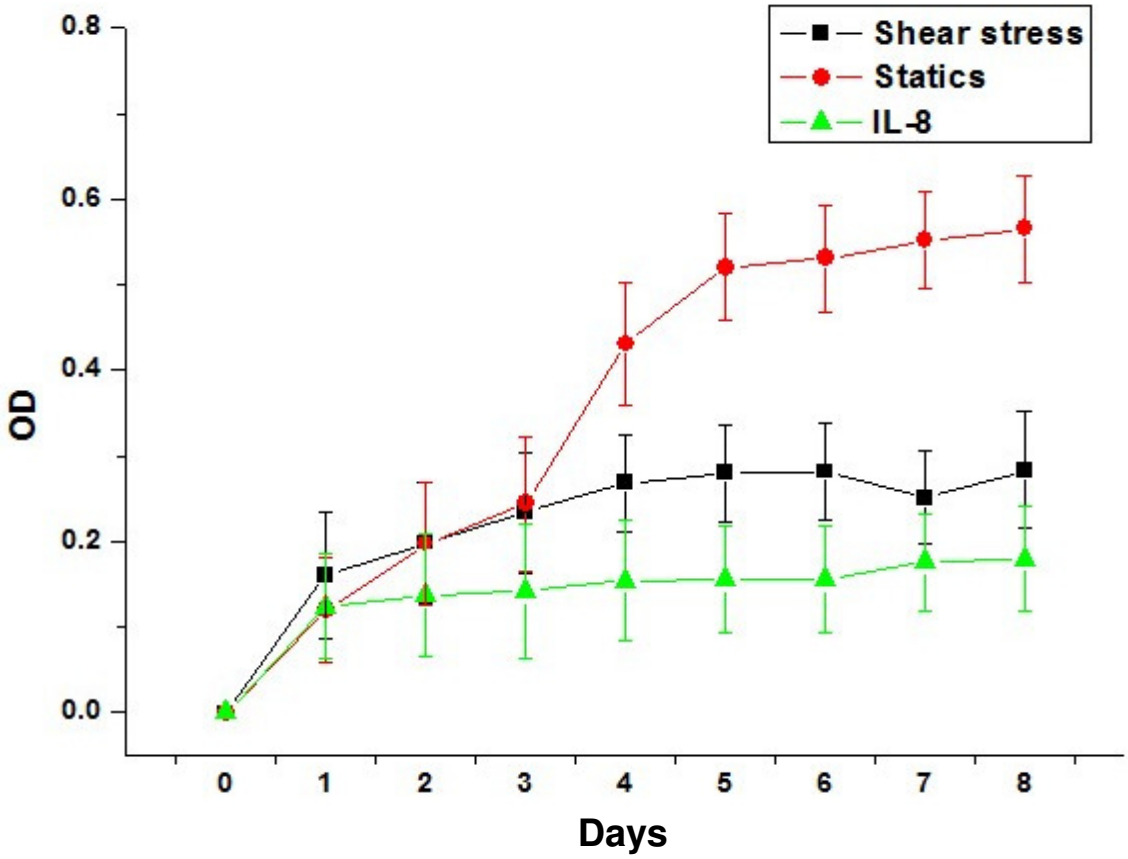

Figure 1. Effect of shear stress and IL-8 can on proliferation of EPCs. EPCs were grown to about 80 to 90% confluence, and cell proliferation assays were allowed to proceed as earlier described.

EPCs stress load

Cells were planted on glass slides and cultured. Upon reaching confluence, a flow system, which was designed in our laboratory (Zhang, 2007), according to McIntire's principle and manufactured by our University's scientific device factory, was used to create laminar flow shear stress on the cell monolayer with the use of a parallel plate chamber. After exposure to laminar flow of 5 or 10 dyne/cm^2 for 2 h, the cells were collected for the experimental assays.

Angiogenesis *in vitro* experiments

In brief, matrigel (100 µL) was added to well of a 24-well culture plate and allowed to polymerize for 30 min at 37°C. After the EPCs were stimulated by shear stress of 5 or 10 dyne/cm^2 for 2 h, cells were resuspended in complete medium and added to the wells containing matrigel. The plates were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Capillary tube formation was assessed at different time by counting the total number of capillary like tubular structures from three randomly chosen fields using an inverted microscope.

Transwell migration assay

At first, transwell filters were equilibrated in serum containing 1640 medium for 2 h. The 1640 medium containing 10% FBS was added into the lower compartments of the migration filters. After the EPCs stimulation by shear stress of 5 or 10 dyne/cm^2 for 2 h, 2 × 10^4 cells were plated per transwell filter. When stimulated by IL-8 (in lower reservoirs), cells were migrated for 6 h at 37°C in 5% CO_2, and were subsequently fixed by immersion of the filters in methanol for 15 min at room temperature. Filters were washed with deionized water, and were stained in 0.2% crystal violet in a 20% methanol/water solution for 10 min. Cells were removed from the upper surface of the membrane with a cotton swab. Cells migrated to the underside of the membrane were counted at 200 × magnification from five random fields per membrane.

Statistical analysis

Data are indicated as mean ± standard deviation (x ± s). For the mean difference between multiple groups, the single factor analysis of variance (one-way ANOVA) was used. Statistical analyses were processed by statistical software SPSS 13.0. $P < 0.05$ indicated significant difference.

RESULTS

Shear stress and IL-8 reduced the EPCs proliferation

As shown in figure 1 the static state, EPCs increased rapidly in first five days, and then trended to a stable

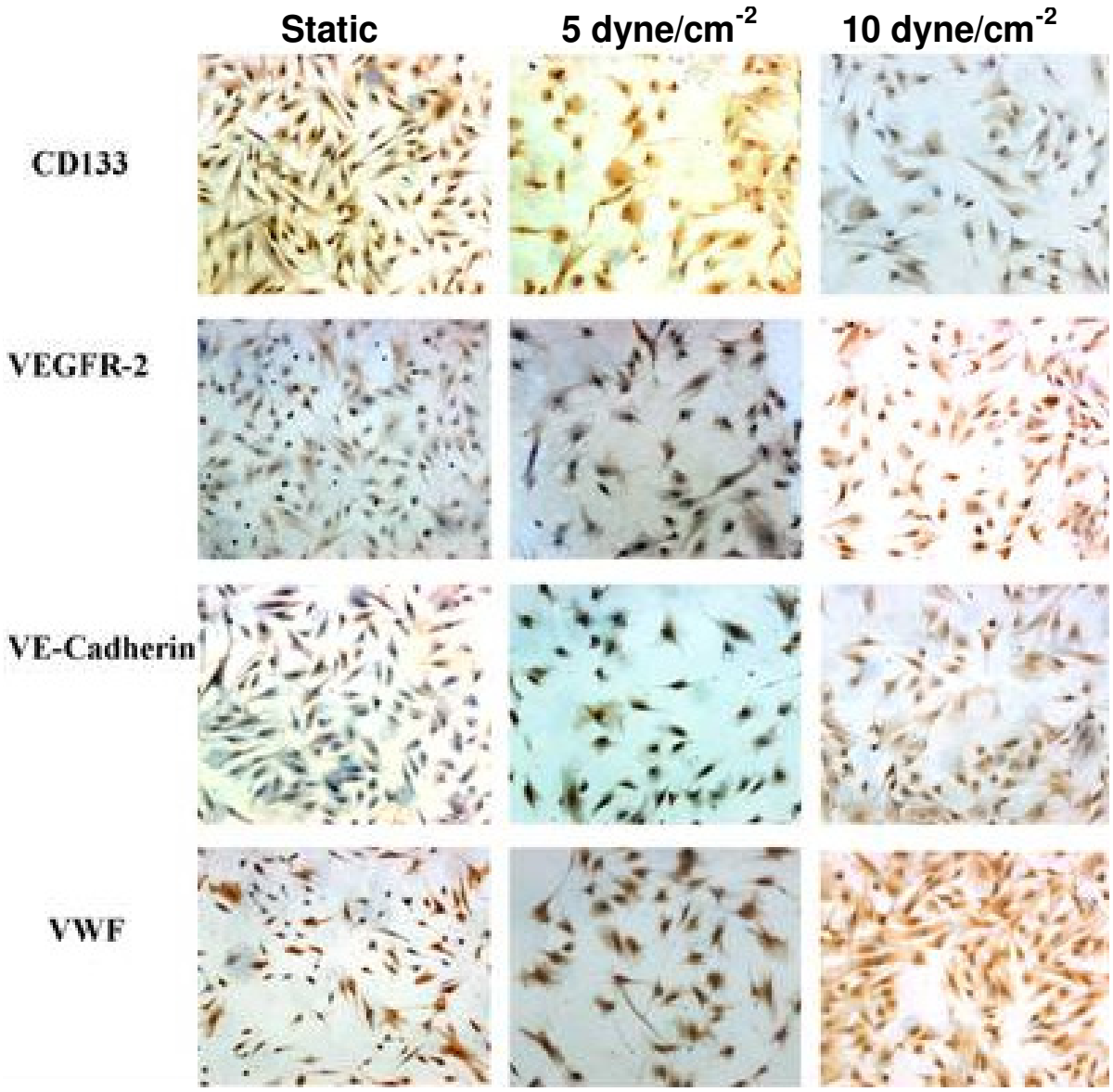

Figure 2. The change of the molecular marker after stimulate by shear stress.

plateau from the sixth day. After stimulation by shear stress (5 or 10 dyne/cm^2) for 2 h, these cells showed a significant growth inhibition from the fourth day. Interestingly, IL-8 significantly decreased the cell growth from the second day. Based on these results, shear stress and IL-8 therefore reduced the proliferation of EPCs, although the mechanism needs further study.

Shear stress prompted EPCs differentiation

As shown in Figure 2, the expression of CD133 and vascular endothelial cadherin (VE-cadherin) in EPCs were positive in static state, but all of the expression of vascular endothelial growth factor receptor- 2 (VEGFR-2) and Von Willebrand factor (vWF) were poorly positive. After stimulation by shear stress of 5 or 10 dyne/cm^2, the expression of CD133 was decreased, but all of the expression of VEGFR-2, VE-Cadherin and VWF were increased than those of static state. Hence, shear stress stimulation enhanced the endothelial lineage marks (VEGFR-2, vWF, VE-cadherin) expression, and decreased the expression of progenitor cells (CD133). The influence of shear stress of about 5 and 10 dyne/cm^2 on the molecular marker expression of EPCs almost had similar trend. However, when stimulated by IL-8 (50,100 and 200 ng/ml), CD34, CD133, VE-cadherin, VEGFR-2 and vWF had no obvious change (data not shown). These results show that shear stress stimulation might prompt the progenitor cells differentiation (to mature

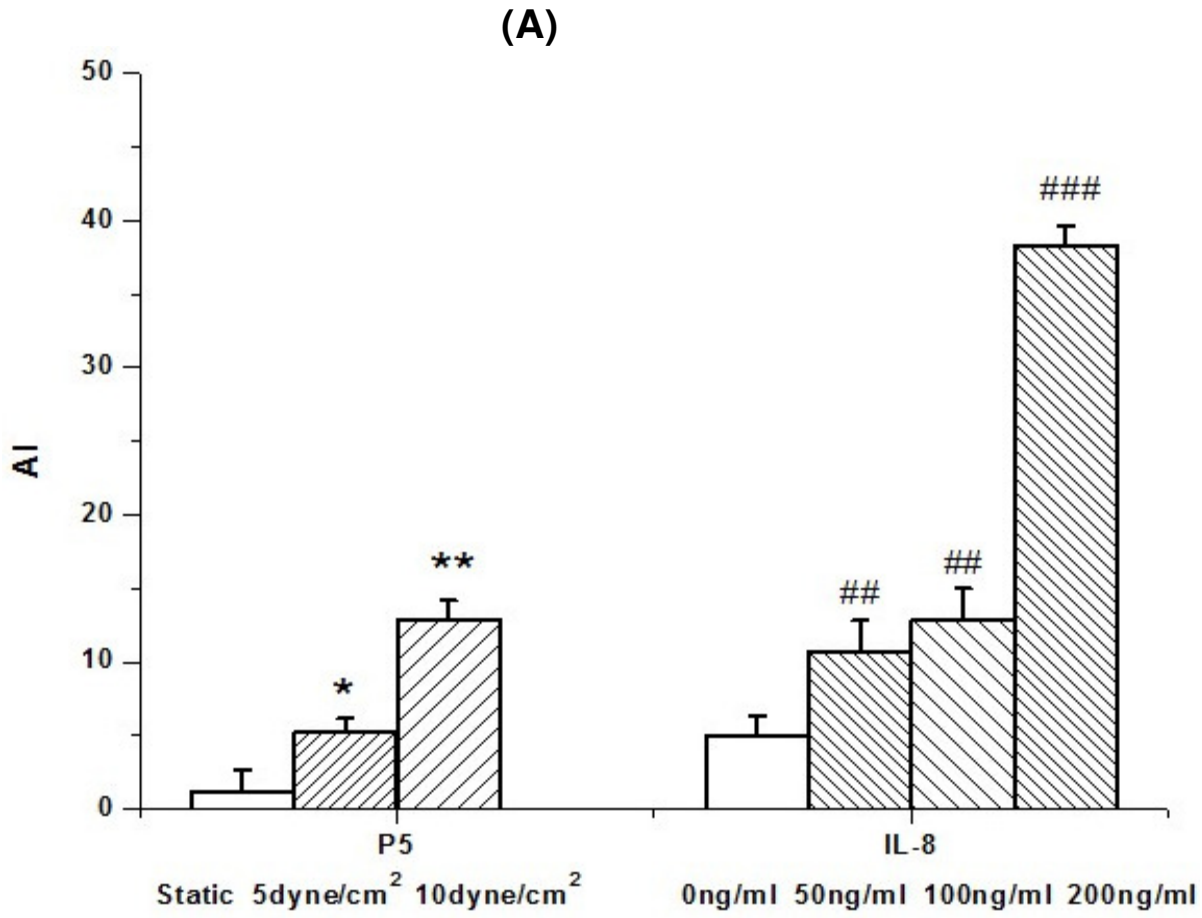

Figure 3. The influence of shear stress and IL-8 on cell proliferation and apoptosis. **(**A) $^{**}p<0.01$, the AI of EPCs stimulated by shear stress about 5 dyne/cm^2 VS that of EPCs under static state; $^{*}p<0.05$, the AI of EPCs stimulated by shear stress about 10 dyne/cm^2 VS that of EPCs under static state; ## $p<0.01$, the AI of EPCs stimulated by IL-8 about 50 ng/ml and 100 ng/ml VS that of EPCs under 0ng/ml; ### $p<0.001$, the AI of EPCs stimulated by IL-8 about 200 ng/ml VS that of EPCs under 0 ng/ml. (B) $^{**}p<0.01$, the PI of EPCs stimulated by shear stress about 5 and 10 dyne/cm^2 VS that of EPCs under static state.

endothelial cells), and IL-8 had no effect on it.

Shear stress and IL-8 stimulation on EPCs cell cycle and apoptotic index

From Figure 3A, when cells were stimulated by shear stress of 10 dyne/cm^2, the apoptosis index of EPCs was increased than that of cells under static state ($p<0.01$). Apoptosis index of EPCs under shear stress of 5 dyne/ cm^2 was also increased than that of cells under static state ($p<0.05$). Hence, both apoptosis index after cells exposed to shear stress increased than that of cells under static state. However, it was observed that the apoptosis index of cells loaded by shear stress of 5 dyne/ cm^2 decreased than that of cells stimulated by shear stress of 10 dyne/cm^2($p<0.05$). In addition, after the EPCs were stimulated by IL-8 of 200 ng/ml, the apoptosis index obviously increased than that of cells stimulated by IL-8 of other concentration ($p<0.001$). Once the EPCs were stimulated by IL-8 of 50 or 100 ng/ml, the apoptosis index significantly increased than 0 ng/ml group *(*$p<0.01$).

Furthermore, when cells were stimulated by shear stress of 10 dyne/cm^2, the proliferation index of EPCs decreased than that of cells under static state($p<0.01$). Proliferation index of EPCs loaded by shear stress of 5 dyne/ cm^2 also decreased than that of cells under static state ($p<0.01$). Therefore, each of the proliferation indexes under shear stress was lower than that of cells in static state, although the proliferation index of cells stimulated by shear stress of 5 dyne/ cm^2 decreased than that of cells loaded by shear stress of 10 dyne/cm^2. Interestingly, there was no significant difference with the proliferation index of EPCs stimulated by IL-8 of 200, 50, 100 and 0 ng/ml groups, respectively.

Base on these results of apoptosis index and proliferation index, it was suggested that shear stress retarded the cells proliferation, but augmented the cells apoptotic.

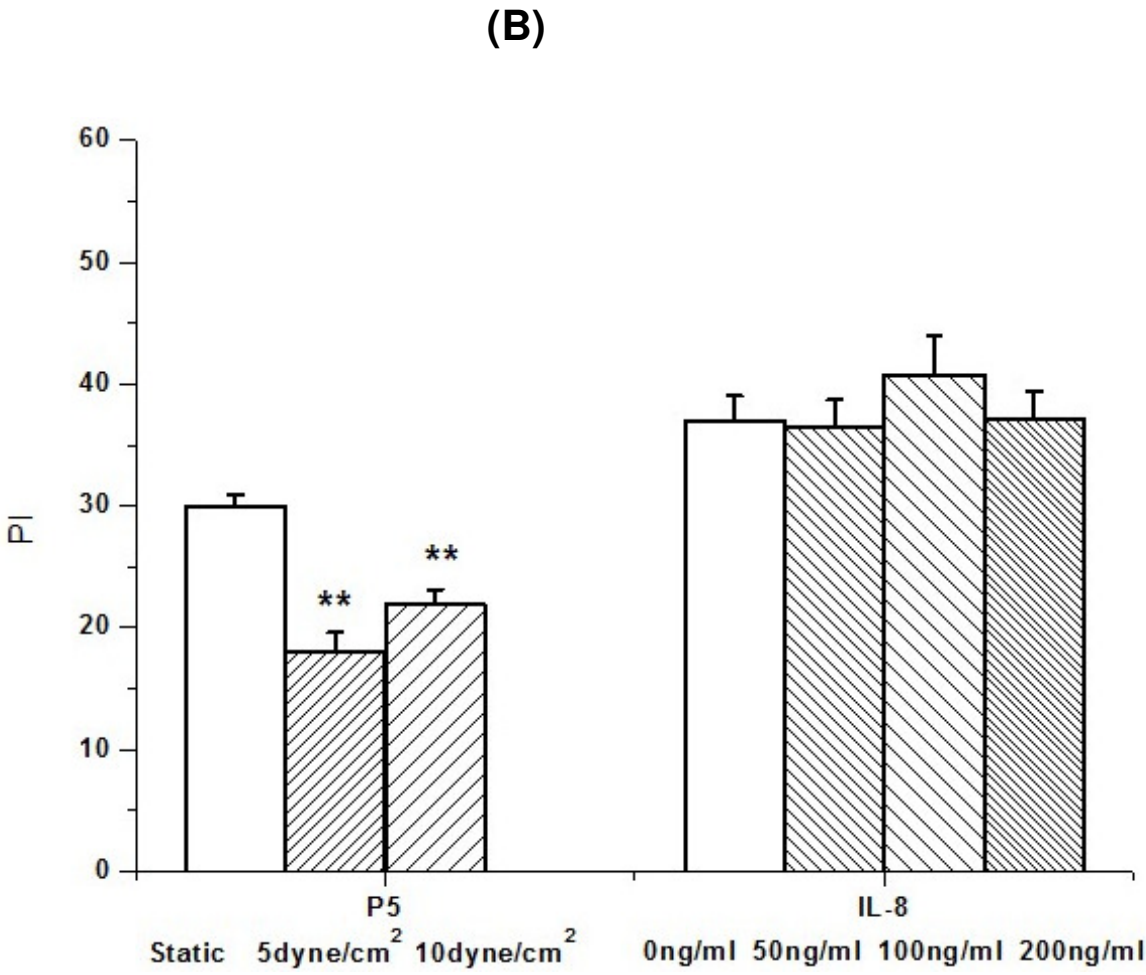

Figure 3. Contd.

IL-8 induced an increase in the cells apoptosis, but had no influence on the cells proliferation. Therefore, shear stress inhibited the growth of EPCs at same time induce cells apoptosis.

10 dyne/cm^2 shear stress and IL-8 accelerated EPCs tube formation

In vitro angiogenesis of human vascular endothelial cells was assessed using a capillary/tube-like network formation assay. An angiogenic parameter- number of capillaries per field was considered as measures of *in vitro* capillary/tube-like network formation. The EPCs under static state begin angiogenesis from 8 h; branch connection become clearer, and cell morphology slightly elongated (data no show). After the cells were stimulated by IL-8 6 h, the tube formation can be clearly seen in the microscope.

In figure 4 the cell morphology looked like spindle shape, and the cell extended along the long axis. The cells exposed to shear stress (5 or 10 dyne/cm^2) had difficulty having tube formation (data no shown). Once the pretreated (shear stress of 5 dyne/cm^2) EPCs were stimulated by IL-8 6 h, the tube formation could not be seen under the microscope until 8 h, while cell morphology was more round than other group and surrounded by thin branches. Moreover, compare to the shear stress of 5 dyne/cm^2, the cells stimulated by shear stress of 10 dyne/cm^2 induced a quick tube formation.

The tube number/filed in 5 dyne/cm^2 + IL-8 group (after the cells under shear stress of 5 dyne/cm^2 2 h, the EPCs stimulated by IL-8 about 6 h) was decreased than that of IL-8 group (the cells stimulated by IL-8 about 6 h alone) (p <0.05), and that of 10 dyne/cm^2 + IL-8 group (after the cells were under shear stress of 5 dyne/cm^2 2 h, the EPCs stimulated by IL-8 about 6 h) was increased than that of IL-8 group (the cells stimulated by IL-8 about 6 h alone) (p <0.05). Therefore, shear stress of 10 dyne/cm^2 stimulation can accelerate the angiogenesis ability of EPCs, while shear stress of 5 dyne/cm^2 inhibited the action.

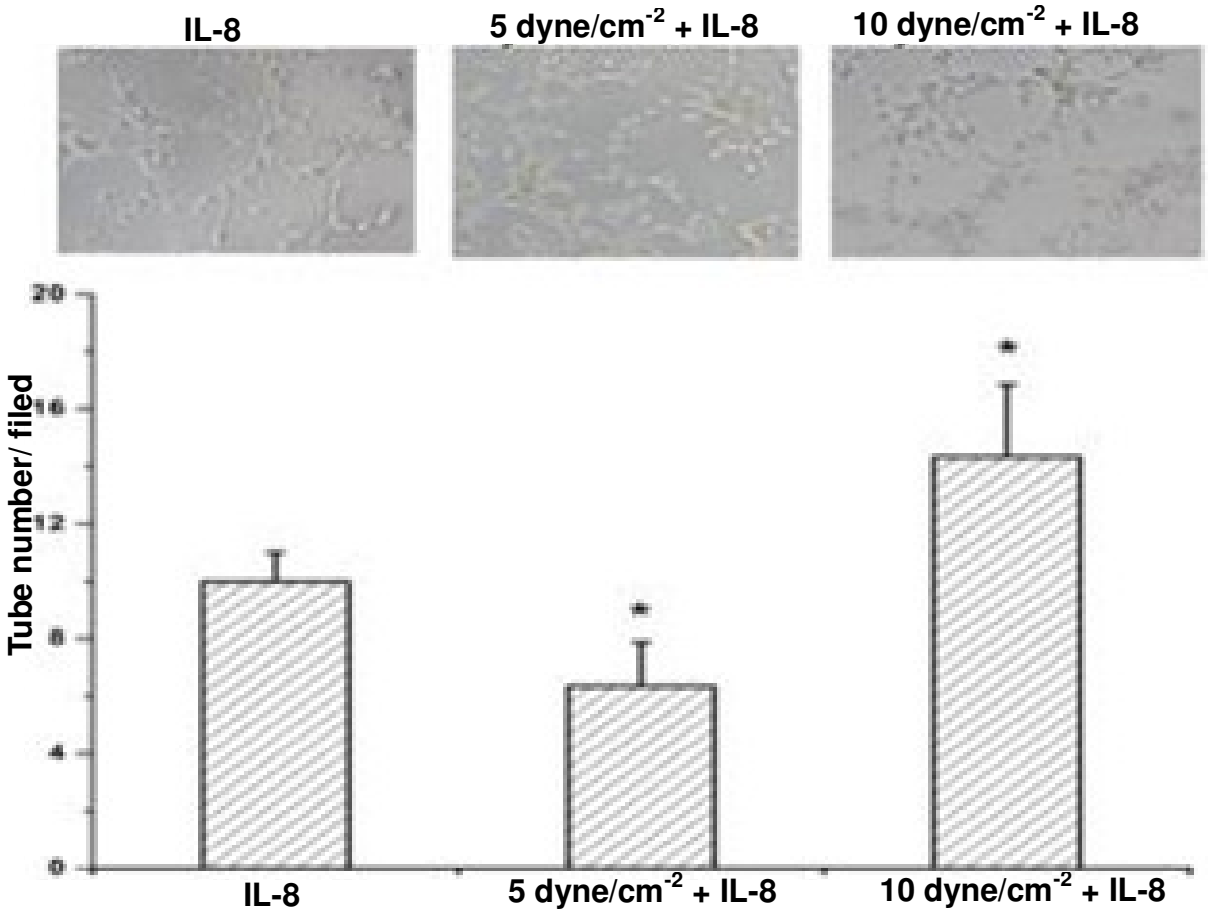

Figure 4. Shear stress and IL-8 influence on the EPCs tube formation. EPCs were grown to 80 to 90% confluence, and stress load allowed to proceed as described earlier. Tube formation was allowed to proceed as described in Materials and methods.* $p<0.05$, the tube number of EPCs under shear stress of 10 dyne/cm^2 or 5 dyne/cm^2 stimulated by IL-8 VS that of EPCs stimulated by IL-8 alone.

This result suggests that the influence of 10 dyne/cm^2 shear stress on endothelial cell function may be a protective effect.

10 dyne/cm^2 shear stress and IL-8 stimulated EPCs cell migration

In Figure 5, the migrated cell number of 5 dyne/cm^2 + IL-8 group (after the cells stimulated shear stress of 5 dyne/cm^2 2 h, the EPCs stimulated by IL-8 about 6 h) decreased than that of IL-8 group (the cells stimulated by IL-8 about 6 h alone) ($p<0.05$), and that of 10 dyne/cm^2 + IL-8 group (after the cells under shear stress of 5 dyne/cm^2 2 h, the EPCs stimulated by IL-8 about 6 h) increased than that of IL-8 group (the cells stimulated by IL-8 about 6 h alone) ($p<0.05$). Therefore, 10 dyne/cm^2 shear stress stimulation can accelerate the EPCs migration ability, while shear stress of 5 dyne/cm^2 inhibited this.

In addition, shear stress can also induce the cell morphology change. Shear stress of 10 dyne/cm^2 induced cell into spindle shape, but shear stress of 5 dyne/cm^2 mainly adjusted cell shape smaller than that of cells stimulated by IL-8 alone. This shape modification might due to the cells which still migrated from upper reservoirs of transwell chamber to lower reservoirs. It follows that 10 dyne/cm^2 shear stress might up-regulate EPCs migration ability on the present of IL-8, while 5 dyne/cm^2 shear stress inhibited this.

10 dyne/cm^2 shear stress and IL-8 promoted EPCs wound healing

As shown in Figure 6, the wound healing distance of EPCs in 5 dyne/cm^2 + IL-8 group (after the cells were under shear stress of 5 dyne/cm^2 2 h, the EPCs stimulated by IL-8 about 6 h) was similar to that of IL-8 group (EPCs stimulated by IL-8 alone). But the wound healing distance of EPCs in 10 dyne/cm^2 + IL-8 group (after the cells were under shear stress of 10 dyne/cm^2 2 h, the

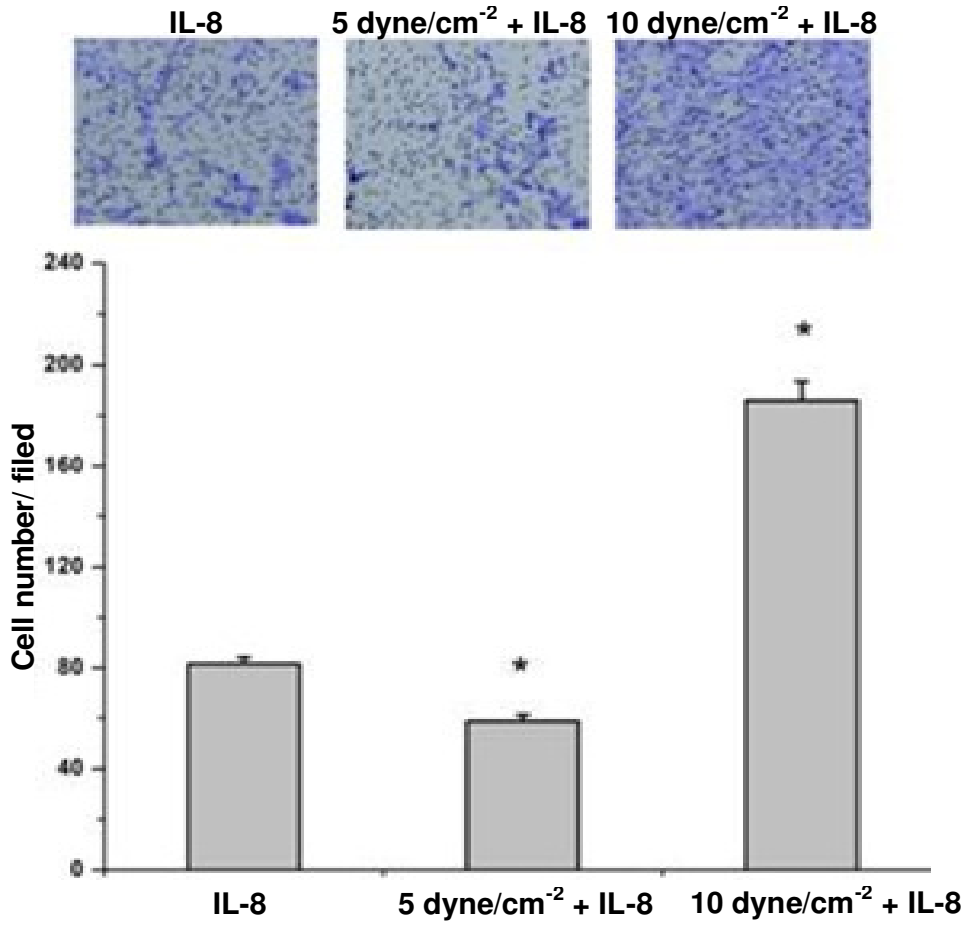

Figure 5. The influence of shear stress on the cell migration (transwell, n = 5). EPCs were grown to 80 to 90% confluence, and stress load was allowed to proceed as earlier described.* $p<0.05$, the cell migration number of EPCs under shear stress of 10 or 5 dyne/cm^2 stimulated by IL-8 VS that of EPCs stimulated by IL-8 alone.

EPCs stimulated by IL-8 about 6 h) was increased than that of IL-8 group (EPCs stimulated by IL-8 alone). Hence, the 10 dyne/cm^2 shear stress promote the EPCs wound healing.

DISCUSSION

Angiogenesis does not only rely on mature partial endothelial cell migration and proliferation, but also on bone marrow EPC chemotaxis to the local (Chao and Hirschi 2010). EPCs are usually moved to the area needing restoration due to vascular injury and the pathological change, by cell differentiation or in the form of paracrine secreting relevant factors to promote angiogenesis. But more and more evidence showed that the EPC recruitment and function execution are actually related to micro-environment of neovascularization, in which fluid shear stress is a key factor (Stolberg and McCloskey, 2009). Angiogenesis is a complex event which requires endothelial cell sprouting, lumen formation and tubulogenesis, and is regulated by the coordinated action of different transcription factors. Their interaction leads to endothelial cell differentiation and acquisition of arterial, venous and lymphatic properties. So, the proliferation, differentiation, cell migration and tube formation of EPCs might relate to both of the physiology and pathology angiogenesis.

Our previous work showed that after endothelial cells were stimulated by 1 to 2 h shear stress, both the IL-8 gene and protein expression were increased. So we selected 2 h as shear stress stimulated time in this research. In this study, the shear stress and IL-8 can inhibit the EPCs grow. Moreover, in previous studies, EPCs are typically identified and enumerated via flow cytometric identification of cells, which co-express the cell-surface antigens CD34, AC133, and VEGFR. However, it was reported that CD34+AC133+VEGFR-2 + cells are HPCs that do not yield EC progeny. So, in our study, the adherent cells, which exhibited an endothelial phenotype were also defined by the acetylated LDL (acLDL) and ulex-lectin uptake? The cells co-express

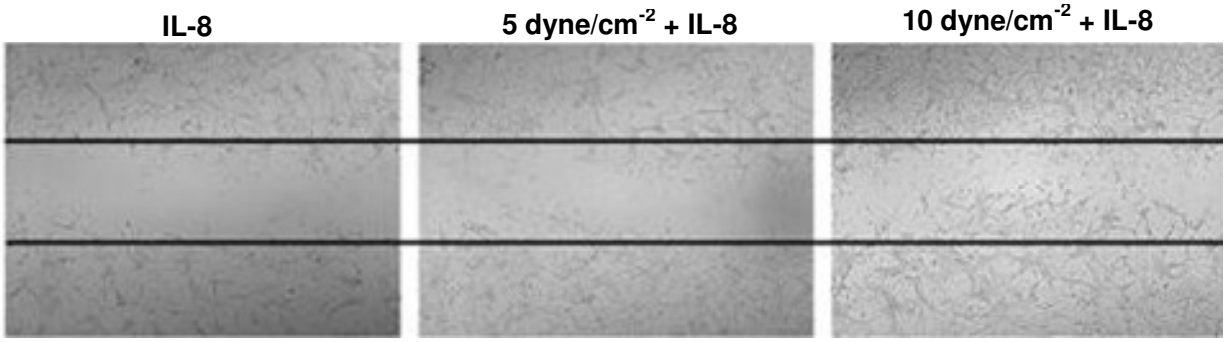

Figure 6. The influence of shear stress and IL-8 on EPCs wound healing. EPCs were grown to 80 to 90% confluence, and stress load was allowed to proceed as earlier described. Then cells were grown in Petri dish and incubated in medium containing 1% FBS overnight. Cell monolayers were then scratched and cultured in medium containing 5% FBS to facilitate cell migration.

CD34, AC133, VEGFR and uptake experiment (+) have been referred to as our target cells-EPCs.Shear stress induces a decrease in the progenitor lineage mark (CD133) expression and upregulated the endothelial lineage marks (VE-cadherin, VEGFR-2, vWF) expression. This result indicated that shear stress stimulation may promote the progenitor cells to differentiate to mature endothelial cells. Ye et al.'s (2008) work has shown that shear stress can promote EPC to mature endothelial cells, which is similar to our results, although, the shear stress we used (5 and 10 dyne/cm^2) was different. And in our study, IL-8 had no effect on the differentiation of EPC cells. In our research, shear stress of 10 dyne/cm^2 can induce EPCs elongation and change to spindle shape, but under shear stress of 5 dyne/cm^2, cell morphology became more round. The study by Yamamoto et al. (2003) also demonstrated EPCs to be sensitive to shear stress, and that EPCs change morphologically and functionally in response to shear stress. EPCs elongated and oriented their long axis in the direction of flow when exposed to shear stress (0.1 to 2.5 dyne/cm^2) for 24 h. Shear stress can stimulate endothelial progenitor cells to mature endothelial cells, which meant that shear stress may be able to drive progenitor cells to postnatal vasculogenesis during wound healing, limb ischemia, or postmyocardial infarction. Furthermore, it was observed that the 5 dyne/cm^2 shear stress decreased the EPCs tube formation and cell migration on present of IL-8, but the shear stress of 10 dyne/cm^2 increased it. So, 10 dyne/cm^2 shear stress can accelerate the EPCs angiogenesis ability, while shear stress of 5 dyne/cm^2 might suppress this. Therefore, the 10 dyne/cm^2 shear stress and IL-8 combined effect might have protective effect on endothelial cell function. Unlike the shear stress that promoted the progenitor cells differentiation into mature endothelial cells, IL-8 had no effect on it. Both of IL-8 and shear stress have promotion effect on the cells apoptotic.

VE-Cadherin is present at endothelial adherens junctions and plays an important role in intercellular adhesion and cell differentiation, growth and migration (Dejana et al., 1999)). VE-cadherin also has been implicated in vasculogenesis and angiogenesis (Bach et al., 1998). Base on our results, we concluded that shear stress might promote the tube formation and wound healing of EPCs by upregulating the VE-cadherin expression. *In vivo*, the blood flow fast at the large vessels which result in high shear stress, while in inflexion of blood vessel, vortex will be formed, thus forming the low shear stress. The shear stress we used in this study was decided by our previous research and other team's study (Olesen et al., 1988; Davies et al., 1986; Ku et al., 1985). Research shows that during the atherosclerosis generation, EC dysfunction is the most important initial factors. Shear stress and IL-8 have combined effects on EC to make the occurrence of atherosclerosis further develop. In addition, high shear stress can promote EPCs reflection to chemoattractive, while low shear stress inhibited this function. Based on these conclusions, we believe that if the vessel is stimulated by high shear stress, this may help to restore barrier function of endothelial cells that contain fundamentally the occurrence of atherosclerosis development.

Conclusion

In this study, we confirmed that both the shear stress and IL-8 reduced the EPCs proliferation. Shear stress stimulation might improve the progenitor cells differentiate to mature endothelial cells by upregulating the endothelial lineage marks. Furthermore, shear stress of 10 dyne/cm^2 and IL-8 enhanced the tube formation, cell migration and wound healing of EPCs, while shear stress of 5 dyne/cm^2 and IL-8 reduced the tube formation, cell migration and wound healing. Hence, our data demonstrated that EPCs are sensitive to shear stress. High shear stress (10 dyne/cm^2) combined with IL-8 can induce the differentiation of EPCs to more matured endothelial cells, which is demonstrated by enhanced proliferation, migration and

angiogenesis. The molecular mechanism will be helpful for finding new targets for treatment of atherosclerotic disease.

Abbreviations

EPCs, Endothelial progenitor cells; **IL-8,** interleukin-8.

ACKNOWLEDGEMENT

This study was supported by the grant from National Natural Science Foundation of China [30600129].

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