

Dear Editor,

We would like to thank you and the reviewers for all of the time and efforts devoted to review our manuscript. The comments are very insightful and greatly appreciated. We have revised our manuscript accordingly, and changes were all highlighted within the manuscript by using red colored text.

Considering that both first authors have contributed greatly to this paper, we hope that the co-first author will be approved. In addition, we have already submitted the manuscript to one of the recommend biomedical editing companies, MedE Editing Group, and got the Non-Native Speakers of English Editing Certificate which will serve to verify that the language of the manuscript has reached grade A.

The point-by-point responses to the comments were given as follows. In addition, the manuscript has been thoroughly checked to minimize typographical, grammatical, and bibliographical errors.

Thanks again for your attention.

Best wishes,
Sincerely yours,
Xiaoming Li, PhD

Comments from editor and reviewers:

Reviewer 1

The topic is interesting and is well described in the manuscript.

Response: Thank you very much.

Reviewer 2

Shear stress is one of the important physical factors to affect stem cell fate and endothelial cells are generally exposed to it as blood flow. Therefore, understanding the effect of shear stress on the endothelial generation. The review article, entitled “ Effects of shear stress on the differentiation of stem cells into endothelial cells”, introduces how shear stress stimulates the signaling and promotes endothelial differentiation with many researches and is helpful to understand them. The reviewer has peer-reviewed according to the criteria checklist and put the comments to modify below.

- The unit “dyne” does not belong to SI unit. The reviewer recommend conversion to the unit “N”. (Checklist no. 6 Units, “Does the manuscript meet the requirements of use of SI units?”).

Response: Thank you for your careful review and valuable suggestions. We replaced the unit “dyne” with the unit “N”. Thanks again.

- Illustration of Figure 1 is unclear. The reviewer could not understand what types of cells were illustrated and where the cells are. In addition, where the shear stress comes from? (different from blood stream?) . Finally, the legend of figure 1 mentioned “signaling pathways in early endothelial differentiation” but there is no illustration about differentiation (what does paracrine factors mean?). Thus, the reviewer recommends modification the illustration. (Checklist no.8 Illustrations and tables, “Are the figures, diagrams and tables sufficient, good quality and appropriately illustrative of the paper contents?”)

Response: Thank you very much for your patience and the fair comments, which are very important for the improvements of the manuscript. In early endothelial differentiation, early EPCs sense the shear stress and upregulate expression of various paracrine factors, which promote EC differentiation of late EPCs. Both cells are in the bloodstream, and they have been labeled in bold in Figure 1. We replaced blood stream in the Figure 1 to clarify shear stress comes from blood stream. Additionally, to illustrate the process of differentiation, we added 2 typical paracrine factors that promoted endothelial differentiation of adjacent cells. Thanks again.

- To promote the research on the effect of shear stress, the reviewer suggests adding the section for the technologies to apply shear stress to the cells, such as micro fluidic system and perfusion culture system.

Response: As the reviewer's good instruction, we have added the technologies to apply shear stress to the cells. On page 5, line 11, we had an introduction of the parallel plate system. On page 6, line 2, we briefly described the structure and principle of the pulsatile bioreactor. We added the description of the cone-plate apparatus on page 12, line 2, and the multiplex microfluidic array on page 14, line 21. Thanks again.

Reviewer 3

This review provides a detailed mechanistic overview of the influence of shear stress (physical stimulation) on the differentiation of stem cells into endothelial cells. The authors have presented all the topics in a systematic manner citing current studies. It is a timely review on a topic of broad interest. This knowledge will be useful to the scientific community for stem cells for pharmacological applications, cardiovascular implants, and so forth. However, there are general, major, and minor comments which the authors have to address before its acceptance. I recommend the publication of this review after the authors address the general, major, and minor comments listed below and in the attached file.

GENERAL comments:

1. Endothelial cells have been abbreviated as “ECs”. But this is not followed throughout the manuscript.

Response: Thank you for your careful review. We have corrected this. Thanks.

2. Abbreviations used are not cited properly throughout the manuscript. Sometimes even after abbreviating after the first appearance, they are written in full (eg. Endothelial cells, embryonic stem cells, vascular endothelial growth factor, etc.) and vice versa. It is advised not to abbreviate if the word(s) appears less than three times in the manuscript.

Response: Thank you for your careful review. We have modified it.

3. “in vitro” and “in vivo” are either in italics or in non-italics form. Please edit as per the journal requirement.

Response: Thank you for your careful remind. We changed all the “in vitro” and “in vivo” into italics and marked them in red.

4. Hours is written either in “hours” or “h” and Days either in “days” and “d”. Please maintain uniformity as per the journal format.

Response: Thanks very much for your valuable remind. We replaced “hours” to “h” and “days” to “d”, and marked them in red.

5. Some sentences highlighted in the attached file are too long and unclear, so please rephrase them (such sentences are present mostly in the signaling pathways subsections).

Response: Thanks very much for your very important comment. These sentences have been rephrased. Thanks again.

MAJOR COMMENTS:

1. The signaling pathways section requires more attention in rephrasing the sentences so that it is easy for the reader to comprehend.

Response: Thank you very much for your valuable comments that are very important for the improvements of the manuscript. We have divided the complex lines of the signaling pathways section into concise and clear sentences. Thanks again.

Specific MINOR comments:

Please see the attached file for all the specific comments.

Page 3

“Patient derived” should be “Patient-derived”

Page 4

Delete “in vitro”

Delete “the”

Page 5

“believed” not a scientific word

“the significantly increased” should be “a significant increase in the”

Page 6

“muscles” should be “muscle”

“larger or smaller” should be “higher or lower”

“significant expression” should be “significant increase in”

“equal” should be “equal to”

“decreases” should be “decrease”

Page 7

“120/80mmHg” should be “120/80 mmHg”

Delete “demonstrated cyclic pressure”

“What's more” Please change to a scientific word. Better to avoid such words in scientific writing.

eg. Furthermore

“5 %” should be “5%”

“markers fetal” should be “markers such as fetal”

“chemical induction factors” should be “chemical inducers”

“CD34 positive” positive can be written in + as a superscript

“human exfoliated deciduous teeth (SHEDs)” should be “stem cells from human exfoliated deciduous teeth (SHED)”

“endothelial growth medium (EGM) endothelial maturation under” I think the authors missed the word "for

Page 8

Delete “enable the”

Delete “mesenchymal”

“ADSCs” should be “ASCs”

Page 10

Delete “to”

Page 11

“sorted out” should be “listed”

“markers smooth” should be “markers such as smooth”

Page 13

“ESC- derived” should be “ESC-derived”

“added” incorrect word. Please use an appropriate word.

“disputes” should be “issues”

Page 14

“positive” can be represented as "+" symbol in superscript

Page 15

“Pathways” should be “pathways”

Page 16

“differentiations” should be “differentiation”

“were lack of” should be “lacked”

“activate” should be “activated”

Add "signals"

Page 17

“wildly” Not a correct scientific word. Kindly change.

“systematized.” Not a correct scientific word. Kindly change.

Page 18

“Human” should be “human”

“promotes” should be “promoted”

“Murine” should be “murine”

Delete “in”

Add "with"

Delete “,”

Add " induced"

Delete “was significantly upregulated”

“and15” add space

Page 20

“to increase the proliferation” should be “to the increase in proliferation”

“enhance” should be “enhanced”

“knockout” should be “knockdown”

“hypoxia condition” should be “hypoxic conditions”

Page 21

“enlarged” not an appropriate word

Page 22

“activation of and” some words are missing after of. Please add and correct it.

“homeobox transcription factors” should be “a homeobox transcription factor”

“the HDAC inhibition Trichostatin A” should be “the HDAC inhibitor Trichostatin A”

Page 23

“what's more” Please change. It is not used in scientific manuscript.

Page 25

“was significant” Please rephrase. Not correct words.

Page 26

“stretch, compressive” should be “and”

“don't” Please avoid words with ' throughout the manuscript. don't can be written as do not.

Page 44

“Tie 2” delete space

“CXCR 4” delete space

Page 46

“tubu” Please correct throughout the table

Response: Thanks so much for your patience and conscientiousness. We have corrected the listed examples in the paper. In addition, the manuscript has been thoroughly checked to minimize typographical, grammatical, and bibliographical errors