

Answering Reviewers Letter (MS No: 46496)

Response for the comments of the editor and referees

Dear Editor and Peer review experts

We are grateful this work ignited your interest and are very grateful for the valuable insights provided by the reviewers. The authors are honored to being able to answer the inquiries raised by formatting and content of our manuscript titled “Induction of differentiation of human stem cells ex vivo: toward large-scale platelet production”-invited manuscript. The manuscript has been carefully revised according to the comments from Reviewer 1 and Reviewer 2. A detailed list of our responses to reviewers’ comments is presented below point by point.

Answer to the Editor

First of all, thank you for submitting your manuscript to the World Journal of Stem Cells. Secondly, please be sure to follow all the steps below to modify the proposed manuscript.

Answer: Thank you for kindly reminding me of the requirements. We have checked all the steps before to submit our revised manuscript.

1. Please provide and upload the approved grant application form(s).

Answer: OK, it has provided as advice.

2. We found that the content of the figures cannot be edited by our staff. Authors have to provide the figures as separate electronic files. Please upload the figures in the following vector or bitmap formats so that we will be able to edit them:

Answer: OK, we have provided the figures as separate electronic files with EPS form.

Answer to reviewers

Reviewer #1

1. The manuscript entitled “Induction of differentiation of human stem cells ex vivo: toward large-scale platelet production” by Lei et al. addresses very important field of regeneration medicine and stem cell technologies specifically large scale production of cellular products. To this end blood components are the most demanded cell types also having advantage of a huge experience in cell transplantation. Platelet transfusion remains the most effective way to treat patients suffering from thrombocytopenia and/or platelet dysfunction caused by different reasons. A better understanding of the cellular and molecular mechanisms of megakaryopoiesis and platelet differentiation may provide optimized strategies for their scalable in vitro production. The paper is well structured and written and contains a lot of modern helpful information both for general and qualified readers. Illustrations are rather comprehensive. There are few minor recommendations.

Answer: We are grateful for the positive, encouraging and loving comments by the reviewer.

2. Megakaryocytic maturation is accompanied by polyploidization. Ploidy is the

number of complete sets of chromosomes in a cell. It is the number of nuclei (16~128 N) or amount of DNA as it were written by the Authors on p. 6 and Fig.1 legend.

Answer: Yes, we have changed “the number of nuclei” to “the number of DNA content” in p.6 and Fig.1 legend. Thanks a lot for the loving guidance. The change has also been highlighted with yellow color in the text.

3. *In vivo*, *in vitro*, *ex vivo* should be written in Italics throughout the text and figure legends.

Answer: Ok, we have rewritten all the “*in vitro*”, *in vitro*” and *ex vivo* with Italics in the text and figure legends. Thanks a lot for the helpful guidance. The change has also been highlighted with yellow color in the text.

Reviewer #2

1. In this review the authors describe the brief history and methods for production of platelet *in vitro*. In particular they focus on iPSCs as a promising resource for future clinical application. In general the review is comprehensive and the problems inherent to use of iPSCs and to differentiation into platelet are well discussed.

Answer: Thanks a lot for the valuable insights and the positive comments of the reviewer for our submitted manuscript. Yes, on behalf of all authors, I would like extend our heart-felt appreciation for the valued comments, and will do the revision accordingly.

2. Minor concerns:

1) Some abbreviations are not defined (for example, TPO, THPO page 5, Ab in page 6, etc).

Answer: Thank you for your suggestion. We have added the full name of some abbreviations in your mentioned. The change has also been highlighted with yellow color in the text.

2) Some texts are difficult to understand. For example, "Usually, a proportion of cultured platelets is often observed in a state of preactivation, with the absence of agonists such as ADP or thrombin," page 13, "We recently found that the rotary cell culture system (RCCS) plays a potential role in megakaryopoiesis and significantly improves the efficiency of platelet generation (Figure 2D), page 15, etc).

Answer: Thanks a lot for the thoughtful and professional comment about this. To avoid the possible confusion to readers, we have rewritten the texts as advice. The change has also been highlighted with yellow color in the text.

3) It would be better to discuss more the limitation and future perspective of the present devices to produce platelet in large scale and to guarantee the quality of platelet for transfusion.

Answer: Thanks a lot for the thoughtful and professional suggestion about this. To make sure the manuscript can present more readability, we have added some discussion about the limitation and future perspective of the present devices to produce platelet, which highlighted with yellow color.