

April 16, 2019

Dear, Jin-Lei Wang

Science Editor, World Journal of Stem Cells

**[Re: Revision of Manuscript ID 45932 Title: Tonsil-derived stem cells as a new source of adult stem cells]**

First of all, we would like to thank you for giving us the opportunity to revise our manuscript. We were pleased to hear your decision dated on March 26, 2019. We would also like to thank the reviewers for their precious time and constructive reviews. We appreciate the vigilant review and valuable recommendations. We believe that the manuscript is substantially improved after making the recommended edits. As suggested, we have now revised our manuscript according to reviewers' comments and suggestions, which we would like to re-submit to World Journal of Stem Cells.

To accommodate the reviewers' comments, we provide point-by-point response to each of the comments, as follows. Revisions in the manuscript marked using highlighted yellow color.

We hope that our responses and revisions are satisfactory to you and the reviewer.

Thank you for your kind consideration.

Sincerely yours,

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## **Reviewer(s)' Comments and responses:**

**[Reviewer ID: 03370303]**

### **Reviewer's general comment**

This review is excellent, showing detailed information regarding how to prepare T-SCs and how to induce their differentiation into various cell lineages. It also gives us useful information regarding the optimal scaffold condition for the differentiation into each cell lineage. This review, which covers broad areas from cell culture technique, tissue engineering and pre-clinical/clinical studies, will contribute to the development and expansion of T-SC-based regenerative medicine. There are a few minor concerns. Before publication in World Journal of Stem Cells, they should be properly addressed.

**Recommend 1:** In page 6, line 12, the words “70-uM cell strainer” should be corrected as “70-μm cell strainer”.

**Response 1:** Thank you for your valuable comment. The typo was corrected.

**Recommend 2:** In page 6, lines 14-15, the words “at a density of 108 cells” should be corrected as “at the density of  $10^8$  cells”.

**Response 2:** Thank you for your valuable comment. The typo was corrected.

**Recommend 3:** In page 15, lines 1-2, please show the non-abbreviated form for the word “BLiM-MSCs”. Is it the abbreviation for “Liver from BM-MSCs”?

**Response 3:** Thank you for your indication. BLiM was accidentally overwritten, we corrected it as BM-MSCs.

**Recommend 4:** In page 18, lines 20-21, please show the reference for the description “T-SCs can also differentiate into various tissue types, such as embryonic stem cells.”. I am afraid that T-SCs could not

be differentiate into embryonic stem cells. If there are no appropriate papers to be referred, the sentence should be replaced by, for example, “T-SCs can also differentiate into cells from all three germ layers.”.

**Response 4:** We appreciated your kind comment. The sentence “T-SCs can also differentiate into various tissue types, such as embryonic stem cells.” means that ‘T-SCs has differential potency like ESCs’. We changed the sentence as your recommendation.

## **[Reviewer ID: 01851506]**

### **Reviewer's general comment**

In this manuscript the authors discuss the potential of the tonsil-derived stem cells (T-SCs) as an alternative source for mesenchymal stem cells (MSCs) in clinical application. As a proof of T-SCs being useful, they cited the literatures demonstrating that T-SCs indeed possess a potential to differentiate into mesoderm such as osteogenic, chondrogenic, adiogenic, and myogenic lineage. Furthermore, T-SCs have a potential to differentiate into ectoderm such as neurogenic and gliogenic, and also into endoderm such as hepatocyte, pancreatic, and parathyroid lineages. The review is intriguing and well written. However, the reviewer has several concerns.

**Recommend 1:** While the authors described the method to isolate the T-SCs in detail in "Isolating and identifying tonsil-derived mesenchymal stem cells" section, it should be kept in mind that this is a review but not a regular article. Therefore, this part should be revised appropriately.

**Response 1:** Thank you for your valuable comment. As you suggested, we revised the methodological details more briefly.

**Recommend 2:** It is helpful for a wide range of readers to discuss the potential contaminating cells in T-SCs defined by the cell surface markers CD73, CD90, CD105, CD29, CD44, CD166, CD58, and CD49e (also negative for CD11b, CD21, CD23, CD35, and CD54). Readers not expert in the field would understand the advantage and limitation of above surface molecules as stem cell markers.

**Response 2:** Thank you for your valuable comment. As you suggested, we added more explanation for the CD markers that used for stem cell markers. The changes in the text were highlighted with yellow color.

**Recommend3:** It is not appropriate to mention that The reason why Tonsil derived stem cells has more differentiation potency than other adult mesenchymal stem cells (Title for Figure 1), as Tonsil tissues consist of two different origin tissues; epithelial cells from endoderm origin and lymphoid tissues from mesoderm origin. The mechanisms underlying the peculiar differentiation potential of T-SCs are much

more complicated than the fact that Tonsil tissues consist of two different origin tissues. It is helpful to show in the Figure 1 where CD73, CD90, CD105, CD29, CD44, CD166, CD58, and CD49e-positive T-SCs reside. Do T-SCs stem from the endoderm and/or the mesodermal (lymphoid) part of the tonsil?

**Response 3:** Thank you for your valuable comment. Actually, research on the TSc's differentiation-potency and mechanism is not enough so far. We could not find and review any previous studies on the expression of CD markers on T-SCs according to the original sites. In order to prevent readers' misunderstanding, we modified the title of figure 1 as follows; "*The possible reason enables diversity in T-SCs differentiation- constitutive features of tonsil*"

**[Reviewer ID: 03478635]**

**Recommend 1:** This review article describes about the new role of tonsil-derived stem cells as the source of adult stem cells. The figures are illustrated very well, although the origin of tonsil in figure 2 is a little bit consuming.

**Response 1:** Thank you for your valuable comment. This article is review. Therefore non-biology or non-medical readers would read this article. We would like present an easy visual information about the potency of T-SCs. We appreciated your review again.

## [Reviewer ID: 02446101]

### Reviewer's general comment

In this review, the authors summarize the details of T-SC isolation and identification and provide an overview of their application in cell therapy and regenerative medicine. The content of this paper is systematic, which has obvious reference value for increasing the understanding of tonsil stem cells. I'm sure that the manuscript really provides some new ideas to the readers. However, there're still some issues which should be addressed.

**Recommend 1:** The limits and shortcomings of T-SC should be added.

**Response 1:** Thank you for your valuable comment. We modified the discussion and emphasized the shortcomings of T-SCs in separated section 10 with adding possibility of T-SCs variation in differentiation capacity. The changes in the text were highlighted with yellow color.

**Recommend 2.** The first paragraph of 6.3 is too long and meaningless, which should be reduced.

**Response 2:** We appreciated your kind recommendation. The paragraph was reduced.

*“Hypoparathyroidism is a rare endocrine disorder, resulting in low serum calcium and increased serum phosphorus<sup>[45]</sup>. Hypoparathyroidism is the only hormonal insufficiency state that does not have a hormone-replacement-therapy approved. Current managements include supplementation with oral calcium and active vitamin D, which cause various life-long adverse effects.”*

**Recommend 3.** A table of conditions for inducing differentiation of tonsil stem cells should be added to facilitate readers' understanding.

**Response 3:** We mentioned the detail differentiation conditions on Figure 2. We appreciated your review again.

**Recommend 4.** The molecular mechanism of tonsil stem cell differentiation should be discussed and added.

**Response 4:** Thank you for your valuable comment. The molecular mechanism of tonsil stem cell differentiation is also of our great concern. We actually considering to handle it within the next research contents with more evidences. Instead, we shortly mentioned that the molecular mechanism in T-SCs differentiation should be identified in the section 10.