

Dear Editor,

We are very pleased to learn from your letter about revision for my manuscript (Manuscript NO.: 55000, Title: Human hair follicle-derived mesenchymal stem cells: isolation, expansion, and differentiation). Thank you for your attention and the reviewer for his helpful comments and advice. We have revised the manuscript according to your suggestions and make response to the comments from the reviewer points by point.

Response to Reviewers #1:

1. A small verb repetition in the phrase “in a pioneering study... demonstrated described “.

Re: Thank you for your kind suggestions. We have corrected the error at line 107.

2. The phrase “subsequent studies is too long and leaves open the statement about MSCs (“represent an emerging MSC”?) Probably source or similar needs to be added.

Re: We have added the source at lines 114-121.

“In 2006, the International Society for Cellular Therapy (ISCT) issued the minimal criteria for characterizing human MSCs. Specifically, cultured MSCs should be adherent fibroblast-like cells, express the surface markers CD105, CD73 and CD90, and lack the expression of CD45, CD34, CD14 or CD11b, CD79 α or CD19 and human leukocyte antigen-DR isotype (HLA-DR). Furthermore, MSCs have osteogenic, adipogenic and chondrogenic differentiation potential in vitro. Therefore, dermal papilla or sheath cells from rat follicles may be a type of MSCs.”

3. Regarding isolation methods from the technical description offered is not extremely clear if a skin biopsy is needed. If so, please mention as such. Collagenase digestion of the skin tissue biopsy, I suppose.

Re: We have added the following description at lines 139-141.

“The human scalp skin is usually obtained by skin biopsy from the scalp of a donor under sterile conditions.”

4. Maybe it will be good to briefly define what means intact/complete hair follicle.

Re: We have added the definition of a complete hair follicle at line 137-139.

“An intact hair follicle usually includes inner root sheath, outer root sheath and connective tissue sheath (dermal sheath) as shown in Figure 1.”

5. Do the second method (basically tissue explant) generate pure populations? From the description it sounds possible that some wells are populated with both keratinocytes and dermal papilla fibroblast?

Re: The primary cells we obtained may not be pure populations, but the purity will be higher with the increase of passage times. We have added the following description at lines 165-172.

“It should be noted that the cells migrated from dermal papilla may contain a small number of Neural Crest Stem Cell-Like Cells. They can form neurospheres under serum-free culture conditions containing N-2, B-27, bFGF, and EGF^[1]. Meiyong Li et al. found that the sphere-forming cells were $1.14 \pm 0.03\%$ of dermal papilla cells^[2].

However, these Neural Crest Stem Cell-Like Cells need to be supplemented with ITS supplement and EGF when cultured in vitro^[3]. Therefore, they will gradually disappear with the increase of passage times under the hHF-MSCs culture conditions.”

6. Relatively to in vitro expansion, are there data about number of passages before senescence occurs in expanded cells? What is the average number of cells that can be derived from one hair follicle in simple 2D without signs of senescence? This would be an useful information if available.

Re: “Vivek K. Bajpai et al. found that hHF-MSCs could be maintained in culture for 11-12 passages (approximately 36 population doublings) before they started to show signs of cellular senescence. In addition to the 8-10 population doublings that occurred during the initial isolation and expansion stage, they may also undergo a total of 44-46 population doublings. It was estimated that a hair follicle could yield approximately 10^{15} hHF-MSCs before senescence occurred to expanded cells.” We have added these descriptions at lines 210-217.

7. Please provide reference to the statement SMC plays a critical role in cell therapy [..]. It is to be kept in mind that very few actual clinical therapy has been clinically approved for such purposes although there are indeed, clinical trials going on. Reference will help understanding what authors are referring to. If it is about preclinical research, state so, if it is about clinical studies please provide source.

Re: Thank you for your kind suggestions. Our statement here is not very appropriate. Therefore, we revised this sentence and added references at lines 271-277.

“SMCs play a critical role in the occurrence and development of prevalent cardiovascular and respiratory diseases, such as atherosclerosis^[4] and asthma^[5], because of their contractile dysfunction. Emerging tissue engineering techniques offer the possibility of reconstructing functional vessel walls by smooth muscle cells^[6]. L. Andrique used smooth muscle cells and endothelial cells to produce functional blood vessels with the correct configuration of lumen, which could also react to vasoconstrictor agents^[7].”

8. In the phrase “in addition Gao et al perhaps the decellularized umbilical arteries were filled with cells and not the other way round.

Re: Thank you for your kind suggestions. We have corrected the error at line 291.

“Gao et al. constructed tissue-engineered blood vessels by filled acellular umbilical arteries with hHF-MSCs.”

9. In the conclusion chapter the authors describe the potential immunepriviledge of hair follicle MSCs. This aspect has not been discussed within the review. It is good to either introduce at least a subchapter or give up this statement in the conclusion.

Re: We have given up this statement in the conclusion (line 358).

10. Cell therapy is seen as a part of regenerative medicine. In my opinion it is not correct to formulate RM AND cell therapy because the former included the later. If one wants to detail can formulate regenerative medicine (cell therapy and tissue engineering) for

example or similar.

Re: Thank you for your kind suggestions. We have change “cell therapy and regenerative medicine” to “cell therapy and tissue engineering” at line 207 and line 375.

Editorial Office’s comments:

Science Editor: (1) Scientific quality: The manuscript describes a review of the human hair follicle-derived mesenchymal stem cells. The topic is within the scope of the WJSC.

(1) Classification: Grade B; (2) Summary of the Peer-Review Report: This is a well-organized well composed manuscript dedicated to review methods of procurement, characterization and current roles of hair follicle derived MSCs. The questions raised by the reviewers should be answered; and (3) Format: There are 2 figures. A total of 48 references are cited, including 26 references published in the last 3 years. There are 5 self-citations (From Bo Wang and Jin-Yu Liu). 2 Language evaluation: Classification: Grade A. A language editing certificate issued by Editage was provided. 3 Academic norms and rules: The authors provided the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by the China National Natural Science Foundation, the Joint Construction Project between Jilin Province and provincial colleges, etc. The topic has not previously been published in the WJSC. 5 Issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval

document(s); and (2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor. 6 Re-Review: Required. 7 Recommendation: Conditional acceptance.

Response to Editorial Office: Thank you for your kind suggestions. We have provided the approved grant application forms of funds. We have provided original pictures.

REFERENCES

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7. Andrique L, Recher G, Alessandri K, Pujol N, Feyeux M, Bon P, et al. A model of guided cell self-organization for rapid and spontaneous formation of functional vessels. *Sci Adv*. 2019;5(6):eaau6562-eaau. [PMID: 31206014 DOI: 10.1126/sciadv.aau6562]