July 8, 2019.

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Dear Dr. Dou, Dr. Wang, Dr. Li, and Dr. Ma:

We would like to thank you for reviewing our manuscript entitled, "Enhanced hepatic differentiation in the subpopulation of human amniotic stem cells under 3D multicellular microenvironment" (Manuscript No. 46865), submitted to the *World Journal of Stem Cells*. We also thank all the reviewers for their time and insightful comments, which helped improve the manuscript. We have revised the manuscript as per the reviewers' comments. We hope the revised manuscript is satisfactory and meets the expectations of the reviewers and editors. We have highlighted the modifications in the revised manuscript.

For editors' comments

As per your comments and suggestions, we revised the manuscript as follows:

1. We added the "Institutional review board statement", "Data sharing", "Copyright statement" and "Article highlights".
2. We expanded abbreviations in the figure title and explained all the abbreviations in each figure legends.
3. We made a separate PDF file with Supplementary Figures.
4. We moved "Acknowledgements" next to "Article highlights".
5. We removed "Institutional animal care and use committee statement" and "ARRIVE guidelines statement", since animal experiment is not included in this manuscript.
6. We placed the original figures in a PowerPoint file.

Reviewer 1; 2019-02-27

Title: Apt. Abstract: Gives a good preview of the intended study model. Introduction: Gives a satisfactory idea of the topic. Basic concepts are well explained. Materials and methods:

Elaborate description of the cell types and tests to evaluate. Very good description of the methodology. Results are well described, Discussion: Very good. Conclusion is satisfactory. Futuristic strategies well documented.

Response

We thank the reviewer for the comments on our manuscript. We are happy to note that the reviewer is satisfied with our manuscript.

Reviewer 2; 2019-05-01

The authors raised questions of possibility using amniotic epithelial cells (AECs) as a cell source for regenerative medicine. This manuscript well-described the characteristics of AECs. Some correction may enhance the novelty of this manuscript. 1. The term 'organoid' should be replaced as "spheroid". As a perspective of my knowledge, organoid means the small organ-like structure that mimics the physiology of specific organ and derived from a pluripotent stem cell. Although AECs considered as a kind of multipotent stem cells, not as a pluripotent stem cell. Therefore, the spheroid more suitable term for use. 2. In Figure 2, providing of larger magnification picture of A, B, C, D, E surely enhanced the understanding of readers. 3. In Figure 4 legend, the authors missed the explanation of D. It should be provided.

Response

We thank the reviewer for the careful review of our manuscript. Our point-by-point responses to the reviewer's comments are as below:

1. The reviewer raised an important point in the definition of "organoids". As the reviewer mentioned, organoids are generally defined as small organ-like structures that mimic the physiology of specific organs. However, the term "organoid" has been used more widely. For example, pluripotent stem cell derived organoid such as liver organoid (Publications from our group, PMID: 23823721 and PMID: 30120080), tissue stem cell derived organoid such as intestinal organoid (Publications from Matthias Lutolf Group, PMID: 27851739 and Hans Clevers Group, PMID: 24315439), or cancer organoid such as pancreatic cancer organoid (Publication from Hans Clevers & David Tuveson Group, PMID: 25557080). Interactions between different cell types, such as stem cells, progenitor cells and terminal cells, within a microenvironment are important for their maintenance. Such a self-organization of the cell types forms small organ-like structures that function similar to higher order tissues or organs. Therefore, we would like to state that organoids do not essentially originate from a Pluripotent Stem Cell (PSC). In case of iPSC derived organoids, the cells are differentiated into several types of progenitor cells that assemble to form complicated heterogeneous structures. Therefore, we would like to denote the amniotic stem cell derived product, in our manuscript, as organoid, since it has a small heterogeneous structure and a physiological function.
2. We agree with the reviewer's comment and thus added images with higher magnification for panels A-E in Figure 1.

3. We thank the reviewer for pointing out this. We have made the corrections and therefore the legend for figure 4 now reads as, “H&E staining is used in A, and PAS staining is used in C.” and “D: ICG tests on AEC sphere and organoid.”.

Reviewer 3; 2019-05-08

In this paper, the authors investigated the role of amniotic stem cells in the regeneration of hepatic cells. They used 3D co-culture and a combination of supportive somatic stem cells to simulate an in vivo microenvironment. The selected subpopulation of adherent amniotic stem cells self-organized ex vivo and generated functional organoids. In general, this study is interesting and helpful to better understand the effects of amniotic stem cells on liver regeneration. The results from this study may provide the guide for stem cell therapy. The manuscript was well designed and the results were presented correctly. Some minor grammatically issues need to be corrected or use standard words, such as 1 d, two-step, multiple-step.....

Response

We thank the reviewer for critically reviewing our manuscript. Since the reviewer pointed out on the word choice, we have checked the Baishideng Publishing Group’s (BPG) guidelines on ‘Common Usage of Quantities and Units’ (https://www.wjgnet.com/bpg/gerinfo/189). According to these guidelines, “d”, which denotes “day(s)”, can be used after Arabic numerals in figures, tables and numerical narration. Therefore, denoting “one day” as “1 d” is appropriate.

Moreover, since BPG’s guidelines do not specify an appropriate style for the use of joint words, such as “two-step” and “multiple-step”, we checked different styles, such as “2-step”, “two-step” or “two step”, to reach at the conventional style. “Two-step” and “multistep” styles have been used conventionally. Therefore, we used this style in the manuscript and changed “2-step” in SFigs. 3 or 4 to “two-step”.

We wish to thank you all for giving us an opportunity to improve our manuscript. We hope these changes would satisfy you to accept our manuscript for publication in the World Journal of Stem Cells.

Sincerely yours,

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