32946-ANSWERING REVIEWERS

Jing-Xin Kong
Scientific Editor
World Journal of Stem Cells

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Manuscript. No. 32946
Article Title: Modifying oxygen tension affects bone marrow stromal cell osteogenesis for regenerative medicine

Dear Dr Kong,

Thank you very much for your kind review and encouragement of our manuscript. We have substantially revised the previous manuscript according to your advice. We believe that the manuscript has been much improved.

We would like to re-submit our manuscript entitled “Modifying oxygen tension affects bone marrow stromal cell osteogenesis for regenerative medicine” to *World Journal of Stem Cells*. Appended are the replies to your comments and the reviewers’ comments, addressed in a point-by-point fashion.

We would be very happy if this manuscript could be accepted for publication.
Thank you very much for your kind consideration. We are looking forward to hearing from you.

Sincerely yours,

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Responses to the Reviewers' comments.

1. Sample sizes for both in vitro and in vivo studies were missing despite the fact that n=5 and n=6 listed in the M&M were believed to be copy number for each analysis.

Response: We thank the reviewer for this comment. We have clarified the sample sizes for each experiment. We have included a comment in the Methods to indicate that the *in vitro* work was performed twice using cells from two different mice. For each assay, 5 or 6 replicates were used for each of the four treatment groups, as indicated by (n = 5) or as (n = 6) in each subsection in the Methods. For the *in vivo* work, 5 discs per treatment group (HN or NN) were implanted at subcutaneous sites on the backs of recipient rats, and harvested for analysis.

2. In the Discussion, the statement in line 292-293 is not consistent with the one in line 296-297. Based on the data presented in line 296-297, the statement "Contrary to our results and previous reports" (line 292) may not be correct. Please advise.

Response: We thank the reviewer for pointing out this confusing comment. We have amended this sentence, as follows: "D'Ippolito and others[11] previously reported an inhibitory effect of hypoxia on osteogenic differentiation of human marrow-isolated adult multilineage-inducible cells."

3. Since low oxygen culture increased cell number, can the authors comment on the promoted osteogenic differentiation? Is it because of increased cell number or enhanced stem cell osteogenic capacity?

Response: We thank the reviewer for this comment. As the reviewer notes, it is unclear whether the increased cell number and/or enhanced MSCs osteogenic ability accounts for the endpoint of increased osteogenesis. We have included this as a limitation, with the following statement "it is unclear whether this increased osteogenesis is solely caused by the increase in cell number or whether hypoxic preconditioning also enhanced the differentiation and mineralization of these cells."

4. The discussion is too short and not comprehensive. Preconditioning strategies have been highlighted in a recent review paper (Biomaterials. 2017 Feb;117:10-23), which might assist the authors to have an in-depth discussion.

Response: We thank the reviewer for bringing this manuscript to our attention. We have

mentioned the findings of this paper in our Discussion. We have also increased the length of our Discussion, making reference to other relevant work in the field, and we have included some of the limitations of our study as another discussion point.