Stem Cells and Cell Therapy Laboratory
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REBUTTAL LETTER

Dear Editor and Reviewers,

In response to the revision required of our manuscript titled: *Suitability and limitations of mesenchymal stem cells to elucidate human bone illness*, for publication in World Journal of Stem Cells, I believe we have addressed the reviewers concerns and added further information besides including appropriate modifications in the manuscript as suggested by the reviewers.

I next reply to the reviewers' specific concerns point by point:

REVIEWER 1:

Authors are encouraged to go through minor comments and make changes suitably.

In this review article, Mitxitorena et al summarized *in vivo* and *in vitro* research findings, results of clinical trials and case studies primary focused on mesenchymal stem cells for the treatment of human bone disorders. Authors also talked about issues with the treatments of bone diseases and focuses on use of mesenchymal stem cell (MSC) therapy. Suitability of cell-based or secretome-based therapy with suitability and limitations are comprehensively covered. I would recommend this article for prospective publication.
Here are some minor corrections, which I believe, will enhance clarity of the manuscript to the readers.

Minor suggestions/corrections:

Line 34 and 96: Instead of "the progenitors of osteoblasts", "osteoblast progenitor cells" are more appropriate.
Line 162: Delete "." Period from the subheading
Line 172: The word "the" after mimic can be deleted
Line 175: the word "of bone" after osteonecrosis" can be deleted. *Osteo* itself means bone.
Line 184: Insert comma "',"' after "supply" and before "which"
Line 201: Change "ability of monitor osteonecrosis" to "ability to monitor osteonecrosis"
Line 203: Change "tissue" to "tissues"
Line 204-205: Revise the sentence.

Line 211: Delete “concerning” and add “of”. Add period “.” after “immune system” and revise sentence thereafter.

Line 228: I think “OVX” should be bracketed instead of “overiectomized”. Make rodent plural to “rodents”.

Line 238: Delete “the” from this sentence before “mechanisms”

Line 241: Spell “OI” to “Osteogenesis imperfacta” here and then follow OI thereafter. I did notice that you have introduced OI before in line 173.

Line 261: ….. “described in the literature” can be deleted without affecting sense of this sentence.

Line 270-271: It seems that these are the gene names and hence should be *italicized*. You italicized the gene name before this manuscript. Please check this in whole manuscript.

Line 275: Delete “a” after word “are”

Line 306: …..”a rare metabolic bone disorder resulting from loss of function mutation in the ALPL gene,” can be deleted. You already described this gene before.

Line 398: This sentence may be modified by deleting, ..”of the tissue with the” and adding “and” before “generation”

Line 446: Insert comma “,” after …..tissue repair”.

Line 484-486: Redundant sentence. Can be deleted.

Line 496-498: For clarity, please add time of subsequent hMSC infusion into bone marrow of HPP patients.

Line 499: Insert “the” in between the words “both and expression”. Insert comma “,” after …. “gene products”

Line 503: Remove bold font from “alveolar bone regeneration”

Line 504: Add comma after “In this clinical study” and delete “the”

Line 514-515: Revise this sentence. Seems like abrupt ending of it.

Table 1: Write a footnote for the signs “+” or “-“ for effectiveness column.

Thanks to the reviewer for the corrections. The manuscript has been edited and improved as suggested.

REVIEWER 2

The manuscript is worthy of publication, and it has been carefully written only from a minor linguistic and grammatical errors, which the researcher/s should review well before publication.

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Thanks to the reviewer for the comments. The manuscript has been edited and improved as suggested.

REVIEWER 3

The manuscript was generally well written because the author covered the various bone diseases, and basic study such as animal models and human experimental disease models. However, non-union after fractures can be intractable in some cases, and major clinical problem for orthopedic surgeons. So, I recommend the author to add a topic for non-union of bones, and its animal model and treatments using cell therapy.

We appreciate the reviewer's comments and have included a topic addressing the role of hMSCs in the pathogenesis of nonunions, and the possibility to treat this condition by autologous MSCs therapy, (lines 496-508).

REVIEWER 4

Summary: This manuscript reported a review on the suitability and limitations of mesenchymal stem cells to elucidate human bone illness. The main contents included the socio-economic impact of bone diseases, advantages and flaws of animal models of bone diseases, mesenchymal stem cells as experimental human (bone) disease models, mesenchymal stem cells as therapeutic tools for bone diseases, etc. The authors discussed the human MSCs (hMSCs) emerge as a suitable tool to study the ethiology of bone disorders at the cellular level, as well as to be use with cell therapy purposes for bone diseases. They focused on the most relevant findings using hMSCs as an in vitro cell model to unravel pathological bone mechanisms and the application and outcomes of hMSCs in cell therapy clinical trials for bone disease. The authors also listed certain limitations of animal models for the study of bone disorders highlight the suitability and benefits of hMSCs for the elucidation of these pathologies. They intended to explain the available strategies based on hMSCs for bone illness new treatments development and future directions in the field for a more accurate knowledge of the cause underlying these human pathologies.

This review may need to address some more issues before being considered for publication.

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1. Some contents are redundant (e.g., socio-economic impact of bone diseases section), and some are irrelevant (e.g., osteomyelitis model, lines 204-217). The authors may need to focus on the topic.

We appreciate the comments of the reviewer. As suggested, we have removed osteonecrosis, osteomyelitis and osteosarcoma models and now we just describe the relevant animal models for the pathologies in which the cells involved in bone remodeling are directly implicated. On the other hand, we think that the topic "the socio-economic impact of bone diseases" is totally needed to put into context the importance of bone diseases in actual societies and health systems.

2. MSCs are mixture population of cells in various differentiation stages which may influence the physiological functions. The characterization of MSCs is needed. Will it influence the suitability as research models for different diseases?

We agree with the reviewer and appreciate the suggested comment. We have included a paragraph pinpointing the high heterogeneity inherent to MSCs and the fact that a recent paper identified the human skeletal stem cell (hSSCs) by an extensive characterization of the expression of specific cell surface markers. These hSSCs show a high osteogenic potential and could be of great interest to be specifically amplified and used for cell therapy purposes (Lines 408-420).

3. The authors discussed some advances in using MSCs for therapeutic purposes, indicating the relevance of MSC in addressing bone disorders. However, MSCs models are cellular level models. More limitations and clinical relevance studies are needed for different diseases.

We thank the reviewer for the comments. 3D culture of MSCs which resembled more physiologically the bone structure, are a promising tool for the elucidation of the pathological molecular mechanisms underlying bone diseases. On the other hand, we

have also highlighted the importance of co-culture of cells (both in 2D and 3D cultures), for example osteoblasts and osteoclasts, in order to mimic the in vivo crosstalk that it is occurring in the bone remodeling process ("2D versus 3D culture of MSCs" section and 523-532 lines).

4. The animal models can provide more information of bone remodeling and interaction between osteoblasts and osteoclasts. How can MSCs overcome this limitation?

We agree with the reviewer regarding the limited capacity of MSCs in resembling bone remodeling, which is driven by a crosstalk between osteoblasts and osteoclasts. There is an approach to mimic this biological process in vitro: by the use of co-cultures of MSCs (or osteoblasts) and osteoclasts. We have now addressed this point in the section "2D versus 3D culture of MSCs".

5. The mechanisms of cytokines and extracellular matrices on MSCs are not mentioned. They are important domains.

We agree with the reviewer in the sense that cytokines and extracellular matrix signaling are very important issues in the biology of MSCs and of bone to a great extent. However, the aim of this work was to review the advantages and disadvantages of using MSCs (in vitro and in vivo) to study (and treat) human bone illness. Thus, we think that a section describing cytokines and extracellular matrix on MSCs would be out of scope. Nevertheless, we will consider the reviewer's suggestion for a future review.

6. To make it clearer for reading, the authors may need to tabulate the contents about MSCs models, listing its own advantages and limitations for each application.

We thank to the reviewer for this comment. We have tabulated the MSCs models related to bone diseases and included subheading in "mesenchymal stem cells as experimental human disease models" section. We think that this will facilitate the reading of the text. We have discussed the hits and flaws of using the different MSCs models in each section.

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We have included a "Conclusion" section with a global overview of the facts mentioned along the manuscript.

7. The authors may also need to provide some suggestion for the improvement of MSCs models.

We thank to the reviewer for this comment. We have included the paragraphs regarding the potential use of hSSCs, as well as a better explanation of the limitations of MSCs 2D cultures and how to overcome these obstacles with the co-cultures of different bone cells and the 3D cell cultures.

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Reply to the editor-in-chief's comments

Dear Editor;

I believe we have addressed the typographical/grammatical errors in our manuscript titled: *Suitability and limitations of mesenchymal stem cells to elucidate human bone illness*, therefore achieving the quality for publication in *World Journal of Stem Cells*.

Clara I. Rodríguez, Ph D.