Dear editors and reviewers:

Thank you for your letter and for the editors' and reviewers' comments concerning our manuscript entitled "**Mesenchymal stem cell-derived exosomes: Toward cell-free therapeutic strategies in regenerative medicine**" (**ID: 55168**). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Responds to the reviewer's comments:

Reviewer #1:

1. Response to comment: Authors have provided a scholarly written work capturing potential of mesenchymal stem cell-derived exosomes as a cell-free therapeutic strategy in accelerating regeneration. A review on their application for various degenerative diseases is very commendable and the highlight on the contribution of this manuscript. The article provides an essential reference for researchers in this field.

Response: We would like to express our great appreciation to your comments on our manuscript. Finally, thank you very much for your recognition for our manuscript. Thank you very much again.

Reviewer #2:

1. Response to comment: This is an interesting study about mesenchymal stem cell-derived exosomes. The MSC-derived exosomes may have great potential for regenerative medicine. The differences of exosomes among differentiation stages of MSCs may be added for the section 4 MSC-derived exosomes.

Response: We would like to express our great appreciation to your comments on our manuscript. Moreover, thank you very much for your recognition for our manuscript.

However, at present, regarding the application of MSC-derived exosomes in various diseases, no application of MSC-derived exosomes at different differentiation stages has been reported. Therefore, we think that it is unnecessary to add the differences of exosomes among differentiation stages of MSCs. Please criticize more. Thank you very much again.

Reviewer #3:

1. Response to comment: My main critique of the manuscript is that it is very heavily focused on in vitro and animal models and essentially fails to mention any human clinical trials which have either completed accrual or are in progress. A brief literature search revealed two recent (2019) papers reviewing exosomes for clinical use, which cover similar material as the current manuscript, but also review a number of current clinical trials utilizing exosomes (Yin K et al., Biomarker Research 2019; Mendt M et al., Bone Marrow Transplant 2019). The Yin paper in particular is similar in format to the current manuscript. A brief search of ClinicalTrials.gov shows 83 registered interventional therapeutic studies (not including basic science, biobanking and biomarker studies) using exosomes. The authors need to both incorporate the findings from published clinical trials, and summarize disease processes being investigated in active clinical trials for their manuscript to provide benefit to the scientific community over what has already been published.

Response: Thank you very much for this question. We have added the content about progress of MSC-Exos in clinical application in section 6. If you think it is not good enough, please criticize more. We will definitely modify and improve it. Thank you very much again.

2. Response to comment: Second, the writing of much of the manuscript related to therapeutic potential of exosomes is not terribly exciting for the reader. Much of the second half of the manuscript is “these authors showed this; those authors showed that”. I think that many of the in vitro and animal study data could be shortened, which would leave room for the authors to mention any clinical trials which pertain to the disease process.

Response: Thank you very much for this question. Indeed, the content of our previous article is only the research progress of MSC-Exos in various diseases *in vivo* and in *vitro*.

However, according to your comment, we have added the content about progress of MSC-Exos in clinical application in section 6. If you think it is not good enough, please criticize more. We will definitely modify and improve it. Thank you very much again.

3. Response to comment: Section 2.1: Reference 25 refers to the International Society for Cellular Therapy position paper. To my knowledge, neurogenic differentiation was not a required characteristic to define MSC in this paper.

Response: Thank you very much for this question. First say sorry. It is surely that neurogenic differentiation was not a required characteristic to define MSCs. Only relevant studies have shown that MSCs can differentiate into neuron-like cells. We have revised in section 2.1. Please criticize more. We will definitely modify and improve it. Thank you very much again.

4. Response to comment: Figure 1: Please define M1 and M2 cells (macrophages) at first use of the abbreviation.

Response: Thank you very much for this question. We have revised in Figure 1 and legend. Thank you very much again.

5. Response to comment: Section 2.2.2: First sentence should be revised to refer to "lodging" of MSC in non-specific tissues. What is meant by "homing to natural 'walls'"?

Response: Thank you very much for this question. We have revised the first sentence of section 2.2.2. Then, homing to natural 'walls' means that the microenvironment of MSCs growth and stable survival, which is the stem cell nest, also indicates the "homing" characteristics of stem cells. If you think it is not good enough, please criticize more. We will definitely modify and improve it. Thank you very much again.

6. Response to comment: Table 2: There are some contradicting statements in the table that need to be corrected, such as Membrane filtration: keeps exosomes intact is an advantage, but deformation is a disadvantage – which is correct? "High yield" is listed as both an advantage and a disadvantage for Precipitation.

Response: Thank you very much for this question. Firstly, in the Membrane filtration, keeps exosomes intact is actually an advantage, which means that the membrane structure of

exosomes is intact, and there is no loss of contents, etc. And, deformation is actually a disadvantage, which means that the structure of exosomes is deformed, the particle size has changed, etc. Moreover, in the precipitation, “High yield” is actually an advantage, we are very sorry to write “High yield” in the disadvantage. We have revised in Table 2. Please criticize more. We will definitely modify and improve it. Thank you very much again.

7. Response to comment: Section 4: First line, should maybe say “ease of isolation” instead of “non-invasive isolation”. Line 4, differentiation of MSCs MAY induce ossification – ossification is a rare event and certainly does not happen in all cases. Section 4.2.1: Can the authors give an example or a reference for exosome production on a large scale using specialized cell lines?

Response: Thank you very much for this question. We have revised the first line of section 4 in the manuscript. It is sure that ossification is a rare event and certainly does not happen in all cases. However, we are only here to suggest that MSCs have the possibility of ossification. Finally, we have added a reference for exosome production on a large scale using specialized cell lines. If you think it is very necessary to expand principles and methods, please criticize more. We will definitely modify and improve it. Thank you very much again.

8. Response to comment: Section 4.2.2: Do the authors intend to say that MSC are not stable at -80C, or that exosomes are more stable at -80C than MSC (which should be kept frozen in LN2 or vapor phase)?

Response: Thank you very much for this question. What we want to express is that exosomes stored at -80°C have better stability compared with MSCs. MSCs are kept in liquid nitrogen. Please criticize more. We will definitely modify and improve it. Thank you very much again.

9. Response to comment: Section 5.2.1: Top line p. 19, please define HuES9.E1 as a human embryonic-derived MSC line.

Response: Thank you very much for this question. We have revised in the manuscript. Thank you very much again.

10. Response to comment: Section 5.3.2, third paragraph middle of p. 23, please revise the

sentence containing “acting as a ceRNA to sponge miR-138-5p can regulate Sirt1” – this is unclear.

Response: Thank you very much for this question. We have revised the sentence containing “acting as a ceRNA to sponge miR-138-5p can regulate Sirt1” in the manuscript. If you think it is not good enough, please criticize more. We will definitely modify and improve it. Thank you very much again.

In all, I found the reviewer’s comments are quite helpful, and I revised my paper point-by-point. Thank you and the review again for your help!

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper.

We appreciate for Editors/Reviewer’ warm work earnestly, and hope that the revision will meet with approval. Once again, thank you very much for your comments and suggestions.

Sincerely yours,

Zhanjun Ma

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