

**Dear editor and reviewer(s),**

Thanks a lot for your great efforts. I'm very excited that I could have a precious opportunity to revise my manuscript. As soon as I received the comments, I began to revise my manuscript immediately. I have read these comments and revised my manuscript carefully and thoroughly according to the every comment of the reviewer(s). And this article has been reedited by one professional language editing company (American Journal Experts, AJE), which is recommended by your journal, and reviewed by a native English-speaker. I will upload the new Editing Certificate as an attached file. If the requirements of editors and reviewers are still not met, please feel free to email me(zuohoud@163.com). Some necessary explanations are listed in the responses. The point-to-point responses are listed as follows:

**Reviewer #1**

**Scientific Quality: Grade C (Good)**

**Language Quality: Grade A (Priority publishing)**

**Conclusion: Major revision**

**Specific Comments to Authors:** Peng et al has summarized the factors influencing osteogenesis and chondrogenic differentiation of MSCs. To make it more specific to OA, as authors planned to, a few issues should be addressed.

**RE:** Thanks for your comments and recognition. We have make some corresponding revisions according to the your comments and suggestions carefully.

**Major:** 1. Authors mentioned the following in the core tips: differentiation function of MSCs also plays an important role in the treatment of diseases. This is also the purpose for writing this review article. Authors should write a section to support this statement. It is to convince the readers, besides paracrine effect, differentiation of MSCs also contribute to OA treatment. Suggest to include clinical data or animal modal to show MSC differentiate into osteocytes and chondrocytes in OA treatment.

**RE:** Thanks for your precious comments. As you said, in the former version, we didn't well in proving that the differentiation function of MSCs also contributes to OA treatment. Therefore, we revised our manuscript and made some necessary changes according to your suggestions. We have added a new paragraph at the end of the introduction (paragraph six), listing clinical experiments and animal experiments, which can prove the chondrogenic differentiation of MSCs in the treatment of OA. At present, no experiment has been performed on the differentiation of MSCs in isolated subchondral bone tissue. However, there are some similar studies that apply MSCs to bone defects, which can prove the osteogenesis differentiation of MSCs. We infer it is also applicable to the subchondral bone defect of OA.

2. As mentioned in the title, abstract and core tips, the focus of this manuscript is specific to OA. Authors should include more evidence/literature on the factors affecting the differentiation of MSCs for OA or OA microenvironment. I would like to emphasise that the keywords here are OA or OA microenvironment. Based on current manuscript, authors have mostly explained or elaborated on the factors affecting osteogenesis and chondrogenic differentiation of MSC in general or in vitro, but did not explain how the OA microenvironment plays a role in differentiation. Thus, for each section of the

manuscript such as Mechanism of osteogenic and chondrogenic differentiation and Factors affecting differentiation (Oxygen concentration, Glucocorticoid, Sources of MSCs), please further explain the topics in relation to OA or OA microenvironment.

RE: Thanks very much for your comments. We have added more content about the OA microenvironment, including hypoxia, inflammation, acidic pH, hypo-osmosis and various cytokines. These factors are common in the articular cavity of OA. In addition, we also listed some other common factors. For example, dexamethasone, which is commonly used in the early treatment of OA. When it is used together with MSCs, its concentration and exposure time need to be controlled. Scaffolds are often needed in MSC therapy, and choosing the appropriate scaffold materials can promote the differentiation of MSCs. And the selection of MSC sources can also have an important impact on its differentiation. Taking these factors into account during treatment can lead to more accurate differentiation of MSCs.

Minor: 3. Suggest to draw a figure of mechanisms/interactions to show how these factors or signalling pathways affect the osteogenesis and chondrogenic differentiation. This is to summarize the section on 'Mechanism of osteogenic and chondrogenic differentiation of MSCs'. Authors could draw one for each differentiation.

RE: Thanks very much for your comments. To help readers to better understand the structure of this manuscript, I draw and add Figure 2 to illustrate the mechanism of signaling pathways and certain factors affecting the osteogenesis and chondrogenic differentiation of MSCs.

4. Authors mentioned that by December 2022, 1141 clinical studies have been registered in clinicaltrials.gov. It is now March 2023. Therefore, the authors should update the figure to the latest.

RE: Thanks very much for your comments. The latest data of April 2023 are updated.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The differentiation function of MSCs plays an important role in the treatment of OA. This review is the first time to report the factors that may affect the differentiation direction of MSCs in the treatment of OA, and provide guidance for more accurate regulation when MSC therapy is applied in the future. However, there are still some issues with this article that need to be addressed.

RE: Thanks for your comments and recognition. We have make some corresponding changes according to your comments and suggestions carefully.

1. It is suggested that authors adjust the writing order in the introduction, such as introducing the current treatment of OA, and then discussing the current research progress of MSCs.

RE: Thanks very much for your comments. We have adjusted the writing order in the introduction by adding an introduction of OA at the beginning, so as to transit to MSC therapy.

2. It is recommended that authors use tables to summarize the specific mechanisms of

**action of factors affecting the differentiation direction of MSCs.**

RE: Thanks very much for your comments. In order to help readers to better understand the structure of this manuscript, I draw and add Figure 2 to illustrate the specific mechanisms of certain factors that affect the differentiation direction of MSCs. We opted for the graphic over the table because we thought it would more clearly show how these factors work.

**3. Several reviews have been published on the influencing factors of MSCs differentiation, and the authors seem to have missed some important factors, such as scaffold materials and bFGF.**

RE: Thanks very much for your comments and suggestions. We reviewed some relevant literature and added two paragraphs (the fifth point of the third part and the second point of the fourth part) to introduce the effects of cytokines and scaffold materials on the differentiation of MSCs.

We have made some changes according to the comments carefully and thoroughly. The version with marked changes was uploaded as the supplemental file. I hope it is satisfactory.

Finally, I express my sincere appreciation to editor and reviewer(s) again. Although I try my best to improve and perfect my manuscript, it still may not be satisfactory completely to all the reviewers. Nevertheless, I'm eager to get a chance to publish this minireview, so that I can deliver some useful information to the readers. Thank you very much. Please don't hesitate to email me, if you have any questions. I sincerely appreciate your efforts and constructive comments. Have a nice day!

Best Regards

Houdong Zuo(zuohoud@163.com)