

## Format for ANSWERING REVIEWERS

January 16, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15855-review.doc).



**Title:** Osteogenic differentiation of amniotic fluid mesenchymal stromal cells and their bone regeneration potential

**Author:** Caterina Pipino, Assunta Pandolfi

**Name of Journal:** *World Journal of Stem Cells*

**ESPS Manuscript NO:** 15855

The manuscript has been improved according to the suggestions of reviewers. All changes made to the manuscript are shown in red.

Postal code and conflict of interests have been added.

The figure has been uploaded in both tif and psd formats.

As certified in the uploaded signed guarantee, we believe that the language of our manuscript has reached Grade A employing a private professional editing service.

Reviewer 1.

- *On p 6 Authors stated that “they AF-MSC represent an intermediate stage between embryonic and adult cells with advantages compared to both.” It is absolutely unclear what particular stage is mentioned. Indeed, there are some stages of embryo development and there are also some stages of stem cells differentiation in adults, whether this sentence relates to the stages of organism development or cell differentiation process.*

This has now been rephrased.

- *It is undebatable that pluripotent cells have enormous differentiation potential, however, it is also obvious that any particular treatment will need corresponding cell type. Modern technologies of adult cell reprogramming have overcome both ethical issues of embryonic stem cells isolation and medical problem of tissue compatibility, therefore Authors have to consider this rapidly growing scientific direction and compare AF MSC not with embryonic but rather with iPS cells.*

We are in agreement with the reviewer's comment, then we added pluripotent stem cells (iPSCs) and compared AF-MSCs to iPSCs.

Reviewer 2.

- *The paper is mainly related to the osteogenic differentiation of amniotic fluid mesenchymal stromal cells (AF-MSC), while the title of this is about AF-MSC in bone regeneration. The authors should distinguish between osteogenesis and bone regeneration.*

We have changed the title.

- *Recently, the mechanisms of osteogenesis are continuously revealed. The summary sentence of “Osteoblastic differentiation begins when the bone morphogenetic proteins (BMPs) bind their receptors activating the transcription factors Runx2 and Osterix, and subsequent downstream osteoblast specific genes, through the*

*activating of Wnt/LRP5 cascade, crucial in bone mass modeling” is not extensive enough. Particularly, the main signaling pathways involved in osteogenesis should be reviewed.*

We have added the main signaling pathways involved in osteogenesis and more references regarding this topic.

- The authors listed amount researches about the osteogenic differentiation in vitro and in vivo. However, mesenchymal stem cells exist in many tissues and most of them have the ability of differentiating into osteoblasts. It is suggested to pay more attention on comparing the ability or the effectiveness of osteogenesis between AF-MSC and other MSC. Further, the possible reasons should also be discussed.*

More information has been provided for the osteogenic ability of AF-MSCs compared to other MSCs.

- In the part of “Tissue engineering approaches for in vivo bone regeneration”, the possible mechanisms for various scaffolds supporting osteogenesis should be involved in this paper. In fact, properties of scaffold such as various chemical groups and roughness both have significant effects on osteogenesis of MSC.*

We have added a better description of the properties of the scaffolds.

Reviewer 3.

- However, the “uniqueness” and advantages of AF-MSCs in comparison to other tissue-derived MSCs in term of their properties and potential clinical application are not well described.*

We have provided more informations regarding the advantages of AF-MSCs in comparison to other tissue-derived MSCs.

- In addition, the text should be proof-read for spelling, grammar and English usage. For example, in “Abstract”, line 4, “clinical” should be “clinically”; line 6, “not raises” should be “does not raise”; and so on.*

According to the reviewer suggestion, “clinical” was substituted by “clinically”, “not raises” was substituted by “does not raise” and english of this review was edited professionally.

Reviewer 4.

- As an overview, this work should present the newest research works in the field of mesenchymal stromal cells. So, please replace some old references, such as reference 10, reference 44, and reference 46.*

We have replaced old references.

- In the part of “introduction”, please add some other application of amniotic fluid stem cells for other clinical applications, such as lung injury and neovascularization.*

We have added other clinical applications of amniotic fluid stem cells.

Thank you again for publishing our manuscript in the *World Journal of Stem Cells*.

Sincerely yours,



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