

## Format for ANSWERING REVIEWERS



January 4<sup>th</sup>, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name:6673-answer reviewer.doc).

**Title:** Osteogenic potential: comparison between bone marrow and adipose-derived mesenchymal stem cells

**Author:** Han-Tsung Liao, Chien-Tzung Chen

**Name of Journal:** *World Journal of stem cell*

**ESPS Manuscript NO:** 6673

The manuscript has been improved according to the suggestions of reviewers:

1<sup>st</sup> reviewer's comment

This review paper compares the osteogenic capacity in vitro and bone regeneration ability in vivo between BMSCs and ASCs. It is a helpful study that gathers most of the scattered papers on this field and provides readers the current progress in understanding the osteogenic capacity of BMSCs and ASCs both in vitro and in vivo study.

But there are still some regrettable issues, in which the queries that the authors should address:

1. This is a well written article, and the title of this manuscript is brief and indicative of the material, which is contained in the manuscript. The abstract could be reduced in length, and some of the details on bone tissue engineering may be reduced or expatiated upon in introduction. The discussion content should be more detailed following each part of the manuscript.

**Answer:** The abstract was rewritten as reviewer's suggestion. We also add some contents in discussion part.

2. It's a pity that there is not a similar table comparing in-vivo osteogenesis potential between human BMSCs and ASCs like Table 1.

**Answer:** Yes, we create a Table for in-vivo osteogenesis.

3. "Investigator" or "Scholar" is recommend for the title of the first column in Table 1 instead of "name".

**Answer:** Yes, we change name to investigator.

4. Column of "Reference(s)" should be supplemented in Table 1.

**Answer:** Yes, we add a column of references in Table 1.

2<sup>nd</sup> reviewer's comment:

Having drawn from their own experience (<https://ww2.eventrebels.com/ERImages/9188/2340122/28583-2-10311.pdf>), the authors offer their insight into the scope of research on osteogenic potential of bone marrow and adipose-derived mesenchymal stem cells. Specifically, they described the comparison of BMSCs with ASCs (in vitro, in vivo) and offered their vision on possible directions in the future. Their summarized table is helpful. Overall, it's well written, informative, and visionary.

One caveat was the authors have not adequately addressed the heterogeneity of BMSCs with ASCs. What's the percentage of either cells with the osteogenic potential? Some minor revision will enhance the clarity, logic flow, and cohesive narrative of their story by incorporating the following specific comments.

### **Specific comments**

1. Title "Tumor stem cells and colorectal cancer" (with a broad spectrum of implication) didn't fully and accurately reflect the major topic and contents of CRC (refer to the following).

**Answers: The title "Tumor stem cells and colorectal cancer" the reviewer mentioned is not correlated to our stem cells topic. Therefore, we do not know how to answer the reviewer's question.**

2. Abstract should be revised to be concise to cover what's about specifically in their review. The way written now is like introduction. They should emphasize the commonality and difference of their osteogenesis potential between BMSCs and ASCs (in vitro, in vivo) here up front and then spring up their vision.

**Answer: Yes, we have revised the abstract.**

3. Page 2, Abstract: "Adult mesenchymal stem cells are considered an appropriate cell sources for BTE because it has no ethic issue derived from embryonic stem cells". Please revise as it's not logical as read. "Sources" should "source".

**Answer: Yes, we revised this sentence.**

4. Page 2, "Bone marrow mesenchymal stem cell (BMSC)" should be "Bone marrow mesenchymal stem cells (BMSCs)."

**Answer: Yes, we changed as reviewer's suggestion.**

5. Page 2, last line:

"We expect the new researchers can have a quick understanding the progress in this filed and design a more comprehensive research based on this review." It's not logical; revise. You can say "We expect the new researchers can have a quick understanding **of** the progress in this filed and design a more comprehensive research based on this review."

**Answer: Yes, we revise as reviewer's suggestion.**

6. Page 5: "tons of paper" should be 'papers'.

**Answer: Yes, we revise as reviewer's suggestion.**

7. Page 5: "However, the low stem cell yield from bone marrow aspirates, painful procedure, and possible complications derived from the procedure and poor mutlipotent ability after extensive passage or at aged people urge scientists to search a better alternative source." It's an awkward sentence, please revise it.

**Answer: Yes, we revise this sentence.**

8. Page 7: "In contrast, the decline in osteogenic potential of ASCs is not so prominent by aging.<sup>[8]</sup>" why?

**Answer: Most recent papers confirmed the less aging effect of cell proliferation and osteogenic effect on ASCs comparing to BMSCs. However, they only described the phenomenon without the explanation the reasons. We only can speculate that bone marrow quality was reduced with aging which results in osteoporosis but old people still can gain fat and become obesity which means the ASCs in fat tissue still proliferate and preserve their differentiation potential well with aging. We also add two more references and contents to strengthen the findings.**

9. Page 8: “In contrast,  $2 \times 10^6$  cells can be isolated from 1gm adipose tissue,” “we can usually draw 1000-2000cc lipoaspirates from patient; there may be  $2 \times 10^8$  to  $4 \times 10^8$  stem cells”. Their calculation was wrong. As the way they stated, it should be round up “ $2 \times 10^9$  to  $4 \times 10^9$ ” not,  $2 \times 10^8$  to  $4 \times 10^8$ .

**Answer: The calculation is correct. It is right that the total cell number is  $2 \times 10^9$  to  $4 \times 10^9$ . But only 10% is stem cells, hence there is only  $2 \times 10^8$  to  $4 \times 10^8$  stem cells.**

10. Page 8: “The bone marrow aspirates yield  $6 \times 10^6$  nucleated cells per ml in average, only 0.001 to 0.01% are stem cells.<sup>[10]</sup> In contrast,  $2 \times 10^6$  cells can be isolated from 1gm adipose tissue, 10% are thought to be stem cells.<sup>[12, 13]</sup>” On page 9, “Although the ASCs pose so many better features for future clinical application in BTE than BMSCs, the osteogenic ability of ASCs is still the most concern to clinical physician.” These two statements are confusing: If they called ASCs “stem cells”, by definition of stem cells these cells should have the osteogenic potential. They should have to differentiate these terms. Page 7 indicates the 4 criteria for MSCs including osteogenic potential. It is better to state “Plastic-adherent fat cells” and using SCs with osteogenic potential.

**Answer: We think there is no conflict between these two sentences. First, the BMSCs and ASCs both have osteogenic potential is correct. However, the ability is different. Most papers think the ASCs have inferior ability of osteogenesis than BMSCs. It is why the clinical physician doubts ASCs can replace the role of BMSCs in bone tissue engineering. However, for avoiding confusions, we make some revision in the sentence.**

**We do not agree that “It is better to state “Plastic-adherent fat cells” and using SCs with osteogenic potential” because it will cause misunderstanding from the original paper.(Reference 5)**

11. Page 11: “Take together, most evidences” – these are not the Standard English expression.

**Answer: Take together was changed to Taken together.**

12. Page 13: “During late osteogenic differentiation, the ASCs have better angiogenesis capacity than BMSCs” – It’s out of context.

**Answer: This just described their findings from the paper. Besides, the angiogenesis is very important for the successful bone tissue engineering. The angiogenesis capacity of ASC can enhance the vascularization of the tissue engineered scaffold and the survival of stem cells and also osteogenesis. We add more contents in the section to verify the importance of angiogenesis on osteogenesis.**

13. Page 14: “direct comparison between both cells are scarce in human study and few in animal study.” It’s not logical as they did show with examples.

**Answer: Yes, we did show the examples but it was as what we described no human study (scarce) to compare between both cells and only 6 papers (few) compared the osteogenesis between BMSCs and ASCs simultaneously in animal study.**

14. Page 16: “6. Future direction and summary” It’s too long. It should be shorten to be concise, straight to the point.

**Answer: Yes, we shortened it by cutting some redundant sentences..**

15. Cut down those repetitive words, e.g., this review paper, etc. You can simply say this review.

**Answer: Yes, we revise as reviewer’s suggestion.**

16. A list of abbreviations will be appreciated.

**Answer: Yes, we do a list of abbreviations.**

17. Table 1. BMSC >ASC under mechanical stimulation should be defined by numbers (percentages).

**Answer: It is hard to quantify how many percentages of BMSCs is superior to ASCs because the analysis in in-vitro experiments composed of many tests such as alkaline phosphatase activity, alizarin red stain, qPCR and so on. The percentage is different among different tests and most papers did not address the percentage. Conversely, the in-vivo study is easy to show the percentage of new bone formation between BMSCs and ASCs. Hence, we make a**

Table III for animal study and show the percentage of osteogenesis between BMSCs and ASCs. But some papers only showed figure to demonstrate the new bone regeneration between these two groups without original data and we did not put percentage on the table in this situation.

Sincerely yours,