Peer Review File

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Review Comments

Comment 1: I suggest to add some additional information about Wnt4 in general, and about its role in bone formation.

Reply 1: Thank you for your valuable suggestion. We have provided the role of Wnt4 in bone formation in the introduction part.

Changes in the text: Page 4, line 59-64.

Comment 2: Besides the downregulated Wnt4 expression, what are the other parameter that makes Inflamed DPSCs different from the normal DPSCs?

Response: We also have provided the information in the introduction part.

Changes in the text: Page 4, line 63-64.

Comment 3: What do you mean about "bone defeat" (line 61)? Didn't you mean "defect" instead? If so, this mistake was made several times throughout the manuscript. Please correct it.

Response: We apologize for the mistakes we made. Actually, it should be bone defect throughout the manuscript.

Comment 4: I think that cell isolation and culture should be in an independent section.

Response: Thanks to the reviewer for this valuable suggestion. We have corrected accordingly.

Changes in the text: Page 6, line 72-83.

Comment 5: Collagenase and dispase enzymes were both 3mg/ml concentration or it is only the collagenase? Where are these enzymes from?

Response: Collagenase and dispase enzymes were both 3mg/ml concentration. We have added the details of both enzymes.

Changes in the text: Page 6, line 76-77.

Comment 6: How the lentiviral transfection (isn't it transduction anyway?) happened? What plasmids, methods, other ingredients were used? Where are they from? Were transduced cells selected after by any method?

Response: We apologize for the initial lack of clarity in our statement. We have added the details in the method part.

Changes in the text: Page 6, line 80-83.

Comment 7: When the flow cytometry analysis happened (passage number), was it after the transduction?

Response: Passage 1 of cells after transduction were used. We also provided the information in the text.

Changes in the text: Page 6, line 85-86.

Comment 8: For the differentiation experiments, what were the conditions? Were these „home-made" media (in this case what ingredients used?) or bought from a company (In this case where from?)? How the results were evaluated?

Response: We apologize for the initial lack of clarity in our statement. The details of induction medium were added in the method part. How the results were evaluated was added as well.

Changes in the text: Page 7, line 92-98.

Comment 9: For the preparation and evaluation of complex Wnt4-DPSCs-IPs/PHBV, what were the dimensions of the PHBV. Were the same conditions used for the transplantations (dimensions, culturing time, etc.) and other cell lines as it is described for the electron microscopy (not microscope-line 83) analysis?

Response: The dimensions of PHBV were added, and same conditions were used for transplantations and electron microscopy.

Changes in the text: Page 7, line 100.

Comment 10: What equipment, settings, software, etc. used for Micro-CT analyses?

Response: Details information of micro-CT analysis were added in the revised manuscript.

Changes in the text: Page 8, line 122-124.

Comment 11: The immunohistochemistry part is rather an instruction than a description of the method used. Need to be rephrased. Incubation time and/or temperature are missing from several steps of the method. What were the proteolytic enzyme, serum blocking solution, first and second antibody? Where are they from?

Response: We apologize for the initial lack of information in our statement. We have rephrased this part.

Changes in the text: Page 8, line 126-132.

Comment 12: What was the software used for statistical analysis?

Response: SPSS 16.0 software (SPSS, Inc., Chicago, IL) was used for analysis.

Changes in the text: Page 9, line 135-136.

Comment 13: The description of cell proliferation and ALP assay are completely missing.

Response: We apologize for the missing information. We have added the description of cell proliferation and ALP assay in the method part.

Changes in the text: Page 7, line 105-110.

Comment 14: Wnt4-DPSCs-IPs expressed positively in STRO-1, CD90, and CD105, negatively in CD34 and CD45. (line 109)… It is the other way around I think, the sentence needs to be rephrased.

Response: Thanks for this suggestion. We have rephrased the expression.

Changes in the text: Page 10, line 143-146.

Comment 15: The results suggested that Wnt4 overexpression did not change the stem cell characteristics of DPSCs-IPs (line 110)… Compared to what? DPSC-IP-Wnt4 to DPSC-IPs or normal DPSCs?

Response: We found that Wnt4-DPSCs-IPs still retain the stem cell characteristics as DPSCs-IPs reported.

Comment 16: the word „multi" used several times instead of multi.

Response: We apologize for the wrong spell. We have corrected throughout accordingly.

Comment 17: in the characterization, Wnt4-DPSCs-IPs compared to Wnt4-DPSCs-IPs on PHBV. It makes no sense, as it should be compared to normal DPSCs on the same surfaces or even DPSC-IPs on the same surfaces (preferably both) to get information about the changes in the cell line. The only information from this comparison that the change of surface does not change these properties…

Response: From Fig.1-Fig.2, we know that Wnt4-DPSCs-IPs retained stem cell characteristics and was potential for osteogenic differentiation. Next, we engrafted Wnt4-DPSCs-IPs on PHBV and want to figure out whether the biomaterial had bad influence on cell proliferation and osteogenic differentiation. From the results of Fig.3, we may found that PHBV didn't change the proliferation and osteogenic differentiation of Wnt4-DPSCs-IPs. We can further performed the complex into animal experiments.

Comment 18: Three days after Wnt4 DPSCs-IPs seeded on PHBV, the proliferation rate was detected (line 117)… The cells did not proliferate before the third day? How do you know that if you do not have results from day 0? Based on your figures there are also data from the first and 7th days. Why these are not mentioned? Moreover, you cannot detect the proliferation rate but calculate it from the detected absorbance measured in case of control and treated cells and Fig 3A. There are only absorbance values which only proportional to the number of viable cells. I would rephrase this section according to the information on the related figure.

Response: Thank you for your suggestion. We have rephrased accordingly.

Changes in the text: Page 10, line 154-157.

Comment 19: Figure 3B shows the ALP activity of Wnt4-IPs alone and on PHBV not only on PHBV as the legend says. It is not osteogenic potential but only one marker, the ALP activity… What are the numbers on the activity axis? Is it OD or percent (neither seems appropriate)?

Response: The numbers on the activity axis stands for % relative to control. Control was Wnt4-IPs in normal culture medium. We have rewrite the axis to make it clear.

Changes in the text: Figure 3B

Comment 20: A large number of porous structures were detected as well as the cell attachment." (line 123) I am not sure, that Figure 3C (and the overall electron microscopy) gives any additional information. Is PHBV a new material as a carrier for cells or why is it important to see the cells on the surface? „A large number of porous structures were detected as well as the cell attachment." I would suggest pictures from the 3 cell lines on the surface to see if there is any difference amongst these (morphology, density for example).

Response: Thank you so much for your suggestion. We gave the cell attachment pictures because different cells have different conditions on PHBV, we need to make sure Wnt4-DPSCs-IPs can attach to PHBV and keep good conditions. We would like to use 3 cell lines to the surface in our further study.

Comment 21: Figure 4A showed…" (line 126)- it is still showing that.
- „the PHBV group demonstrated physiological bone repair" (line 126). I disagree. Physiological bone repair means bone repair without foreign materials, cells, or agents. PHBV group serves as a control for the effect the applied carrier. But it really would be good to see the physiological bone repair on the figures.
- „Osteocalcin expression was detected, the immunohistochemistry results showed that osteocalcin expressed at a higher level…" You did not expect to detect osteocalcin expression or why is it written this way? Please rephrase for clarity
- On figure 4B the graph shows significant alterations. It would better to stress these significant changes in the text as well.

Response: Thanks so much for this suggestion. We have corrected the expression of PHBV group. As the reviewer suggested, we have changed the way of expressing osteocalcin levels. We also stressed the significant changes of micro-CT data in the results part.

Changes in the text: Page 11, line 163 to 165. Page 11, line 171-175. Page 11, line 167-171.

Comment 22: I believe that it should be completely rewritten based on the rephrased, reevaluated results, however, I would like to point out some of the most notable mistakes.
- „a possible strategy to improve the impaired osteogenic potential of DPSCs-IPs was figured out,…"(line 138) I don't think the phrase „figured out" is appropriate.
- „Our results showed that after loading Wnt4-DPSCs-IPs on PHBV, the proliferation and osteogenic differentiation ability of the complexes were not statistically different from Wnt4-DPSCs-IPs, which proved the biological safety and osteogenic potential of the complexes in vitro." (line 156) I don't think, that proliferation and osteogenic differentiation have anything to do about biological safety.

Response: According to the reviewer's valuable suggestions, we have rephrased the expressions.

Changes in the text: Page 12, line 180-181, line 194-196, line 202-203.

Comment 23: The results of in vivo experiments showed that the even PHBV group had bone repair potential proving that PHBV is biologically safety and the alveolar bone itself has a certain self-repairing." (line 160) First, bone repair potential also doesn't have anything to do about biological safety. Second, the „certain self-repairing ability" possibly called physiological bone repair which is not demonstrated in the manuscript as the PHBV group is anything but that. And finally, without the physiological control, you do not know or conclude anything about the bone repairing potential of the PHBV group.

Response: We used PHBV group as the control group because that the biomaterials themselves may have bad influence on bone defect. Figure 4A showed that PHBV group has no bad effect on bone regeneration, so that we may first concluded that the biomaterial was safe, that is why we used "biological safety" in the manuscript. According to the reviewer's suggestions, "without the physiological control, you do not know or conclude anything about the bone repairing potential of the PHBV group", we agree, so we rephrased the way of expression.

Changes in the text: Page 13, line 204-206.