Peer Review File

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Reviewer A

The work by Chu et al. regarding anatomical bone-defect repair by MSCs and scaffolds is an intriguing study and provides some interesting results. The paper is relatively well written and the conclusions are supported by the data provided in most parts. However, the paper would benefit from addressing the following points:

1) How did the authors determine that the cells used for seeding their scaffolds were MSCs?

Thank you for your suggestion. MSCs are abundant in bone marrow and we determined the combination of MSCs and porous β-TCP through the comparison of the number of CFU/ALP+ in bone marrow before and after SECCS and through the observation of spread and differentiation of cells in the particles. If MSCs did not adhere to the scaffolds, there would be CFU/ALP+ no significantly less than that in the bone marrow before SECCS. In order to clear this, we have cited our previous results in the articles.

2) How were the MSCs tested for their multipotency and cultured?

We have tested their multiple differentiation ability including osteogenic, chondrogenic and adipogenic differentiation in our previous work and we have added the references in the article. [10 Chu W, Gan Y, Zhuang Y, et al. Mesenchymal stem cells and porous beta-tricalcium phosphate composites prepared through stem cell screen-enrich-combine(-biomaterials) circulating system for the repair of critical size bone defects in goat tibia. Stem Cell Res Ther 2018; 9:157.]

3) Is it appropriate to give 6 significant numbers in the amount of MSCs replanted?
There were 5 goats in the experimental group and therefore 5 numbers in the amount of MSCs replanted.

4) Were any other cells tested in this model, such as fibroblasts? How would they work in compared to MSCs?
Thank you for your inspiring question. Actually, there were other bone marrow cells involved and we had tested the bone marrow nucleated cells. As our focus was on the MSCs, we didn't compare other cells' effect on bone repair with that of MSCs in detail. We are willing to study the relationship between such cells and MSCs to find potential working mechanism of bone repair in this study and we added the limitation of lack of working mechanism in the last paragraph.

5) What was the level of inflammation in the repair sites? Was this affected by the MSCs at all?
According to the hyperplastic soft tissue, there were actually inflammation which may be caused by the protruded frame end when the outer protective complex was degraded. As we focused on the bone repair effect, we did not detect the inflammatory indicators in this study and we couldn't conclude the extent to which MSCs contributed to the alleviation of such inflammation. However, in order to remind our readers of such risks, we added the limitation in the last paragraph.

Reviewer B

1.Title: OK
Thank you for your comment.

2. Abstract:

2.1 Please include a short summary of the number of animals used, age and breed, and how the bone defect model was established.

We have added the information in the abstract.

2.2 Histomorphology should be changed to histomorphometry on page 4 line 50.

Thank you for your careful review, we have corrected it accordingly.

3. Introduction:

3.1 The introduction is brief and clear. However, the relevance of the applied assessment methods should be briefly explained in the last paragraph.

In order to maintain the logic continuity, we discuss the relevance in the part of methods and discussion.

3.2 Also, a brief explanation on why the lateral half of the goat distal femur was chosen as the defect site and the relevance of the bone defect model to the study objectives should be included.

Thank you for your suggestion, we give a brief explanation about the model in the part of discussion because we believe such arrangement could make the introduction as concise as possible.

4. Methods:

4.1 Please provide the breed, and age range of the animals used in this study. Did all the animal survive till the end of the observation period or was there any loss recorded?

According to your suggestion, we have provided the detailed information. All the animals survive till the end of the observation period.

4.2 Briefly explain how the mechanical testing was done to determine the compression test among the three implant groups.

We have added it in the article.

4.3 Also, the protocol for Van Gieson's picro-fuchsin stain should be briefly described.

We have described the protocol briefly.

5. Results:

5.1 Please provide information on the post-operative care and recovery of the animals after the femur fracture. How was the weight bearing at the fractured limb during the 9-month observation period? Were the animals allowed and able to move freely at the meadow or was their movement/activity reduced due to the fracture?

We included the information on the post-operative care and recovery in the part of methods. All animals including those in the control groups were able to move at the meadow, but they avoided stepping on the ground completely with the fractured limb after a relatively long walk. We believe it may be caused by the joint damage and we have discussed this in the part of discussion.

5.2 Figure 2D on page 26 is not shown?

Thank you for your reminder, we have corrected it.

5.3 Please provide high resolution images of the X-ray and CT scans shown in figure 7 on page 29. The figure A and B should be well enlarged for better viewing.

We have replaced the figure 7 with a high resolution images.

5.4 I recommend that the (micro?)-CT images should be used to assess the amount of newly formed trabecular bone in-growth within the frame between the three implant groups.

Thank you for your recommendation. Actually, we had tried the use of the micro-CT, but the samples were too huge to be put into the device.

5.5 In addition, it is necessary to perform mechanical testing at 9-month post-fracture to determine the bone biomechanical strength at the bone-implant interphase of the SECCS-processed β-TCP/MSC implants compared to the control and blank groups.

Thank you very much for your constructive suggestion. It would be more convincing to include the data of biomechanical strength; However, the frame was fixed by the customized plate and screws, so the mechanical strength was mostly taken by the fixing system. What's more, the removal of fixing devices would destruct the new bone

covered on the plate and affect the stability at bone-implantation interface, thus influencing the mechanical results. In view of the in-growth bone, we believe the histology and histomorphometry would be more accurate.

6. Discussion:

6.1 Please show relevant data or supporting references to justify your explanation that the significant decrease seen in the number of MSCs after treatment with SECCS indicates that the MSCs successfully adhered to the porous β-TCP material. A successful adherence of the MSCs to the porous β-TCP implant should reflect high osteogenic capacity and not a decrease as your CFU/ALP+ result revealed. An additional staining such as the Alizarin-Red staining and quantification should be employed to further validate the osteogenic competence of the bone marrow MSCs before and after treatment with SECCS via osteogenic induction assay.

The use of SECCS was to combine MSCs in bone marrow with the porous β-TCP to improve the osteogenesis of β-TCP by leveling up the number of MSCs within it. The decrease of the number of CFU/ALP+ in bone marrow from Figure 5A (before SECCS) to Figure 5B (after SECCS) indicates the successful combination of MSCs and β-TCP, we have provided the supporting reference. In our previous work, to identify the combination of MSCs and β-TCP as well as the osteogenesis of MSCs within β-TCP, we performed ALP staining for the SECCS-processed β-TCP (the contents of calcium and phosphate make it cannot perform Alizarin-Red staining directly) and there was obvious expression of ALP on those particles, we included those references accordingly.

6.2 Please discuss the limitations of this study in the last paragraph.

We have rewritten the last paragraph accordingly.

7. Conclusion: OK

Thank you for your comment.