Peer Review File

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Reviewer A:

Comment 1: There are too many grammatic mistakes; it is hard to read the manuscript. It is advised that to send the manuscript to be reviewed and edited by a writing center or a professional writer.

Reply 1: We thank the reviewer for their advice. The manuscript has been reviewed and polished accordingly.

Comment 2: Line 27, the author wrote that (3D printed PLA-HA scaffolds were cylinder scaffolds with mean pore size 27 of 500μm and 60% porosity), please clarify the sentence, what do the authors mean the difference between 500μm and 60% porosity?

Reply 2: We thank the reviewer for their insightful comments. Pore size is a scalar quantity that describes the diameter of a hole. The porosity of a material is the percentage of the pore volume of a block material relative to the total volume of the material in its natural state. To facilitate bone formation, bioactive scaffolds should have a porous structure and porosity which are conducive to bone repair, vascular access, and adequate oxygen supply[1]. Large pores of 200–500 μm in the scaffold improve bone growth and capillary formation[2]. Using our method, the scaffolds were constructed with large pore sizes and porosity. Our previous study indicated that the scaffolds we used produced satisfactory results[3].

Changes in the text: We have added the related information (line220-226)

Comment 3: It is advised that the authors write in their own language, paraphrase the manuscript to avoid plagiarism. A similar language of this manuscript is detected in other manuscripts that are already published.

Reply 3: We thank the reviewer for their advice. The manuscript has been revised and polished accordingly.

Comment 4: The introduction has two hypotheses. Please revise the introduction to have one strong hypothesis

Reply 4: We thank the reviewer for their suggestion. We have revised the manuscript accordingly.

Changes in the text: We have modified our text as advised (line91- 92).

Comment 5: Lines 116, it is unclear how the authors were able to load the eBM into the scaffold. Although the figure demonstrates the procedure, it would be highly beneficial to please expand this method section for the reader to understand the procedure clearly.

Reply 5: We thank the reviewer for their advice. We have added the relevant information as advised.

Changes in the text: We have modified our text as advised (line134-137).

Comment 6: Line 122, the authors mentioned that at zero weeks, what do the authors mean by zero weeks? It will be visible if the authors determine which day do they take the x-Ray at zero weeks?

Reply 6: We thank the reviewer for their advice. ‘0 weeks’ is indeed an inadequate description. ‘0 weeks’ means that the defect site was examined by X-ray immediately after the operation. The manuscript has been revised accordingly.

Changes in the text: We have modified our text (line142).

Comment 7: Lines 130 and 131, the authors used 12% EDTA to decalcify the specimens for 4 to 5 weeks. Did the author note any detonation in the specimens' mechanical or physical properties due to the decalcification process's length?

Reply 7: We thank the reviewer for their insightful comments. We used 12% EDTA to decalcify the specimens for 4 to 5 weeks to meet the needs of histological sectioning. This is a widely applied approach; many previous studies have used 12% EDTA to decalcify the specimens[3-5].

Comment 8: The authors used qualitative analysis methods to evaluate and score the new bone formation. Although this method is acceptable, it would be highly advised to use quantitative analysis to measure the quantity of new bone formation, mainly when the number of animals per time-point is low.

Reply 8: We thank the reviewer for their advice. We realize that the number of animals per time-point is low; however, due to the limited funding and animal ethics principles, the research team was not authorized to conduct more animal experiments in the present study. This is a preliminary study; however, we intend to perform more animal experiments to promote the translation of the research results in the future.

Comment 9: The authors used only H and E staining to stain the new bone formation,

H and E staining is not the optimal or the best stain to quantify a new bone formation. Von-kossa and other specific stains for new bone formation are highly recommended in this type of study.

Reply 9: We thank the reviewer for their insightful suggestion, and we agree that the Von-Kossa measure is a good measure to identify new bone formation, especially of mineralized bone tissues. However, Von-Kossa staining has to be performed in hard-tissue slices, which were not reserved and prepared in the present animal experiment. Due to the limited funding and animal ethics principles, the research team was not authorized to conduct more animal experiments in the present study in the short term. However, we will adopt these measures in the further research.

Comment 10: The authors indicate soft tissue formation in the negative control group compared to the positive control group and the experimental groups. Furthermore, the data shows that the positive control group shows new bone formation. This may indicate that the scaffold plays a role in minimizing soft tissue and forming new bone formation. It is highly advised that the authors discuss this point in detail.

Reply 10: We thank the reviewer for their insightful comments; we have revised the manuscript accordingly.

Changes in the text: We have modified our text as advised (line248-250).

Comment 11: Line 177, the authors indicate that the eBM does have cartilage, bone tissue, and intact pieces of vasculature. This may give a mixture of soft and hard tissues already implanted in the defect side, although the procedure is autologous. It is unclear if this practice is practical for transferring to the clinic and tried on patients in the future.

Reply 11: We thank the reviewer for their insightful comments. While eBM has been used clinically and possesses an excellent property for bone repair, it does not possess the gross anatomical structure to guide bone repair and regeneration. We believe that PLA-HA composite can serve as a scaffold to provide the mechanical support needed for seeded eBM in the construction of a complex scaffold to facilitate bone repair and regeneration.

Reviewer B

The authors of this publication have described the application of 3D printed polylactide-hydroxyapatite (PLA-HA) scaffold seeded with enhanced bone marrow (eBM) for the treatment of critical sized bone defects in a rabbit model. The results demonstrate that eBM seeded scaffolds had a significant enhancement in bone volume and new bone formation in the defect compared to control (empty defect) and scaffold-only groups. The results demonstrate the promise of this cell-scaffold combination for treating critical size bone defects.

The authors need to address the following points prior to further consideration for publication.

Comment 1): The adapted reamer-irrigator—aspirator technique to cultivate eBM in rabbit requires characterization for the cell's types present in the aspirate. Did the authors perform either cell differentiation (chondrogenic, osteogenic) and flow cytometry to show the cell types in the rabbit eBM?

Reply 1: We thank the reviewer for their suggestion. The cell types present in the aspirate have been studied previously[6, 7]. Additionally, cell differentiation (chondrogenic and osteogenic) was also performed in a previous study[3].

Comment 2): Did the authors only evaluate eBM in a 3D printed scaffold without HA? This needs to be an additional control group to show the effect of the eBM alone on bone regeneration due to the rabbit models having a good self-repair potential and note the effect of the eBM in PLA-HA scaffolds.

Reply 2: We thank the reviewer for their insightful comments. eBM alone was previously identified to have certain regenerative potential. In this present study, we sought to use the regenerative potential of eBM to develop a satisfactory bone repair biomaterial. To achieve the best bone repair results possible, we adopted a PLA-HA scaffold as the eBM loading material. Additionally, due to the limited funding and animal ethics principles, the research team was not authorized to conduct more animal experiments in the present study in the short term. Nevertheless, we are very grateful to the reviewer for their insightful comments, and should we perform further research at the next stage, these comments will definitely be taken into account.

Comment 3): For figure 5, how did define new bone formation using the ImagePro software? Please define and write in more detail about how this was completed.

Reply 3: We thank the reviewer for their advice.

Changes in the text: We have added the related information as advised (lines 146–149).

Reviewer C

The paper is related to study the effect of enhanced bone marrow (eBM) in conjunction with three-dimensional (3D) Polylactide–Hydroxyapatite (PLA-HA) scaffolds on repairing critical size bone defects in a rabbit model.

Some comments:

Comment 1: Figure 2 is two photos from digital camera and one from SEM.
In the SEM figure, neither the microstructure nor the morphology of the material can be seen. Please include * or any other sign to indicate where the HA is and where the PLA is. Put a photo at lower magnification to see the morphology. How have the authors determined the pore size and porosity data? By the SEM photo of figure 2 no. Authors are requested to include mercury porosimetry and helium pycnometry to determine the different porosity range and pore / s size. Please include an SEM photo showing porosity and some other pores. The two digital camera photos do not add anything. Indicate the size of the test mataerial in the text (diameter and length) and delete these photos. Instead put more SEM photos to appreciate the microstructure and morphology of the implants as stated in the figure caption, since they are not currently observed.

Reply 1: We thank the reviewer for their suggestion. In this study, μCT and SEM indicated that 3D-printed PLA-HA scaffolds were hollow cylindrical structures similar to a long bone, possessing a porous wall structure with a pore size of 500 μm and 60% porosity. Pore size and porosity data were tested by μCT. The 2 digital camera photographs were deleted. A lower magnification photograph of the scaffold tested by μCT is shown in Figure 2. Figure 2 has been modified as advised.

Changes in the text: We have modified the text as advised (see Figure 2, and lines 395–397).

Comment 2: In line 194 the authors indicate "long bone possessing porous wall structure with a pore size of 500 and 60% porosity," In the SEM figure this porous wall structure is not observed. The authors are kindly requested to provide proof of

this.

Reply 2: We thank the reviewer for their insightful comments.

Changes in the text: We have modified our text as advised (figure 2)

Comment 3: Put a SEM photo of the PLA-HA / eBM microstructure to see the difference between both materials and that the BM have effectively adhered to the PLA-HA.

Reply 3: We thank the reviewer for their insightful comments.

Changes in the text: We have modified our text as advised (Figure 2D)

Comment 4: In line 96-99 the authors indicate “High-resolution Micro-CT (μCT, Switzerland) was used to assess porosity and average pore size of the scaffolds; Data were analyzed by μCT. "Please include detailed figures of pores and porosity. There must be a deviation and a ± in the results. Or are all pores the same? Put a photo that shows the data of porosity and pore size.It is also not clear for me the choice of the time periods (4 and 8 weeks), could the authors please explain in the M&M section? Include the commercial name of the equipment used in the characterization of the implants. Put magnification in figure 4 A

Reply 4: We thank the reviewer for their insightful comments. We have modified the text as advised.

Changes in the text: We have modified the text as advised (see Figure 2D, line 31 line 171, and line 228).

Comment 5: In line 195-196 the authors indicate "which not only provide the

adequate room for eBM, but offer the surface of attachment for MSC to proliferate and differentiate."If the material that the authors have developed is not good to put with eBM, why do the authors continue with the study?

Reply 5: We thank the reviewer for their comments. In the sentence that read, 'which not only provide adequate room for eBM, but also offer the surface of attachment for MSCs to proliferate and differentiate', the authors' original intent was to state, " scaffolds can provide adequate room for eBM and an attachment surface onto which MSCs can proliferate and differentiate". To eliminate any potential misunderstanding, we have modified the text accordingly.

Changes in the text: We have modified our text as advised (line229-234)

Comment 6: Given this information, my question is how much eBM is impregnated in PLA-HA before being implanted? The authors are requested to give proof of this.

Reply 6: Our method was as follows: eBM was injected into the hollow tunnel of the scaffold with a 10-ml syringe. Subsequently, the scaffold material was soaked in the tube for eBM and mixed well for 10 minutes. The whole scaffolds were then filled with eBM.

Comment 7: Starting on line 201 is a repeat of the results, without giving any reason for them. Without comparing with other authors… .. The discussion does not exist. The authors limit themselves to presenting their results without commenting on them or giving reasons for their behavior. Authors are kindly requested to re-write the discussion section, both in depth and length comparing with other authors and giving reasons for their results.

Reply 7: We thank the reviewer for their insightful suggestion; we have revised the

discussion section accordingly.

Comment 8: In line 219 the authors indicate “In the present study, we just preliminarily study the effect of eBM in conjunction with PLA-HA. In the following days, the study will be performed to choose the better material structure. The work is so preliminary that I would wait to have everything before dividing into many minor publications what could be a much better paper. On the other hand, the authors do the opposite of what should be done in the development of a material. First you have to obtain and characterize the material, then in vitro testing and finally in vivo testing. The authors have started the house from the roof.

Reply 8: We thank the reviewer for their comments; we have deleted the improper description in the revised manuscript.

Reviewer D

Zhiqing Liu and colleagues report about the findings of an animal model aiming to explore the effect of enhanced bone marrow in conjunction with three-dimensional Polylactide –Hydroxyapatite scaffolds on repairing critical size bone defects of the radial bone. They conclude that eBM can enhance the capacity of PLA-HA for bone repair and regeneration by radiological, μCT, histological methods. Therefore, eBM in conjunction with 3D PLA-HA scaffolds may be a promising way for the treatment of critical size bone defects.

In summary, this is well structured manuscript of a well conducted animal study with a relevant clinical topic.

some specific comments:

Comment 1: please correct the grammar if the first sentence of the discussion

Reply 1: We thank the reviewer for their advice. The manuscript has been reviewed and polished accordingly.

Comment 2: conclusion: …radiological, μCT, histological methods. Please correct …radiological, μCT and histological methods.

Reply 2：We thank the reviewer for their advice.

Changes in the text: We have modified the text as advised (see lines 275–277).

Comment 3: Figure5: in (A) three 3D and longitudinal sections are shown. To which treatment group do they belong? Please clarify in the figure or the caption.

Reply 3: Figure 5 has been modified as advised.

Changes in the text: We have modified our text as advised (figure 5, line 412-416)

Comment 4: line 146: with a pore size of 500 and – please correct: with a pore size of 500 μm and

Reply 4: We thank the reviewer for their insightful comments.

Changes in the text: We have modified our text as advised (line171)

Reviewer E

Comment: Authors tried to demonstrate the bone regeneration capability of 3D-printed PLA-HA in conjunction with reaming debris of bone marrow. The theme was relevant to the scope of Annals of Translational Medicine. The bone regeneration capability seemed to be enhanced by addition of eaming debris of bone marrow. But,

I don't really understand the difference between it and autologous bone. There was no novelity in this manuscript. I did not find any scientific significant.

Reply 1: We thank the editor for their insightful comments. Traditionally, the most common harvesting site for autologous bone graft has been the iliac crest. However, there have been concerns about iliac crest bone grafts owing to the limited bone volume and the considerable donor-site morbidity[8]. Recently, a reamer-irrigator-aspirator device (Synthes, Inc., West Chester, PA) has become available to surgeons as an alternative way of collecting bone graft material[9]. In fact, eBM is an alternative autologous bone graft harvested from the bone cavity, which overcomes some of the drawbacks of iliac crest bone grafts and has superior osteogenic properties compared to the iliac crest bone graft[6, 8, 10]. We have modified the text to make this clear.

Changes in the text: We have modified the text (see lines 47–51)

Reference

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