Dear Editor,

Thank you for the opportunity to revise and resubmit our manuscript to World Journal of Stem Cells. We have addressed the reviewers' comments (please see below) and have re-formatted the document according to World Journal of Stem Cells standards. We believe these amendments have significantly improved our manuscript and it will be now acceptable for publication in your journal.

Kind regards,

Elena Jones

Reviewer 1

Comment:

The authors define the main findings from multiple manuscripts that examine the benefits of platelet lysate in wound healing; however, the collective body experimentation is limited. The submitted manuscript has the following concerns: - The manuscript contains a significant number of one-sentence paragraphs. It significantly disrupts the flow and appears to be a series of loosely connected facts.

Response:

We thank the reviewer for this valuable suggestion. One short paragraph (on chemokines in PL) has been re-written to include a concluding sentence. Other short paragraphs have been merged with the paragraphs below.

Comment:

The entire document should be rewritten with better transitions so that it flows and is more coherent.

Response:

We have improved coherence by adding concluding sentences to the paragraphs describing chemokines present in PL and PL-loaded nude scaffolds. Sentences relating to ABO blood group antigens have been moved to section **THE USE AND BENEFITS OF HUMAN PLATELET-RICH PLASMA AND PLATELET LYSATE AS FCS REPLACEMENTS.** In the end of the manuscript we also added an overall concluding statements relating to the lack of clinical studies that used PL for bone repair.

Comment:

While there is a great deal of information here it is poorly organised. It is difficult to see what in vitro and in vivo work has been performed. Is this based on pre-clinical work or clinical studies?

Response:

We endeavoured to structure our manuscript and organise information by using several Tables and Figures. We used the word 'preclinical' in Table 2 heading in our original submission however we have now further emphasised this by also adding the word 'preclinical' in the corresponding manuscript text. Also we now addressed this in Table 1 where preclinical studies have now highlighted in bold. We have also added overall concluding statements to emphasise current scarcity of clinical studies in this field.

Reviewer 2

Comment:

There are some studies try to establish standard clinical-grade PRP or plus MSC in recent years. These important reviews and original articles should be included, for example, Tissue engineering Part B, Reviews. 2014;20(3):200-5. New biotechnology. 2015;32(1):199-211. Journal of translational medicine. 2015;13:232. Immunology letters. 2015. Stem cell research & therapy. 2015;6:6.

Response:

We thank the reviewer for pointing this out. These important reviews have now been cited

12. Shih, D.T. and T. Burnouf, *Preparation, quality criteria, and properties of human blood platelet lysate supplements for ex vivo stem cell expansion.* N Biotechnol, 2015. **32**(1): p. 199-211. [PMID:21951067 DOI:10.1089/ten.tec.2011.0308].

22. Stroncek, D.F., et al., *Establishing a bone marrow stromal cell transplant program at the National Institutes of Health Clinical Center.* Tissue Eng Part B Rev, 2014. **20**(3): p. 200-5. [PMID:24368014 DOI:10.1089/ten.teb.2013.0529].

23. Luzzani, C., et al., *A therapy-grade protocol for differentiation of pluripotent stem cells into mesenchymal stem cells using platelet lysate as supplement.* Stem Cell Res Ther, 2015. **6**: p. 6. [PMID:25582222 DOI:10.1186/scrt540].

24. Riordan, N.H., et al., *Scalable efficient expansion of mesenchymal stem cells in xeno free media using commercially available reagents.* J Transl Med, 2015. **13**: p. 232. [PMID:26183703 DOI:10.1186/s12967-015-0561-6].

Comment:

In addition, the classification and comparison of individual studies should highlight the main findings and give clear conclusions, which may help readers to know the current status and unsolved problems in this field.

Response:

In response to this comment, we have added several concluding sentences (to sections on chemokines present in PL and on PL-loaded nude scaffolds) as well as overall concluding statements, which now read: 'Finally, while the existing pre-clinical evidence on the use of PL-expanded MSCs or PL-coated scaffolds is encouraging, clinical studies investigating the benefits of PL-based products for improving bone regeneration are still lacking. Such evidence is definitely required to progress further in this field'.

Comment:

Reference #21 concluded that PL-serum, similar to PL-plasma, can substitute for FBS in hMSC cultures, so, it did not support the authors' worry about the use of heparin.

Response:

We agree with the reviewer and the sentences in question have been re-written. The last sentence in this section now reads: 'These concerns are avoided with the use of the human origin heparin or by preparing PL from serum instead of plasma(21)'.

Comment:

Most of the orthopaedic surgery that needs artificial grafts are delayed or optional operation. The scaffold could be pretreated with PRP days before or with autologous MSCs simultaneously weeks before the operation. The authors' concern on instant-available PL-loaded scaffolds is not a common situation.

Response:

To address this comment, we have now re-written relevant section and removed a sentence on instantly-available PL-loaded scaffolds. Sentence is question now reads:' This can be split into two long term areas of investigation, primarily, large scale production of allogenic PL and secondly, fast small scale production of autologous PL exclusively for use in acute surgery'.

Comment:

A lot of missing spaces between words and after punctuations.

Response:

This has now been addressed.

Reviewer 3

Comment:

Bone regeneration following trauma, tumour resection and degenerative bone disease is a key clinical problem. How to improve bone regeneration efficiency is a hot research topic. So far, the use of autograft bone or its substitutes supplemented with bone-specific growth factors and/or osteogenic cells such as mesenchymal stem cells (MSCs) is common strategy. Recent studies that human platelet lysate (PL) is an effective alternative to FCS, displaying enhanced proliferation ability while keeping osteogenic differentiation capacity. Limited pre-clinical investigations support in vitro finding and potential clinical application. Moreover, PL-coated

scaffolds without seeded MSCs demonstrated equal new bone formation and vascularisation in vivo. Together, platelet lysates contain rich bioactive factors that act synergistically to facilitate MSC attachment, proliferation and differentiation, thus it provides an exciting medium for expanding mesenchymal stem cells and functions as scaffold coating for bone regeneration. The review by Dr. Ala Altaie summarized the advancement in basic research and preclinic study on bone regeneration using PL. The review also analysed the questions about quality control and direction on how to transform this research into clinical application. For my opinion, it is a high quality review.

Response:

We thank the reviewer for his positive appraisal of our work.

Reviewer 4

Comment:

I recommend to the authors to present a summary of factors present in PL as a table.

Response:

In response to this comment, we have modified Table 1 to include an additional column on PDGF levels in reported PL preparations, which is one of the better reported growth factors present in PL. Regarding other factors, the available information is very scattered and inconsistent due to using variable PL preparation methods as well as growth factor measuring methods. The other main growth factors, chemokines and attachment factors present in PL are listed in the text.