Reviewer's code: 02446319

SPECIFIC COMMENTS TO AUTHORS

Thank you for great manuscript about Regenerative Therapy of Platelet Rich Fibrin Combined with Allogenic Bone Marrow-Derived Stem Cells on Rats' Critical Sized Mandibular Defects. Your results is almost histological data. if possible, I think it's more valuable manuscript if there are some Radiologic data (CT) .

This paper was extracted from a thesis produced by Muhammad A. Awadeen as a part to fulfill his requirements of obtaining MSC degree in Oral Biology. Actually, it is not in our plan to do a radiological analysis, but the supervisors asked the candidate to involve a CBCT image at least for the rats' critical sized mandibular defects according to the request of the respected reviewer.

Reviewer's code: 02567328

SPECIFIC COMMENTS TO AUTHORS

In this manuscript the authors report data regarding the use of Platelet rich fibrin (PRF) combined with mesenchymal stem cells (MSCs) for mandibular defects. The use of PRF alone or in association with different type of MSCs for bone defects and regeneration are already reported in literature. The results described in the manuscript are interesting but does not add anything new.

The authors added a paragraph in the introduction section reporting the rational to doing such preliminary study (previous studies had been reported on the use of a combined therapy of MSCs with PRF concentrate for the treatment of articular cartilage defects, mandibular reconstruction and regeneration, alveolar bone defects and clefts, tibial bone defects and bone remodeling. However, none of them performed their experimental work on critically sized defect model.

It would be interesting if the authors identified how the presence of the MSCs makes it more effective bone regeneration in the presence of PRP. In the text the authors state" PRF membranes released autologous growth factors gradually expressed a stronger and more durable effect on proliferation and

differentiation of rat osteoblasts". MSCs improve release of these growth factors or they themselves produce other factors that in sinergic way promote bone regeneration?

This paper was extracted from a thesis produced by Muhammad A. Awadeen as a part to fulfill his requirements of obtaining MSC degree in Oral Biology. The shortage in our study regarding this respected point was acknowledged in the conclusion section.

The article must be improved by adding new data, so there are only preliminar data and a deepening is required . Moreover: - To which passage were the MSCs used? -For complete MSC characterization, it is necessary demonstrated also their ability to differentiate in mesengenic lineages - Why the authors did not use alizarin red as a dye to demonstrate bone formation? Alizarin Red is used to determine (qualitatively and quantitatively) the presence of calcific deposition by cells of an osteogenic lineage also at early stage.

BMSCs were seeded on PRF at passage 3 (clarified in the revised version). The in-vitro differentiation of BMSCs to osteogenic lineage has been performed using alizarin red and an inverted microscopic image has been added in the revised version.

Reviewer's code: 00504800

SPECIFIC COMMENTS TO AUTHORS

The manuscript by Awadeen et al. demonstrates that platelet rich fibrin (PRF) membranes seeded with MSC has the potential to heal critical-sized bony defects, using a rat mandibular model. Overall the manuscript is easy for the reader to follow. The study is statistically well powered and the description of the analysis is good (with one question, noted below). I think the manuscript is worthy of publication once several issues are addressed.

All concerns about the manuscript have been resolved and addressed in the revised version.

1. For the most part, the differences between treatment groups appear to be distinct, and the statistics well done. I am not a statistician, but I question the

use of the LSD post-hoc test. The LSD is most liberal post-hoc ANOVA test, meaning that it will find the most comparisons between groups to be significant. A good example of this is in Table 2, Week 1, LSD comparing groups II and III in which a difference between 0.9 +/- 0.03 and 0.88 +/- 0.02 was found to be significant, which is hard to believe. Is there another post-hoc test (such as Tukey's) which is more stringent that could be applicable here?

Row data were fed again to the SPSS software and two-way ANOVA statistical test was performed followed by Tukey's post-hoc test. Therefore the post-hoc test for two group comparison revealed non-significant difference between group II and group III at 1 week examination period, corrected in the revised version.

2. Is it known which passage the BMSC were at the time of thawing, and at the time of the in vitro experiments? It is not clearly stated in the Methods how long, or for how many passages, BMSC were cultured after thawing.

BMSCs were cryopreserved at the second passage (clarified in the revised version). The sub-cultured primary cells were purchased after six months of cryopreservation (clarified in the revised version). BMSCs were seeded on PRF at passage 3 (clarified in the revised version).

3. The characterization of MSC surface markers (page 9) is not well written, and the analysis does not clearly demonstrate consistent MSC marking. At first it sounds as if all antibodies are FITC conjugated, but then some PE conjugates are noted.

After consulting the person who in charge for cytometric performance and analysis, He indicated that two flow cytometers were used for characterization of BMSCs. FACS was used for analyzing CD34 and CD45 in a double staining method while BD Accuri C6 flow cytometer was used for analyzing CD14, CD19, CD44, CD105 and CD90 (clarified in the revised version).

For the histograms in Figure 2, CD34 and CD45 are usually <5%, CD90 and CD105 are usually >95%; see Alge DL et al., J Tissue Eng Regen Med 2010, among many others, for examples.

A paragraph was added at the end of the discussion section clarifying the reason of higher profile in the negativity of CD34 and CD45 and a lower profile in the positivity of CD14, CD19, CD44, CD105 and CD90.

In Figure 2D, a vast majority of the cells shown in the scatter plot are positive for CD34, CD45 or both, and I don't understand how the authors can state that only 6.8% of the cells are negative for these markers.

The dot histogram was included mistakenly and the original one has been added in the revised version.

The histograms for CD105 and CD90 are very poorly positive compared to other studies (for example, Alge DL et al. 2010). The results shown are not consistent with what others have reported for rat BMSC.

A paragraph was added at the end of the discussion section clarifying the reason of higher profile in the negativity of CD34 and CD45 and a lower profile in the positivity of CD14, CD19, CD44, CD105 and CD90.

Adding isotype control curves to the histograms would also be helpful.

The kind of isotype control was added in the revised version (Normal rat IgG-Peridinin chlorophyll protein complex was used as an isotype control to differentiate non-specific background signal from specific antibody signal).

4. Figures 3-5: While in some cases it is clear to the reader where the bony defect is located, if the authors could draw a circle to delineate the defect areas from the neighboring normal bone, particularly for group III where new bone growth is apparent, it would be helpful.

A circle was drawn up for figures 3-5 delineating the defect areas from the neighboring normal bone, particularly for group III at 1, 2 and 4 weeks' time periods.

5. Four weeks is a relatively short period for bony defect healing. Is there a reason (other than cost and expediency) that the authors sacrificed the animals at 1, 2 and 4 weeks instead of, say 2, 4 and 8 weeks, to see more complete bone healing?

The title was modified to be more appropriate with the short follow up period

to be "Early Therapeutic Effect of Platelet Rich Fibrin Combined with Allogenic Bone Marrow-Derived Stem Cells on Rats' Critical Sized Mandibular Defects" instead of "Regenerative Therapy of Platelet Rich Fibrin Combined with Allogenic Bone Marrow-Derived Stem Cells on Rats' Critical Sized Mandibular Defects".

Minor comments/corrections: Line 192: "Fluorescein" should be "fluorescence"

Corrected in the revised version.

Lines 288-301: This would be easier for the reader to follow if the authors consistently called the groups I, II, and III, and gave more specific figure information - for example, at end of line 291 say (Fig. 4A-C); say (Fig. 4D-F) at the end of the sentence on line 297.

Modified in the revised version.

Line 342: …when they ARE used as a…

Added in the revised version.

Line 347: …growth factors , WHICH gradually…

Added in the revised version.

Line 355: Please revise the sentence beginning "A progressive polymerization…", it is confusing as written

The sentence was re-written to be more clear and easy understandable.

Line 368: …membrane IS superior…

Added in the revised version.

Line 369: …proliferation , and IS suitable…

Added in the revised version.

Line 387: performed is not the right word; detected would be better

Corrected in the revised version.

Line 397: The statement that macrophages decreased after the 4th week of the study is not correct, since the study only went for 4 weeks – please revise.

Corrected in the revised version.

Line 399: Delete “Glynne”

Deleted in the revised version.

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JOURNAL EDITOR-IN-CHIEF'S REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 47129

Title: Early therapeutic effect of platelet rich fibrin combined with allogenic bone marrow-derived stem cells on rats' critical sized mandibular defects

Journal Editor-in-Chief (**Associate Editor):** Shengwen Calvin Li

Country: United States

Editorial Director: Jin-Lei Wang

Date accepted review: 2019-09-06 21:01

Date reviewed: 2019-09-06 21:02

Review time: 1 Hour

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	[ ] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Rejection
[ ] Grade D: Fair		[Y] Minor revision
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Major revision

JOURNAL EDITOR-IN-CHIEF (ASSOCIATE EDITOR) COMMENTS TO AUTHORS

EIC comment: English language and style are fine-tone/minor spell check required for clarity. There are numerous typographical/grammatical errors (also incorrect punctuation with abbreviation) throughout the Manuscript (108 examples as marked by red track, but not an exhaustive presentation, are attached for your reference in a separate e-mail to the WJSC Editorial Office).

All required changes edited by the EIC regarding English language editing have been accepted in the revised version. Thanks for great efforts improving our manuscript.

Data presentations should be enhanced for readability by addressing specific critiques as below. Specific comments:

1) Fig. 2: they should use arrow-heads, pointing out the areas. Scale bars should be used.

Arrows have been added for figure 2A and a scale bar has been added for figure 2B.

2) Fig. 3: Scattering dot plots? Selection of a population?

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Selection of population has been added for dot plot (Figure 3D); CD34-PE (6.8%) and CD45-FITC (6.8%).

3) Fig. 4: Can they double-check the marks? E.g., O: in H showing intense staining, while in O in G showing blank/empty spot? Any references or alternative confirmation? Ideally, they should use arrow-heads, pointing out the areas. .Scale bars should be used.

Arrow heads have been added instead of letters for clarification and scale bars have been added to all the figures that contained in the panel of figure 4.

4) Fig. 5: Can they double-check the marks? E.g., O: in G showing intense red staining, while in O in I showing blank/empty spot? Any references or alternative confirmation? Ideally, they should use arrow-heads for pointing out the areas. Scale bars should be used.

Arrow heads have been added instead of letters for clarification and scale bars have been added to all the figures that contained in the panel of figure 4.

5) Fig. 6: Ideally, they should use arrow-heads, pointing out the areas. Scale bars should be used.

Scale bars have been added to all the figures that contained in the panel of figure 6.

6) Can they use bar illustrations for better clarity for Tables 1, 2, and 3?

Bar charts have been added in the final version of the manuscript.

7) Discussion: First paragraph – "a therapeutic treatment" - "Tautological phrases such as final outcome, red in color, and I personally express the same information twice. Avoid using repetitive words when writing, especially in formal situations."

Corrected in the revised version of the manuscript.