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PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 80209

Title: Optimal concentration of mesenchymal stem cells for fracture healing in a rat model with long bone fracture

Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05935626
Position: Peer Reviewer
Academic degree: DDS, Doctor, MD
Professional title: Doctor
Reviewer's Country/Territory: Indonesia
Author's Country/Territory: South Korea

Manuscript submission date: 2022-09-27
Reviewer chosen by: AI Technique
Reviewer accepted review: 2022-09-30 16:17
Reviewer performed review: 2022-10-03 16:51
Review time: 3 Days

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

I would like to congratulate the authors for this manuscript. The study is interesting. I have some comments: Methods: Regarding the dose, how many volumes (with and without MSCs) was administered into the fracture site? Is the volume based on previously published study? If so, please refer the study. Regarding the model of purposely making the fracture of femur, was this model based on previously published study? if so, please refer the study. Following the fracture, were fixation and stabilization of fracture done or not? If they were done, please add in the methods. If not done, please give the reasoning. Since saline was used as scaffold / carrier of MSCs, how did you manage the MSCs to remain at the fracture site and not leaked into the surrounding areas? For the western blot and RT-qPCR analysis, which group and how many animals were allocated for these evaluations? Results: Regarding figure 1 and 2, please add arrows to point out, and re-aligned the figures in full (proximal-distally). Regarding figure 3 and 4, please arrange the figure to be in the same direction and same magnification, and please point out where the fracture was located. References: Please use the latest references. Please recheck and correct the mistyped words. Line 69: At 2 weeks post-fracture...; Line 85: osteogenesis and...

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Reviewer's code: 03712811
Position: Editor-in-Chief
Academic degree: MD, PhD

Professional title: Director, Full Professor

Reviewer's Country/Territory: Italy

Author's Country/Territory: South Korea

Manuscript submission date: 2022-09-27

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-09-27 21:56

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Review time: 6 Days and 13 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In this study, the Authors aimed at defining the proper and optimal concentration range at which mesenchymal stem cell (MSCs) may prove effective in promoting fracture healing in a rat model of nonunion long bone fracture. They used three different MSC concentrations, referred to as Low (L) (2.5 × 106), Medium (M) (5.0 × 106), and High (H) (10.0 × 106), injected directly into the fracture site, and compared the healing outcome with reference to the control group (C), injected with saline. An inter-group (M, H, and L) comparison was also performed. Micro-computed tomography (CT) was used to assess new bone formation, in terms of bone volume (BV) and percentage bone volume (PBV). Histological analysis was performed to evaluate a fracture healing score. The protein expression of factors related to MSC migration (stromal cell-derived factor 1 [SDF-1], transforming growth factor-beta 1 [TGF-β1]) and angiogenesis (vascular endothelial growth factor [VEGF]) was evaluated using western blot analysis. Real-time PCR was used to investigate the gene expression of bone morphogenetic protein-2 (BMP-2), TGF-β1 and VEGF. The Authors found that: (i) BV and PBV were significantly increased in groups M and H, as compared to group C at 6 weeks post-fracture, (ii) Significantly more cartilaginous tissue and immature bone were formed in groups M and H than in group C at 2 and 6 weeks post-fracture, (iii) at 2 weeks post-fracture, SDF-1, TGF-β1 and VEGF expression were significantly higher in groups M and H than in group L, (iiii) BMP-2 and VEGF expression were significantly higher in groups M and H than in group C at 6 weeks post-fracture, (iiiiii) There were no significant differences in expression levels of chemokines related to MSC migration, angiogenesis and cytokines associated with osteogenesis between M and H groups at 2 and 6 weeks post-fracture. The Authors

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conclude that a concentration of 5.0 × 106 MSCs was optimal to promote fracture healing in a rat model of long bone fractures. The issue approached by the Authors is no doubt of relevance within the field of regenerative medicine. A major point is the observation that there is a clear-cut "watershed" among the MSC concentrations used, where L MSC started a rescuing, but incomplete repair, which was fully executed at M and H MSCs. In fact, it appears that L-related improvement at 6 weeks post-fracture didn't yield union, while union of fracture fragment with immature or mature bone was evident in a concentration-dependent fashion, in the presence of M, and even more H MSCs, as it is suggested by the histological analyses, and by the calculation of the respective histological scores. As an important point, the Authors should clearly report whether, in spite of the histological scores, they observed a complete ossification at the fracture site, an observation of major clinical implication. There are some major points that are not addressed in this study, which should be addressed in a revised version: - The Authors did not assess at what extent the injected MSCs were retained within the recipient tissue. For instance, Huo Z et al., used MSCs carrying a reporter gene to detect engrafted donor cells in recipient mice tissues and fractured bone. In the absence of data showing the putative concentrations of injected MSCs within the fractured recipient tissue, it's difficult to correlate the observed outcome, such as fracture healing, to the concentration of MSC in the delivery buffer. This point should be addressed in the Discussion section, and listed among the limitations of this study. - While the protein expression level of SDF-1, TGF- β1, and VEGF, and the relative western blots were shown from specimens at 2 weeks post-fracture, the Authors did not show similar protein expression analyses from specimens at 6 weeks post-fracture. For this time point, only mRNA expression data are provided. This is a relevant point. In order to attribute the major rescuing effect of M and H MSCs to the indicated growth factors, since the highest histological scores with these cell concentrations were achieved at 6 weeks (at 2 weeks, with both M and H

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MSCs union had not occurred yet), the Authors need to show protein expression data at the later observational point of 6 weeks. Providing gene expression results at this time point is interesting, but it is well known that quite often changes in gene expression are not matched by concomitant changes in protein expression levels. - Another important issue is that the changes in VEGF mRNA expression are not substantiated by experiments showing whether increased VEGF gene expression was associated to an enhanced vascularization at the fracture site. Showing these data at the histological level is extremely relevant, as it may create an effective link between the concentration of the injected MSCS, and the extent of bone healing. This point again reminds the importance of assessing the protein expression level at 6 weeks, showing that the observed increases at 2 weeks were not a transient phenomenon. On the whole I believe that the manuscript may be reconsidered after a major revision process, taking into account the above reported criticisms and suggestions.

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Stem Cells*

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Position: Peer Reviewer
Academic degree: DDS, Doctor, MD
Professional title: Doctor
Reviewer's Country/Territory: Indonesia
Author's Country/Territory: South Korea

Manuscript submission date: 2022-09-27
Reviewer chosen by: Jing-Jie Wang
Reviewer accepted review: 2022-11-02 14:55
Reviewer performed review: 2022-11-02 16:17
Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[Y] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Thank you for the revision and improvement of the manuscript. However, please correct the mistyped words in the author contribution section: provised, and in the core tip section: osteogesis.