

Response to Reviewers Comments Manuscript # 85023

Reviewer #1:

Specific Comments to Authors: Comments to the Author: This paper describes the effects of different concentrations and different time spans of zinc on hUC-MSCs cell proliferation, division and various cell function. Markers of different physiological processes were used to verify effects on cell cycle, proliferation and migration functions.

Thanks to the reviewer for understanding the importance of the topic. We acknowledge the reviewer for critically analyzing our work and suggesting improvements.

Reviewer #2:

Specific Comments to Authors: Hello In this study, the researchers investigated the effect of different concentrations of zinc on mesenchymal stem cells, and their results are interesting. They found that Zinc enhances the cell adhesion, migration, and self-renewal potential of hUC-MSCs. Some things are suggested to improve this manuscript.

Thank you very much for the in-depth review of the manuscript and for highlighting the shortcoming, which helped us to improve the manuscript. We have incorporated all the changes as mentioned and suggested by the reviewers. Concerns raised by the reviewers have also been justified. The reviewers will now find the manuscript substantially revised concerning the suggestions.

Changes made in manuscript are highlighted in blue color.

- 1- The introduction section of the manuscript is long and it is suggested to be summarized. We have modified the introduction section and the background information was reduced.
- 2- In the methodology section, it seemed better that the researchers used mechanically and enzymatically digested tissues instead of chopped pieces of tissue to increase the number of cells obtained. We have optimized the protocol for the isolation of MSC from umbilical cord tissue. This method perfectly works, and we have characterized the isolated cells according to ISSCR criteria. This method is previously reported in several publications and cited in the method section. A few references are given below.

Aslam, I. Khan, F. Jameel, M.B. Zaidi, A. Salim, Umbilical cord-derived mesenchymal stem cells preconditioned with isorhamnetin: potential therapy for burn wounds, World J. Stem Cells. 12 (2020). <https://doi.org/10.4252/wjsc.v12.i12.1652>.

S. Khalid, S. Ekram, F. Ramzan, A. Salim, I. Khan, Co-regulation of Sox9 and TGF β 1 transcription factors in mesenchymal stem cells regenerated the intervertebral disc degeneration, *Front. Med.* 10 (2023). <https://doi.org/10.3389/fmed.2023.1127303>.

N. Beeravolu, I. Khan, C. McKee, S. Dinda, B. Thibodeau, G. Wilson, M. Perez-Cruet, R. Bahado-Singh, G.R. Chaudhry, Isolation and comparative analysis of potential stem/progenitor cells from different regions of human umbilical cord, *Stem Cell Res.* 16 (2016) 696–711. <https://doi.org/10.1016/J.SCR.2016.04.010>.

3- It is not explained in the methodology section how many times the cells were passaged. We have added this information in the methodology section 2.1. hUC-MSCs upto passage 5 were used in the experiments.

4- To increase the study's validity, it is better for the researchers to mention the references based on which they isolated and cultured mesenchymal stem cells and determined their characteristics.

The references for the isolation and characterization of MSCs are cited in the revised version.

Aslam, I. Khan, F. Jameel, M.B. Zaidi, A. Salim, Umbilical cord-derived mesenchymal stem cells preconditioned with isorhamnetin: potential therapy for burn wounds, *World J. Stem Cells.* 12 (2020). <https://doi.org/10.4252/wjsc.v12.i12.1652>.

S. Khalid, S. Ekram, F. Ramzan, A. Salim, I. Khan, Co-regulation of Sox9 and TGF β 1 transcription factors in mesenchymal stem cells regenerated the intervertebral disc degeneration, *Front. Med.* 10 (2023). <https://doi.org/10.3389/fmed.2023.1127303>.

N. Beeravolu, I. Khan, C. McKee, S. Dinda, B. Thibodeau, G. Wilson, M. Perez-Cruet, R. Bahado-Singh, G.R. Chaudhry, Isolation and comparative analysis of potential stem/progenitor cells from different regions of human umbilical cord, *Stem Cell Res.* 16 (2016) 696–711. <https://doi.org/10.1016/J.SCR.2016.04.010>.

5- In the immunophenotyping section, it is better to report the companies from which the antibody was purchased.

We added the antibodies description in the methodology section. A kit (Cat # 562245, BD Bioscience) was used for the immunophenotypic characterization of MSCs. Similarly, the details of antibodies used for immunocytochemical staining of MSCs were also added.

CD73 (550256, BD Pharmingen, USA), CD117 (32-9000, Zymed Laboratories, Inc., USA), CD29 (MAB-1981, Chemicon International, USA), CD105 (560839, BD Pharmingen, USA), Stro1 (14-6688-82, Molecular Probes, Invitrogen, USA), Vimentin (V6389, Sigma-

Aldrich, Inc., USA), Lin28 (PA1-096, Molecular Probes, Invitrogen, USA), and CD45 (CBL415, BD Pharmingen, USA).

- 6- It is better to report the results as standard deviation (SD) instead of the standard error of the mean (SEM).

Standard deviation is added to the results.

7. In the discussion section, it is better for the author to focus on zinc and its research on stem cells and discuss less about the umbilical cord. The discussion is long.

The unnecessary details from the discussion were removed and only zinc is focused. The paragraph which discussed the isolation and characterization of MSCs was removed from the discussion.

- 8- In the conclusion section, the Authors predicted: "The results also indicate the significance of the careful consumption of zinc supplements as a lower concentration of it positively regulated growth, but its higher concentration may retard or negatively affect growth and development." This study was conducted in vitro, and this prediction cannot be convincing.

This statement is removed in the conclusion section.

- 9- It is better to mention the limitations of the study in the discussion section.

A paragraph about the limitation of the study is added to the discussion section.