

## Cover Letter

May 20, 2021

Dear Editor,

Thank you for the review of our manuscript entitled “**Recent advances in stem cell therapy for neurodegenerative disease: Three dimensional tracing and its emerging use**”. We are glad that reviewers found significant merits in our work. They have also made specific suggestions to improve our manuscript. We have now thoroughly revised our manuscript according to reviewers’ suggestions, which are summarized point-by-point below (red color). We trust that our manuscript is now significantly improved and is suitable for publication in the *World Journal of Stem Cells*.

Sincerely yours,

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Reviewer #1:

1. Firstly, this review has stated the advantages and disadvantages of several stem cell tracing and imaging techniques. However, it is better to make a conclusive suggestion that what kind of stem cell therapy studies are suitable for these techniques. With conclusive suggestion, this review will be more readable and relevant to general audience.

3. Thirdly, this review lumped nearly all kinds of stem cells in the part of “STEM CELL THERAPY IN NEURODEGENERATIVE DISEASE”, however, it is better to discuss which kind of stem cells might be the best choice in therapy for neurodegeneration diseases based on published studies and what the differences are in the therapeutic effects of different kinds of stem cells.

*Answer> Thanks for your suggestion. We deeply agree that a conclusive suggestion is effective in understanding the effects of stem cell to the general audience. But, there are already many review papers published and shared in this WJSC journal, so we further approached and focused on techniques that can implement a three dimensional image for the proof of concept (PoC) of stem cells based on these stem cell function. Please understand our goals for reviewing stem cell tracing.*

2. Secondly, the authors mentioned that cell tracing strategies help to answer fundamental questions regarding the cell conditions, that is, dose, time, phase. However, the authors lack relevant studies to support this statement.

*Answer> We are sorry for not being able to fully convey our conclusion and implication in this paper. We attempted to convey that three dimensional positioning technique of stem cells may play an important role in setting the cell condition for a therapeutic point, not suggesting specific condition (page 18, lane 470-473).*

Reviewer #2:

1. Tittle. In this MS, the authors used half of the space to introduce the recent progress on stem cell-based therapy on neuro-degenerative diseases, including the Alzheimer's disease, Parkinson's disease, Huntington's disease. However, only small portion of the MS was used to summarize the three-dimensional imaging for stem cell tracking related progress. The title should be changed to a more accurate one which will be coincident with the content.

*Answer> Thank you for your suggestion. We deeply agree that our title of manuscript did not focus whole contents. So we changed our title as stem cell therapy (main topic) and its tracing technic (sub-topic) (page 1, lane 4-5).*

2. The authors did not give a clearer definition about 3D image. Under the subtitle STEM CELL TRACING IN DEGENERATIVE BRAINS, the authors mentioned the imaging technology it is also part of the 3D image field.

*Answer> We are sorry for causing confusion of 3D image. But, we mentioned that 3D image technique, i.e. tissue clearing method, may use to clearly define physiological and pathological mechanism of brain, furthermore tissue clearing after stem cell labelling can also define the degenerative brain (page 16, lane 419-423; page 18, lane 455-458).*

3. The structure and flow of this review MS need to be re-organized. Introduce the 3D image on stem cell tracing systematically, including the origin, development, shortcomings and so on.

*Answer> Thank you for your constructive suggestion of our manuscript. As mentioned, the 3D image technique on stem cell tracing carefully suggested future perspective as a new way to implement 3D imaging in the brain through stem cell tracing using the intact tissue clearing technique. In other words, it is the graft of new technology, and we disclosed some of our current research strategies. Therefore, the reviewer's suggestion, including origin, development and shortcoming, do not exist, so we presented to an information (origin, development and shortcoming) of tissue clearing method by replacing a 3D imaging technique of stem cell therapy. Now, the research paper to be prepared and submitted by our*

*team will be the first research case on stem cell 3D tracing using tissue clearing. Once again, we appreciate the reviewer's suggestion, and we will try to achieve practical research results for this review.*

Reviewer #3:

1. I believe it would be useful to cover also the use and principles of in vivo imaging and particularly for imaging of transplanted NSCs using a two photon laser scanning microscope. There is bibliography on the subject (e.g. Nature 2016 Nov 10;539(7628):248-253. doi: 10.1038/nature20113.)

*Answer> We deeply agree your comment and reference, so added this matter to our manuscript for the advanced imaging of three dimensional tracing for the stem cell-transplanted brain (page 17, lane 448-449; page 30, lane 877-879).*

2. At the beginning of the sections ‘Stem cells and Alzheimer’s disease’ and ‘Stem cells and Parkinson’s disease’ it is necessary to add a paragraph describing the pathology of each condition with main manifestations regarding aggregation, oxidative stress, inflammation etc. as applicable. It will help the reader to follow the restorative potential of NSC transplantation described next in the section.

*Answer> Thank you for your comments. We also widely feel the same way as the reviewer’s suggestion. In this manuscript, pathologic changes of diseases were briefly described (lane 134-137, lane 185-191, lane 249-255) because their aspects were very diverse under the different conditions and the molecular mechanisms that accordingly act are very complex. We predicted that if the various conditions were explained, the mention of NSC transplantation functioning accordingly would increase exponentially and would require a complex understanding of the reader. Please understand our intention to briefly mention this matter in the manuscript.*

3. Pages 15-18 Stem cells derived from pluripotent ‘embryonic’ stem cells (ESCs), which are more lineage-committed adult stem cells or reprogrammed ‘embryonic-like’ pluripotent stem cells (PSCs) have been used as a therapeutic source in neurodegenerative diseases [7,8]. more lineage-committed adult stem cells : this is not correct terminology. These are not adult stem cells. What do the authors mean ‘more lineage committed’?

*Answer> We sorry for confusing our manuscript to understand because of error. This sentence was changed into correct word (page 4, lane 76).*

4. Pages 160-162 Multifunctional stem cells such as MSCs and progenitor cells can be isolated from various tissues in the adult human body and can only differentiate into cells of the first derived type. Multifunctional stem cells: do the authors mean multipotent stem cells? can only differentiate into cells of the first derived type.: It is not clear what the authors mean when they say 'cells of the first derived type'

*Answer> We changed this sentence to understand clearly MSC's function (page 6, lane 120-123).*

5. Pages 166-67 Because organizations with ineffective recovery systems cannot easily return after injury or extensive degenerative events Do the authors meant to say recover?

*Answer> This meaning refers to abnormal tissues that are difficult to recover.*