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Dr Ying Dou  
Science Editor, *World Journal of Stem Cells*

Dear Dr Dou

Thank you for the careful consideration of the manuscript (*Manuscript ID: 04023799*) and all valuable suggestions to its improvement. To address all comments systematically and in full, I revised the text and illustration thoroughly.

The main changes and additions are as follows:

- ✓ The running title has been provided.
- ✓ The author contributions have been added, as requested.
- ✓ The text has been formatted accordingly to guidelines.
- ✓ Each reference has been checked to confirm that there is no repeat, with both PMID and DOI numbers being added to.
- ✓ The illustration has been revised for colour clarity and also uploaded as a separate file, in an editable format. Apologies for the confusing colour coding.

I went to great length to deal with the comments raised by Referees in full. In brief, the main changes have included adding more detailed information to the revised text (red coloured), as suggested by Referee 2.

My point-to-point reply to Referees is appended below and also uploaded as a separate file.

I hope that the revised manuscript will be suitable for publication in *World Journal of Stem Cells*. Thank you.

Kind regards,

Dr Olga Kopach

**“Monitoring maturation of neural stem cell grafts within a host microenvironment” by Kopach O.**

## **Reply to Referees**

### **Reviewer #1:**

“The manuscript by Dr. Kopach is a timely and well-written review of the importance of host microenvironments on the maturation and function of injected stem cells.!

*I thank the Referee for the careful consideration of my work and appreciate the encouraging comment.*

### **Reviewer #2:**

“The author provides a short and general overview outlining the significance of the host microenvironment on the differentiation and integration of neural stem cell grafts. While this is an important and specific aspect the author remains rather general in its concepts without providing details on methodology and findings how to monitor the maturation of NSCs...”

*I thank the Referee for the careful consideration of the manuscript and do appreciate the comments towards its improvement. I have revised the text carefully and rearranged thoroughly to streamline the ‘details on methodology and findings’ on how monitor the maturation of NSC. I did address precisely this issue, in some detail, in the original manuscript. The additions to the revised text are as follows (the last paragraph of page 8): “Functional studies have been carried out in organotypic hippocampal slices, aimed at [...]. The experimental data from electrophysiological recordings, combined with electron microscopy and immunohistological approaches, have revealed that NSC-derived hippocampal neurons have matured electrophysiological properties, and have functionally integrated into the host circuits [...]. Next, a morphological comparison has been performed with regard to the synapses which NSC-derived neurons constituted with nearby endogenous cells...” Also, more detailed explanations of findings on the*

*maturation of NSC are as follows (page 9): “Based on the experimental data from a direct comparison between electrophysiological parameters, [...] (~70% of grafted NSCs differentiated into glia), opposing the reduced neuronal lineage (a drop from ~70% to ~30% in the proportion of NSC-derived neurons; see Figure 1)”.*

*To address the methodology, in more detail, the text has been modified to reflect these considerations as follows (page 7): “Evidence-based advances of the *ex vivo* brain tissue preparations have attracted attention to this experimental approach as an alternative to *in vivo* studies. It fulfils[...]”. A few more examples include (page 8): “[...] In this context, once again organotypic brain slices perfectly fit these studies”; (page 9): “In the post-ischemic environment (organotypic hippocampal slices subjected to ischemic conditions – oxygen-glucose deprivation<sup>[40]</sup>), NSC grafts have been largely differentiating into glia, with a prompt rise in NSC-derived oligodendrocytes, followed by astrocytes. Notably, [...]”*

*“The manuscript would benefit of providing more introductory information of the potential therapeutic mechanisms of NSC (e.g. paracrine interactions with host tissue, extensive migration).”*

*I do appreciate this comment and have put more attention to reflect that different mechanisms contribute to the therapeutic effects of NSC-based therapy. The text in its current form provides the explanations of ‘the potential therapeutic mechanisms of NSC’ as follows (page 10): “Promoted glial lineage implies the glia-mediated neuroprotective and neurotrophic supports of the oxygen-glucose-deprived endogenous neurons as the first steps of defence against the ischemic impairments. [...] the NSC-derived oligodendrocytes may constitute endogenously-driven neuroprotection by providing a metabolic supply (paracrine signaling action), for instance, via [...]. In addition to this mechanism, the revealed impact of oligodendrocytes on astroglial development<sup>[39, 48]</sup> may explain [...] to provide trophic support. The astrocytic-mediated surveillance of neurotoxic inflammation<sup>[50]</sup>, together with a high capability to taking up glutamate and potassium<sup>[51]</sup> are essential to lower excitotoxicity within the post-ischemic tissue.”*