RESPONSE TO REVIEWER 1

We thank Reviewer 1 for the constructive comments that helped us to improve our manuscript. Our responses are listed below. Corresponding changes in the manuscript have been highlighted in yellow.

GENERAL COMMENT:

The review is well written, it is interesting for experts and those interested in the subject, it is updated and deserves to be published.

RESPONSE: Thank you for your positive evaluation of our review article.

COMMENT 1:

INTRODUCTION

In TWO particular areas of the brain, neural stem cells (NSCs) are present. These cells may replace the loss of DAergic neurons in PD because they have a few key characteristics that allow them to differentiate into neurons, astrocytes, or oligodendrocytes.

RESPONSE: corrected.

COMMENT 2:

In a particular area of the brain, neural stem cells (NSCs) are present. These cells may replace the loss of DAergic neurons in PD because they have a few key characteristics that allow them to differentiate into neurons, astrocytes, or oligodendrocytes. ***These two sentences need explanation and references. Written like this, they do not agree well with later texts. Could be removed or changed.***

RESPONSE: We thank you for the comment. Please refer to the improved paragraph

Endogenous neural stem cells (NSCs) exist throughout life and are found in specific areas of the human brain. NSCs exhibiting abilities to self-renew and differentiate into neurons, astrocytes, or oligodendroglia are responsible for restoring brain function under normal circumstances. The regeneration of DAergic neurons from stem cells is considered an alternative treatment for PD [15].

COMMENT 3:

EMERGENCE OF NSC IN ADULT BRAIN AND ALTERNATIVE SOURCES OF NSC

RESPONSE: corrected.

COMMENT 4

NSC … are found in a TWO specific brain region (Figure 1A). The subgranular region of the dentate gyrus and the lateral wall of the subventricular zone both contain a significant amount of adult endogenous NSCs [24]

RESPONSE: corrected.

COMMENT 5

EXPERIMENTAL APPROACHES TO NSCs TRANSPLANTATION

The heterogeneous population of membrane-bound exosomes and extracellular vesicles (EVs) is a part of the secretome system [43]

RESPONSE: corrected.

COMMENT 6

More references and comments

- ***Comment on studies on cell therapy + gene therapy***

RESPONSE: Thank you for the valuable comment. Please refer to:

1. Newly added paragraph in the INTRODUCTION

This review focuses on the therapeutic potential of NSCs in PD. Accordingly, we overviewed approaches of their use in experimental studies and clinical trials and discussed challenges related to their application alongside the pros and cons of NSCs-based therapy in PD. Given the state-of-the-art accomplishments of stem cell therapy, gene therapy, and nanotechnology, we shed light on the perspective of complementing the advantages of each process by developing nano-stem cell therapy, also using genetically engineered NSCs.

2. Rephrased and newly added content in section EXPERIMENTAL APPROACHES TO NSCs TRANSPLANTATION

To increase trophic factor secretion, genetically engineered human NSCs were developed [36]. Stereotaxic transplantation of hNSCs (HB1.F3 clone) secreting stem cell factor into the 6-OHDA-lesioned striatum of rats has been demonstrated to result in functional improvements and ameliorated Parkinsonian behavioural symptoms. It was accompanied by the activation of endogenous neurogenesis in the subventricular zone, alongside the preservation of TH-positive cells of the nigrostriatal pathway [37]. Similarly, human olfactory bulb NSCs genetically engineered to express hNGF ameliorated the cognitive deficits associated with 6-OHDA-induced lesions in PD model rats. Notably, transplanted cells exhibiting enhanced survival and differentiation rate migrated to damaged areas to promote repair or neuroprotection through cell replacement, integration, and/or neuroprotection [38].

3. Consistently added study by Marei et al. [38] in Table 1
4. Newly added content in the section **PROSPECTS FOR NSC-BASED THERAPY**

Advances in the development of stem cell and gene therapies create new opportunities. The combination of stem cell and gene therapy could be a technical breakthrough that increases the therapeutic effectiveness of stem cells. Engineered cells overexpressing genes involved in DA synthesis or neurotrophic factors might increase their functional capability and solve differentiation and survival issues, thus improving gene therapy's efficacy [77]. Taking into account the safety of cell-based therapy the cells can be reprogrammed to avoid as many adverse effects as possible, including immune reactions and tumours [78].

5. Comment in the section **CONCLUSION**

Currently, combining stem cell therapy, including genetically engineered cells, with nanotechnology approaches to complement each other's advantages provides new insights into the improvement of the therapy protocol and efficacy.

COMMENT 7

PROS AND CONS OF THE USE OF NSCs IN PD

*Although several clinical trials have been studied using different NSCs for PD (Clinical Trials. gov; NCT02452723, NCT02795052, NCT03815071, NCT03128450, NCT03309514, NCT03724136, NCT01898390) [54]. **A table recording clinical trials, types of cells used, and interim results should be included.***

RESPONSE: Please refer to the updated paragraph and Table 3. Since interim results are not available for all presented trials, we included the aims of the studies in Table 3.

Despite these great efforts, there is still a gap between experimental therapeutic approaches and their translation into clinical practice. Although several, as depicted in Table 3, clinical trials have been studied using cell-based treatments for PD (Clinical Trials. gov; NCT03119636, NCT05635409, NCT02452723, NCT03815071, NCT03128450, NCT03309514, NCT01898390, NCT02795052, NCT00976430, NCT03724136, NCT03684122) [62] these days, the main obstacles restricting the clinical use of stem cells refer mainly to ethical concerns, immune response, tumorigenesis, and toxicity [31].

COMMENT 8

ACKNOWLEDGEMENTS

*We would like to thank their appreciation to all the scientists whose previous work contributed to this review article. **This is a very grateful acknowledgment to the researchers who preceded the authors of this work.***

RESPONSE: We appreciate the feedback.

RESPONSE TO REVIEWER 2

We thank Reviewer 2 for the insightful comments that helped us to improve our manuscript. Our responses are listed below. Corresponding changes in the manuscript have been highlighted in yellow.

GENERAL COMMENT:

The authors submitted a manuscript summarizing the challenges, pros and cons, and prospects of neural stem cells in the treatment of Parkinson's disease (PD). PD is one of the most common neurodegenerative diseases caused by specific degeneration and loss of dopamine neurons in substantia nigra of the midbrain. Cell replacement therapy is a strategy to achieve long-term motor improvements by preventing or slowing disease progression. Several promising cell sources offer authentic and functional dopaminergic replacement neurons. These cell sources include fetal ventral mesencephalic tissue, embryonic stem cells, neural stem cells, mesenchymal stem cells from various tissues, induced pluripotent stem cells, and induced neural cells.

RESPONSE: Thank you for the insightful reading of our manuscript.

COMMENT 1:

The paragraph organization of the manuscript is not very well organized. The "PARKINSON'S DISEASE AS A CHALLENGE" section and the "EMERGENCE OF NEURAL STEM CELLS" section could be simplified and combined with the "INTRODUCTION" section.

RESPONSE: In the revised manuscript, we deleted the headline PARKINSON'S DISEASE AS A CHALLENGE and its content we included in INTRODUCTION (please refer to the reworked introduction section) and a newly added paragraph at the end of the section:

This review focuses on the therapeutic potential of NSCs in PD. Accordingly, we overviewed approaches of their use in experimental studies and clinical trials and discussed challenges related to their application alongside the pros and cons of NSCs-based therapy in PD. Given the state-of-the-art accomplishments of stem cell therapy, gene therapy, and nanotechnology, we shed light on the perspective of complementing the advantages of each process by developing nano-stem cell therapy, also using genetically engineered NSCs.

According to another recommendation, EMERGENCE OF NEURAL STEM CELLS has been rephrased to EMERGENCE OF NSC IN ADULT BRAIN AND ALTERNATIVE SOURCES OF NSC.

COMMENT 2:

In the "PROS AND CONS OF THE USE OF NSCs IN PD" section, the description of ESCs and iPSCs can be deleted because it is not relevant to the topic (NSCs) of the manuscript. Individual English errors in formatting and grammar need to be corrected.

RESPONSE: We slightly reshaped the paragraph. We have retained a very brief description of ESCs and iPSCs to highlight the advantages and disadvantages of NSCs compared to other stem cell therapies, which clinical development is now presented in Table 3. Nevertheless, we are open to suggestions for further improvement of this section.

COMMENT 3: *Individual English errors in formatting and grammar need to be corrected.*

RESPONSE: corrected.