ROUND 1

March 12, 2021

Name of journal: World Journal of Stem Cells

Manuscript NO: 62663

Title: Stem cell therapy in ocular pathologies in the past 20 years

Dear Editor of WJSC,

On behalf of the other authors and myself, I would like to extend my gratitude for the efforts and time spent reviewing our submission. The Reviewers make excellent points and offer valuable suggestions to improve the manuscript. **Please find the responses in bold font under each of the comments made by the reviewer below, which can also be found in red font in the revised manuscript:**

Reviewer 1 *(code: 05084565)*

In this review, the authors reviewed stem cell therapy for the ophthalmic disorders. Major concerns

1. The review is not well organized. The review is presented as the "what, why, which, how, when and where" structure, which seems to be not very satisfactory since it results in many repeated contents in different parts and less logical of the context. Table 1 should be modified or removed according to the change of the structure. In my opinion, detailed and well-organized Tables presenting the clinical trials of different stem cells in different ophthalmic diseases might be more meaningful than the outline Table. Besides, some information seems to be not very consistent with its subtitle, for example, most of the information in part 4 seems to be not very consistent with the title of this part, and some information seems to be not appropriate to be put in this part, such as the last two paragraphs, which might be more appropriate to be put in the last part of the manuscript or in the corresponding section of the part 5.

1. The goal of this invited paper was to provide a useful review of the pertinent literature in a clear manner. The Reviewer has provided good suggestions to obtain this

aim. We have edited the paper to avoid repetition throughout the various sections of the manuscript to render the message in context with the subheadings. In accordance to Reviewer 1, Table 1 has been removed and substituted with Tables 2 and 3, which lists clinical trials based on ophthalmic diseases. The information in parts 4 and 5 has been modified to render the sections more pertinent with the subtitles, as suggested. Limitations and future prospectives have been modified, corrected and better explained, as suggested.

2. It is confusing that the authors considered only studies referring to humans and the title of part 4 is "how … in clinical research", while they mentioned in the abstract "literatures have reported limited success of clinical trials to date", and they actually referred few clinical trials and repeatedly mentioned "in animals" in part 5, such as "Most … are on animal models", "limited … in animals", "Promising … in animals, … have not been confirmed in human trials to date"," only a few studies on animals have been reported in literature", and so on.

2. Our apologies for the confusion. All mention of studies throughout the manuscript has been changed to include both animal and human studies. Tables 2 and 3 include the type of study for the references cited.

3. There are too many reviews of the stem cells (especially in the parts of "what are stem cells" and "which stem cells can be considered for treatment in ophthalmology"), the current situation of stem cell therapy (in the introduction) and the basic knowledge of eyes and ophthalmic diseases (in part 5).

3. In accordance to the Reviewer, less important reviews have been deleted throughout the paper. For example, previous references 34, 36 and 47 have been removed and/or changed.

4. The category of stem cells seems to be not precise enough and there are repetition and confounding in different categories.

4. Modifications have been made throughout the text to improve precision, avoid repetition and correct possible inaccuracies. The paper has been shortened by about 10 % with the intention of making concepts clearer and more concise.

5. Much information is provided, while not logical (the whole context and in each single part and the above and the following sentences) and rigorous (for example, most of the information in the first paragraph of part is not specific to stem cell therapy for ophthalmic disorders, and injection seems to could be considered as a approach of transplantation) enough makes the keypoint seems to be not prominent enough and the reading not fluent enough.

5. We have tried to address these issues with the specific modifications in the text to render the information pertinent to each subheading and to improve the quality and fluency. We have eliminated redundant sections and corrected inaccuracies, especially in the sections regarding treatments.

6. The abstract, core tip and the key words are not recapitulative enough.

6. These sections have been modified to render them more relevant, with the aim of summarizing the key concepts.

7. There are many grammatical (preposition, word form, phrases & collocations, etc.) and sentence pattern and structure errors. For example, a native English speaking (should be speaker); in centred on; the main goals … include the possibility of preventing …, restoring …, recreating…, and regenerate (should be regenerating) eye tissues; in addition to …, …, dentistry etc(I think there should be an "and" before dentistry); and so on. Minor concerns 1. As the authors mentioned, this manuscript is not a meta-analysis, thus the "MATERIAL AND METHODS" part and Table 2 seem to be not necessary. In addition, "MATERIAL" should be "MATERIALS". 2. Of the phrases "ocular pathologies" and "eye pathology", it might be more appropriate to change the word "pathology/pathologies" to "disease/diseases" or "disorder/disorders" or change the phrase to "ophthalmopathy" when describe the disease; the phrases "animal clinical trials" and "animal trials" need to be reconsidered and the concepts of "animal experiment" and "clinical trial" should be distinguished. 3. In the subtitle "when can stem cells be used and in what ocular pathologies", "when can stem cells be used" is the same meaning as "in what ocular pathologies can stem cells be

used". 4. There should be references for some parts of the manuscript, and more appropriate references should be chosen for some parts of the manuscript. For example, in the second paragraph of the introduction, there is only one reference and it could be changed to a more appropriate one.

7. The grammatical errors and editing have been addressed in accordance to the useful points made by the Reviewer. We have corrected the sentences mentioned by the Reviewer. We have modified the Introduction section as requested, and inserted 7 references before the section explaining the aims of our review.

Reviewer 2 (*code: 02897448)*

Stem cells play an important role in the treatment of refractory eye diseases and bring hope to patients. It is very necessary and valuable to summarize the research results and development trend of stem cell therapy in this field. I'm very glad that this manuscript summarizes the results of stem cell therapy in ocular pathologies in the past 20 years, which provides some useful information for researchers and clinicians.

Many thanks for the positive comments made by the Reviewer.

1. However, based on the analysis of these existing research results, the authors should also put forward their own points. Some issues should be interpreted carefully before this manuscript is acceptable for publication considering the high standards of World Journal of Stem Cells. 1. In "3. Which stem cells can be considered for treatment in Ophthalmology", the authors introduce many types of stem cells used in eye diseases in this manuscript. However, the authors should discuss what are the main criteria for selecting stem cell type in clinical application, and which stem cell type has the most clinical application prospect, rather than just in animal experiments.

1. The issues regarding the important factors in the clinical application of specific stem cells in humans have been added in this section 3.5. Other considerations on this element have been added in points 4 and 5.

2. In "4. How can stem cells be used in clinical research", the authors introduce several methods of stem cell therapy in the treatment of ophthalmology, but the summary is not comprehensive enough. Due to the existence of inflammatory factors and other adverse factors in the damaged tissue microenvironment, the survival rate of directly transplanted stem cells is very low. In addition, three-dimensional grafts with stem cells might be better to be implanted for the treatment of certain diseases. Therefore, biomaterial scaffolds play an increasingly important role in the treatment of eye diseases with stem cells. The authors only mention the use of biomaterials and nanomaterials in the manuscript, but do not provide some necessary information about the main materials, scaffold types, research results, key problems and the development trend. Therefore, it is suggested that the authors summarize the combination of biomaterials and stem cells used in the treatment of eye diseases in the revised manuscript.

2. In accordance to the suggestions made by the Reviewer regarding how stem cells can be used, additional information has been added on pages 21 and 22, in the paragraph "*future prospectives*".

3. In "5. When can stem cells be used and in what ocular pathologies", the authors introduce the application of stem cells in ocular pathologies, however, it is better that the authors can point out which stem cell type might be more suitable for the treatment of a specific disease based on the analysis of the existing research results. This is very helpful to choose the most suitable stem cell type in clinical application.

3. Mention has been made regarding which stem cells are suitable in specific diseases, which has also been added in Tables 2 and 3.

4. In "6. Limits and why not", the authors suggest that researchers should pay attention to the possible side effects of stem cell therapy. It is suggested that the authors should summarize the side effects that have been found in stem cell therapy, and give some suggestions about how to reduce these side effects and improve the safety of stem cell therapy. In addition, the authors may put forward their own points about the possible methods to reduce the high cost of stem cell therapy, which is very helpful to promote the large-scale clinical application of stem cells.

4. In accordance the suggestions made by the Reviewer, additional information has been added on pages 20, 21, paragraph 6 in the section entitled "*Limits and why not*".

Reviewer 3 (*code: 02909322)*

This review is well organized to present a brief and clear overview of the main types of treatments based on stem cells in the field of human ophthalmic pathologies following these subtitles: 1. What are stem cells; 2. Why use stem cells in ocular pathologies; 3. Which stem cells can be considered for treatment in ophthalmology; 4. How can stem cells be used in clinical research; 5. When can stem cells be used and in what ocular pathologies; 6. Limits and why not; and 7. Future prospective. Up to now, the brief overview about stem cell therapies on the ophthalmic disease have been rarely summarized.

Many thanks for the positive comments regarding our manuscript.

However, several minor points should be noted as below: 1) Some important experiments in other research groups should not be omitted and need to be discussed. For example, in "5.1 Ocular surface, cornea and limbus" section, as to talking about MSCs, Ma et al. were the first to expand MSCs on HAM and subsequently transplant the construct onto the ocular surface of LSCD rats (Ma Y., Xu Y., Xiao Z., et al. Reconstruction of chemically burned rat corneal surface by bone marrow-derived human mesenchymal stem cells. Stem Cells. 2006;24(2):315–321.) ; In 2011, Reinshagen et al. injected enriched MSCs under an AMT in LSCD rabbits (Reinshagen H., Auw-Haedrich C., Sorg R. V., et al. Corneal surface reconstruction using adult mesenchymal stem cells in experimental limbal stem cell deficiency in rabbits. Acta Ophthalmologica. 2011;89(8):741–748.) Gu et al. succeeded in differentiating rabbit-derived bone marrow MSCs into corneal epithelial-like cells (Gu S., Xing C., Han J., Tso M. O. M., Hong J. Differentiation of rabbit bone marrow mesenchymal stem cells into corneal epithelial cells in vivo and ex vivo. Molecular Vision. 2009;15:99–107.)

1. We apologize that these important experiments that have been overlooked in the first draft. We have added them in the revised manuscript on pages 12 and 13, in paragraph 5.1.

2) As we know, retinal degeneration is one of the dominant causes of irreversible vision impairment. So, the table about stem cell-based clinical trials for RD treatment should be provided, and some clinical trials as the following published review should be cited (Wang Y, et al. Stem/progenitor cell-based transplantation for retinal degeneration: a review of clinical trials. Cell Death Dis. 2020.).

2. In accordance to the Reviewer, pertinent clinical trials, which also include RD treatments, have been added, in addition to a new Table 3.

Once again, the valuable comments and assistance with our paper is greatly appreciated. We look forward to your final decision regarding our modifications, with hopes that all concerns have been addressed in an appropriate manner.

Kind regards,

Marco Zeppieri, Giovanni Miotti, and Pier Camillo Parodi.

ROUND 2

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The Reviewer makes excellent points and offer valuable suggestions to improve the manuscript. Please find the responses in bold font under each of the comments made by the reviewer below, which can also be found in red font in the revised manuscript:
Reviewer (code: 05084565)

1. About the category of stem cells in "3. Which stem cells can be considered for treatment in ophthalmology", I think "3.3 Multipotent stem cells: mesenchymal stem cells (MSCs) " and "3.4 Eye stem cells" belong to "3.2 Adult stem cells", and multipotent stem cells are not only include mesenchymal stem cells.

Our apologies for the confusion. We have modified this section in "3.2 Adults stem cells" in paragraphs 3-4 with the aim of clarifying the categorization of adult, multipotent and eye stem cells. In the modified version, we always refer to the possible use in ocular pathologies. Corrections can be found in red font as follows: "The previous view of adult stem cells has been that the differentiation potential was strictly limited to cell lineages found within the tissue of origin studied in tissues such as skin and bone marrow. During the past few years, this view has been changed. Several studies have shown apparent plasticity of adult stem cells, like the ability to differentiate to cell types other than the tissue of origin [9]. This remarkable finding challenged long-held assumption that truly multipotent or pluripotent stem cells did not persist beyond early stages in embryogenesis. Multipotent stem cells can be inserted in this context because they express markers of pluripotency previously seen only in embryonic stem cells or pregastrulation embryos [9]. The most commonly studied multipotent SCs in ophthalmopathies are Mesenchymal stem cells (MSCs)."

2. In the previous manuscript, Table 1 was "Outline of the review", which was removed in the revised manuscript, but the last sentence of the first paragraph of the "MATERIALS AND METHODS" was still "The detailed outline of the review can be found in Table 1". In addition, in the revised manuscript, it seems more appropriate to change Table 1 to Fig 1.

Many thanks for reporting these minor details. Mention regarding the outline has been deleted in the Materials and Methods section. A new Figure 1, which has been transformed from Table 1, has been modified as suggested. The following has been added in the Materials and Methods and Figure Legend sections regarding the figure and tables, as suggested: "The details regarding the selection of papers considered in the manuscript are listed in Figure 1." "A summary of the most significant studies and conclusions is reported in Table 1 and Table 2." "FIGURE LEGENDS Figure 1: The selection of PubMed literature published from 2000 to 2020, which was considered in the manuscript." Once again, the valuable comments and assistance with our paper is greatly appreciated. We look forward to your final decision regarding our modifications, with hopes that all concerns have been addressed in an appropriate manner. Kind regards, Marco Zeppieri, Giovanni Miotti, and Pier Camillo Parodi.