

Response to Reviewers – Manuscript 40966

Dear Editor,

Thank you for the opportunity to revise this manuscript. We have amended the manuscript according to the tracked changes indicated in the 40966-edited file and offer point-to-point answers to the reviewer's below. All changes to the manuscript have been highlighted in yellow.

Editor's Changes:

Provide running title: Running title inserted.

Add postcode: Postcode added.

Informed consent: Statement of informed consent expanded and consent file supplied.

Audio Core Tip: Audio core tip provided.

Article Highlights: Article highlights have been added according to the guidance.

Reviewer 1:

The manuscript by Orozco et al. discusses the anti-inflammatory potential of human corneal stroma-derived stem cells. This is a well-written, highly informative manuscript; the authors have addressed an important topic, namely the use of adult stem cells to restore the corneal epithelium. Indeed, progress in corneal stem cell research provides encouraging perspectives regarding their use in regenerative medicine. Moreover, stem cell-based approaches hold the potential to combat the worldwide shortage of donor corneas. The editing of manuscript needs to be revised. Non-standard abbreviations should not be employed in the abstract; however, if used, abbreviations would need to be spelled out in full at first use. I recommend this paper for publication after suggested revisions.

Author's Response:

We thank the reviewer for their kind comments and recognition of the importance of the manuscript. We have adjusted so the abstract so that all non-standard abbreviations are defined at first use.

Reviewer 2:

The manuscript illuminates that an in vitro injury model was developed using hCEC culture, allowing the initial testing into the anti-inflammatory potential of CSSC. Additional information about identification of CSSC is needed.

Author's Response:

We have added a sentence to the introduction to reference all the characterisation and identification that has previously been performed on CSSC.

Response to Reviewers – Manuscript 40966

Dear Editor,

Thank you for the opportunity to revise this manuscript. We have amended the manuscript according to the tracked changes indicated in the 40966-edited file and offer point-to-point answers to the reviewer's below. All changes to the manuscript have been highlighted in yellow.

Editor's Changes:

Provide running title: Running title inserted.

Add postcode: Postcode added.

Informed consent: Statement of informed consent expanded and consent file supplied.

Audio Core Tip: Audio core tip provided.

Article Highlights: Article highlights have been added according to the guidance.

Reviewer 1:

The manuscript by Orozco et al. discusses the anti-inflammatory potential of human corneal stroma-derived stem cells. This is a well-written, highly informative manuscript; the authors have addressed an important topic, namely the use of adult stem cells to restore the corneal epithelium. Indeed, progress in corneal stem cell research provides encouraging perspectives regarding their use in regenerative medicine. Moreover, stem cell-based approaches hold the potential to combat the worldwide shortage of donor corneas. The editing of manuscript needs to be revised. Non-standard abbreviations should not be employed in the abstract; however, if used, abbreviations would need to be spelled out in full at first use. I recommend this paper for publication after suggested revisions.

Author's Response:

We thank the reviewer for their kind comments and recognition of the importance of the manuscript. We have adjusted so the abstract so that all non-standard abbreviations are defined at first use.

Reviewer 2:

The manuscript illuminates that an in vitro injury model was developed using hCEC culture, allowing the initial testing into the anti-inflammatory potential of CSSC. Additional information about identification of CSSC is needed.

Author's Response:

We have added a sentence to the introduction to reference all the characterisation and identification that has previously been performed on CSSC.

Reviewer 3:

The authors described a new in vitro model for testing of effects of corneal stroma-derived stem cells (CSSC) on corneal epithelial cell line (hCEC). The manuscript represents preliminary studies summarizing basic results. It looks like a description of results for diploma work or thesis (description of results of testing four different media, detailed description of fixation of amniotic membrane, etc). A simple statement that four different media were tested and one was used for next study should be sufficient. Similarly, description of history of amniotic membrane in Introduction is not necessary. Everybody in ophthalmology, who is interested in this work, knows well amniotic membrane.

Details:

1. Abbreviations CSSC and AM must be explained in the first sentence of Abstract.
2. M and M, paragraph Co-culture: How many hCES were seeded per well, also the volume of medium should be provided.
3. Hours should be written in the same way. Somewhere is hours, in other sentences h (see for example legend to Fig. 2).
4. Could authors exclude a possibility that cytokines in supernatants (Fig. 7) of co-culture experiments are produced by CSSC under influence of hCEC and not by hCEC?
5. Authors showed that co-cultivation of EtOH injured hCEC with CSSC significantly decreased toxicity and improved hCEC viability. Could authors speculate about the mechanism of this protective effect of CSSC?
6. More recent references (2017/2018) could be included. 7. References must be checked and corrected by the authors. The majority of names of journals are in a full form, other (as ref. 16) are in abbreviated form.

Author's Response:

Thank you for your review. We felt that all the results presented in this manuscript add to the body-of-work and scientific story, and thus should still be included. The cell types used in this study had not previously been cultured in the four types of media used, and the viability results shown in figure 2 could be used to inform the work of others when choosing the optimal media for co-culture. The description of how the amniotic membrane was fixed into holders for culture of cells is a novel technique, and could be of valuable assistance to researchers working with amnion for the first time, as due as it can be a difficult material to handle.

We do not believe that there is any harm in leaving the description of amniotic membrane in the introduction. Although researchers in ophthalmology will most probably have knowledge of amniotic membrane, this publication may have impact outside of this field and little harm can come from a refresher of the history of amnion.

In response to your numbered points:

1. We have adjusted the manuscript so that the corneal-stroma-derived stem cells (CSSC) and amniotic membrane (AM) are defined in the abstract.
2. Cell seeding densities have been added to the co-culture section, although these were mentioned previously in the individual descriptions of the culture of cells.
3. All uses of hours have been abbreviated to h to improve consistency.
4. We cannot definitively say whether the cytokines produced in the co-culture experiments were produced by the hCEC or the CSSC. However, all co-culture experiments were performed with non-co-culture controls of both hCEC and CSSC, injury and no-injury and all cytokines levels were corrected for background levels produced in these controls. In co-culture the total levels of cytokines during injury are significantly reduced compared to non-co-culture of the hCEC, indicating that something in the system is having an anti-inflammatory effect, and this could correlate with the addition of the CSSC. Figure 4 demonstrates that CSSC produce much less IL-6 and IL-8 in response to inflammatory stimulus than hCEC do, indicating that it is less likely that these cytokines are produced by the CSSC. The PCR that was performed was specific to the hCEC, so does not indicate any change of cytokine gene expression by the CSSC, supporting the evidence from the protein assays.
5. We have not yet published results from mechanism-of-action studies, however, we could speculate that the improved cell viability is a results of trophic factors released into the media by the CSSC, but further studies are needed to identify the actual proteins/targets.
6. As far as we could tell all references were in the correct abbreviated form with the exception of the original reference 19 (now 23), which has now been corrected. References 16-20 have been added to include more up to date work studying the effect of MSCs in in vivo corneal injury.