

September 12, 2019

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 49995-Manuscript File.docx).

Title: Sphere-forming corneal cells repopulate dystrophic keratoconic stroma: Implications for potential therapy

Author: Himanshu Wadhwa, Salim Ismail, Jennifer J McGhee, Bert Van der Werf and Trevor Sherwin

Name of Journal: *World Journal of Stem Cells*

Manuscript NO: 49995

The manuscript has been improved according to the suggestions of reviewers and editor as follows:

1 Format has been updated according to the 'Guidelines and Requirements for Manuscript Revision: Basic Study' document

2 Revision has been made according to the suggestions of the reviewers

Reviewer 1 (02728252)- No suggestions by this reviewer. We thank the reviewers for taking the time to review our manuscript and for their positive and constructive comments.

Reviewer 2 (03976790)-

1. p. 8: Petri dish (not "petri")

'petri' has been corrected to 'Petri' as suggested on page 9 of the manuscript

2. p. 10: Material and methods. The authors used several primary antibodies in order to visualize different markers of the cells. They used indirect immunofluorescence with a second antibody giving a specific staining. Results can be appreciated on the figures. Please specify which secondary antibody is specifically assigned to the primary antibody.
The specific secondary antibody assigned to each primary antibody has been added to page 10-11 of the manuscript as requested.
3. p. 14: Results. - Repopulation of keratoconic tissue surface: there are a lot of results, so a table summarizing these results could be useful for the reader
The cell viability results of this section have been placed into a summary table (Table 2) on page 46 as suggested
4. P. 18 to 27: Discussion. The discussion is very long with sometimes very long sentences, and could be shortened.
The discussion section has been revised according to the comments of each of the reviewers and adjusted to contain more concise sentences as suggested.
5. References: I did not find references 32, 35, 36, 37 in the text. Please check the references.
References have been checked as suggested. Reference 32 is cited on page 12 and references 35, 36 and 37 are cited on page 19 of the manuscript

Reviewer 3 (00410685)-

1. Page 13: Cells were seen to have migrated radially in all directions from the centre of spheres as early as Day 1. Page 14: At all three time points, labelling of ABCB5 appeared to be concentrated within spheres and not outside (Figure 3a-c, red) - indicating presence of stem cells within spheres alone Page 16: The extent of cell migration from the centre of spheres increased from Day 3 to Day 7 in 11/16 implanted spheres. Page 17: Cells within implanted spheres, as well as cells adjacent to but outside of the sphere (Figure 6c-d) stained positively for proliferation marker EdU at Day 10. Please quantify the data reported by image analysis and include more data in order to increase confidence on the relevance of the data.
The immunohistochemistry results were by necessity qualitative and would be very difficult to quantify meaningfully due to the nature of the observations of stem cell

markers such as ABCB5 observed solely within spheres and not observed outside of the spheres. Similarly, EdU detection was performed to identify regions of cell division rather than to quantify rates of cell division. We believe that analysis of the images to quantify the data would deliver a high level of bias and would not provide an acceptable level of accuracy as to the true expression levels. Therefore, we have used ddPCR which is highly sensitive and reliable for generating quantitative data to support the qualitative findings of the immunohistochemistry results.

2. Being a key issue for future development in the field, if possible, can the author discuss and/or speculate more on the current status of the research on signaling molecules that might be required to direct differentiation of spheres?

Rather than aiming to identify signaling molecules that drive differentiation of sphere cells seeded/implanted onto corneal matrices, we have relied upon the inherent properties of the spheres to detect the matrix onto which they are placed and react accordingly by producing cells of the correct lineage for that particular surface. We have added discussion on this to the manuscript on pg23-24 as requested.

Reviewer 4 (02467578)-

1. Please provided the No. of approved certificate by ethics committee.
Ethics reference No. (NTX/07/08/080/AM06) has been added to page 7 of the Materials and Methods section of the manuscript as suggested
2. The authors designed the different time points, please provided the rational cause.
We selected the day 0, 1, 3 or 4, time points based primarily on previous work with spheres placed on collagen which indicated that these times were the critical points where an increase in cell migration and proliferation occurred. The day 14 time point was used as an experimental endpoint due to the observation of complete tissue matrix repopulation. This rationale has been added to page 9 of the manuscript.
3. The discussion should be concentrated on the results.
The discussion section has been carefully revised to integrate more closely with our results as suggested.
4. The present conclusion should be revised in accordance with the results.

The conclusion has been revised to more closely reflect the results as suggested

3 Revision has been made according to the suggestions of the editor

1. Please revise the manuscript according to the review report and my comments. And answer all of the reviewers' comments carefully (point-to-point).

The manuscript has been revised according to the editor and reviewers comments as requested. A point by point response to all of the reviewers comments have been provided in the section above.

2. You need to provide the grant application form(s) or certificate of funding agency for every grant, or we will delete the part of "Supported by...".

A certificate of funding has been provided for each of the funding bodies cited, as requested.

3. Any article describing a study (basic research) involving animal subjects is required to have the institutional animal care and use committee (IACUC)'s institution name (such as the Genovese Institute) and protocol number (such as 14-9347-39G or EN-21549) stated explicitly in the title page section.

This study did not use animal subjects. This statement has been added to the title page of the manuscript.

4. In order to improve the quality of Basic Study manuscript, authors should download and complete the ARRIVE checklist to ensure that the manuscript meets the requirements of the ARRIVE Guidelines. Authors must state on the title page of the manuscript that the ARRIVE Guidelines have been adopted (see below). Authors must upload the PDF version of the completed ARRIVE checklist to the system.

This study did not use in vivo animal experiments. This statement has been added to the title page of the manuscript.

5. Please write the article highlight section accordingly. Please don't copy from the main text.

Article highlights section has been included at pages 29-31 in the manuscript as requested.

6. Please read these four important guidelines carefully and modify your figure(s) accordingly:

First, all submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes. Second, for line drawings that were automatically generated with software, please provide the labels/values of the ordinate and abscissa in text boxes. Third, please prepare and arrange the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed by the editor. Fourth, in consideration of color-blind readers, please

avoid using red and green for contrast in vector graphics or images. For pictures with multiple parts, please create text box in the upper left corner with uppercase letters A, B, etc. ; please use SmartArt, text box and shape to draw the flowchart directly in PowerPoint;

Figures have been modified as editable images and prepared in a PowerPoint file as requested

7. Please don't include abbreviations in the title of the figure/ table. Please explain all the abbreviations in the figure/table legends: full name (abbreviation). Please explain all the abbreviations of each figure/table under each piece of figure/table legends. Please don't include any *, #, ...in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters.

Abbreviations have been removed from figure and table titles and explained in legends as requested.

Superscript numbers and letters have been used for illustration and statistical significance as requested

Thank you again for publishing our manuscript in the *World Journal of Stem Cells*.

Sincerely yours,

Professor Trevor Sherwin, PhD

Department of Ophthalmology

University of Auckland

Private Bag 92019

Auckland 1020

New Zealand

Email: t.sherwin@auckland.ac.nz

Tel: +64-9-9236466 Fax: +64-9-3677173