BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells
Manuscript NO: 32866
Title: Reconstructing human endothelium in damaged corneas using embryonic stem cells
Reviewer's code: 02254242
Reviewer's country: Reviewer_Country
Science editor: Jin-Xin Kong
Date sent for review: 2017-02-08
Date reviewed: 2017-02-13

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[Y] Grade A: Priority publishing	Google Search:	[Y] Accept
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[ ] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[ ] No	

COMMENTS TO AUTHORS

The manuscript provides evidence for the attachmentof hESC to a human Descemet's membrane. The author's have demonstrated that there is growth of the hESCs. However, the growth on that membrane is limited. This is acceptable as a first step into the use of these cells in corneal transplantation. A larger study of proteins and gene expression would have been more convincing, but could be presented in further studies.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells
Manuscript NO: 32866
Title: Reconstructing human endothelium in damaged corneas using embryonic stem cells
Reviewer's code: 02445899
Reviewer's country: Reviewer_Country
Science editor: Jin-Xin Kong
Date sent for review: 2017-03-27
Date reviewed: 2017-03-29

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[Y] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[ ] No	[Y] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[ ] No	

COMMENTS TO AUTHORS

General comment Thank you for submitting your article to the WJSC. This is an interesting piece of work that could form a valuable addition to the current corneal repair/regeneration literature. It is my view that, at this stage, both the study and article lack detail and depth and as such should not be accepted for publication in its present form. However, to the credit of the authors they do acknowledge that the differentiation protocol, etc. are in need of optimisation. If this optimisation were to be undertaken and more convincing evidence of the identity and functionality of the CEC-differentiated hESCs shown then the article should be re-submitted for consideration. If possible have the manuscript reviewed by a native English speaker to improve the grammar. Specific comments/queries Other markers should be tested on the 'differentiated' (& control) cells e.g. CK13 &/or 19 for conjunctiva and CK12 for corneal epithelium? I would like to direct the authors to the work of Okumura et al., 2014 (IOVS 55(11): 7610-18) and Yamaguchi et al. 2015 (Current Eye Research 40(12):

1211-17) for additional potential CEC markers, CD73-neg and ITGA3-pos, respectively. M&Ms: far too much detail of the tissue processing – this is fairly standard & could be shortened. p6: what is the logic of adding FBS to a culture medium already containing the SR supplement? Please explain. p6: Is the PBS used for the cell washes with or without Ca and Mg? Please clarify. p8, line 1, 5 & 10, etc.: They are tissue sections not tissue slides. Please correct. p10 line 5: There is no evidence that the supposed CEC-differentiated hESC are functioning in vivo – is this a typo? There is certainly no evidence that these cells can maintain fluid balance in an intact, in vivo –or ex vivo - system. Typos p7, line 11: replace 'a ocular stick' with 'an ocular stick' p7, line 13: 'replace gentile' with 'gentle' p7, line 15: replace 'motions' with 'motion'

As the reviewers point out this is the first report that hESC can differentiate into endothelial-like cells in the human cornea in vitro. Since there is no antibody towards corneal endothelium we have shown that the endothelial-like cells are very similar to corneal endothelial cells both morphological and in immunohistochemical tests.

As also pointed out one obvious next step would be gene expression analyses, which is ongoing and time consuming. Time consuming is also ongoing animal studies to investigate the physiology and function of the endothelial-like cells.

In the meantime we feel it is important to show that basic science is addressing the question: Can we have access to, at least in theory, unlimited number of human corneal endothelial cells.

The M&Ms: has been shortened

The PBS used has been specified (PBS $^{Ca-/Mg-}$)

Suggested corrections in the text have been performed.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells
Manuscript NO: 32866
Title: Reconstructing human endothelium in damaged corneas using embryonic stem cells
Reviewer's code: 00504335
Reviewer's country: Reviewer_Country
Science editor: Jin-Xin Kong
Date sent for review: 2017-03-27
Date reviewed: 2017-04-04

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[ ] No	[Y] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[ ] No	

COMMENTS TO AUTHORS

The authors described the first experment focused on cultivation of hESC on human cornea. The results show that hESC attached to the cornea and that 10 of 18 samples expressed OCT-4 , 9 of 18 cases expressed PAX-6 and 14 of 18 cases were positive for NaKATPase. Three of 18 samples were positive for corneal epithelial marker CK-3. This is interesting, but highly preliminary study. Since there are such remarkable differences among individual samples, could the authors inform, how many times the experiments were repeated? (It means, how many diferent batches of hESC were prepared and in separate experiments transferred onto the corneas? Were such differences observed also among individual separate experiments?) 1. The title says „ Reconstructing human endothelium…“ In fact, no evidence for reconstruction of the endothelium is given. The data show only that hESC adhered to the cornea and partially changed their expression profile. 2. Is there direct evidence that hESC differentated into corneal endothelial-like cells? Neither PAX6 nor NaKATPase are markers of corneal

endothelium. CK-3 is a marker of corneal epithelium. 3. Why hESC were before transfer onto the cornea allowed to initiate differentiation? Is it possible that described changes in the expression profile of hESC could occur after differentiation step even without transfer of hESC onto the cornea? 4. The second sentence in Discussion says, „ ..our study shows that the construct with healed human endothelia is working in vivo…..". No evidence for this statement (working in vivo) is given. 5. Legend to Figure 1 – a magnification should be given. 6. References should be prepared in the same style. For example, ref. 1 -title of the journal is in abbreviated form, ref. 8 – title of the journal is in full name, etc..

Each batch of HESCs was used for transplantation on to 1-3 corneas. The variation was not correlated to the batch used. Although the quality of the corneas varied slightly. We cannot correlate the outcome to the type of tissue, ie the keratoconus buttons with almost normal thickness and the decompensated buttons with increased thickness.

We suspect that the "pre-differentiation" period is the key to solve the problem of variation. It seems that just adding ROCK inhibitor is not sufficient for 100% success of differentiation but other still unknown factors may be used to elevate the efficiency of the differentiation to corneal endothelial cells.

The title is changed

During the establishing and characterization of the used stem cell line it was noticed that the cells were prone to spontaneously differentiate to neurectodermal cells and finally to neuronal cells (not published). As human cornela endothelial cells are of the same origin (neurectoderm) we took advantage of this by allowing the cells to pre differentiate (for a short time period). We have also done some trials with "fresh" stem cells but in those cases the expression of OCT4 did not decline as much as wen using pre differentiated cells. The pre differentiated cells do not express PAX6, ZO-1.

Suggested corrections in the text have been performed and a bar has been inserted in Fig.1.

References are checked and corrected.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells
Manuscript NO: 32866
Title: Reconstructing human endothelium in damaged corneas using embryonic stem cells
Reviewer's code: 00505245
Reviewer's country: Turkey
Science editor: Jin-Xin Kong
Date sent for review: 2017-03-27
Date reviewed: 2017-04-09

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[Y] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[ ] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[ ] No	

COMMENTS TO AUTHORS

This is a well written manuscript evaluating the results of an attempt to get human embryonic stem cells (hESCs) to differentiate into corneal endothelial like cells in vitro on human corneas that had been partially or completely cleared of all existing endothelial cells. The overall aim was to further develop techniques for reconstructing the corneal endothelium. I think this is a valuable study since there are no published studies aimed at making hESCs differentiate into human corneal endothelial cells, as the authors also stated in their manuscript. I have only one comment: In the Discussion section, the authors state that the process needs further optimization. What are the suggestions of the authors for researchers interested in further experiments? For example, could some growth factors be added into the culture medium of the hESCs? A paragraph could be added to the Discussion section regarding this issue. As a result, I believe that the manuscript deserves publication in the 'World Journal of Stem Cells'.'

We suspect that the "pre-differentiation" period is the key to solve the problem of variation. It seems that just adding ROCK inhibitor is not sufficient for 100% success of differentiation but other still unknown factors may be used to elevate the efficiency of the differentiation to corneal endothelial cells.