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The effect of human amniotic epithelial cells on urethral stricture fibroblasts

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1st Editorial decision

5-Jun-2019

Ref.: Ms. No. JCTRes-D-19-00006
The effect of human amniotic epithelial cells on urethral stricture fibroblasts
Journal of Clinical and Translational Research

Dear Dr. KN,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jul 05, 2019.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The paper addresses a potentially novel approach to improve the treatment of USD and optimize the availability of grafts that express a non-fibrotic genotype and phenotype. Human-derived USF cells are used a template test system, while HAMECs are explored as potential alternative graft material. The secretome-rich medium derived from HAMEC cell culture is used to assay the effects of this medium on fibrotic behavior of cultured USF cells.

This is the first study to demonstrate the possible utility of HAMECs in the USF treatment setting. The most basic parameters are assessed using a scratch assay and transcriptomic analysis of TIMP and alpha-SMA. The preliminary conclusions are supported by the data. The manuscript is very clearly written and presented in the most logical manner. The shortcomings of the work are clearly addressed in the Discussion section.

Firstly, the most important information missing from the Discussion section is:

1) How is the envisaged procedure reduced to practice? I presume the HAMECs are isolated from placentas, screened for infections and critical genetic mutations, and then grown onto scaffolds that are subsequently grafted into the patient's urethra. Please describe this procedure in more detail and provide argumentation regarding the similarity in cell biology between a culture dish and a scaffold environment. The latter should attest to the validity of using cell monolayers as an appropriate method to assess paracrine effectors of HAMECs grown on scaffolds. Please revert to literature for corroborative evidence. Furthermore, the HAMECs were grown to P1, which seems unrealistic when the methods is used in the clinical setting. The authors need to address genotypical and phenotypical changes when the HAMECs are grown beyond P1. Do they, based on available literature, expect changes in secretome composition (and therefore differential effects on USFs) with increasing population number? How does cryogenic storage of HAMECs or CM affect the fibrosis-ameliorating effects of the cells and medium?

2) Elaborate on which research and validation steps need to be undertaken to bring this concept to clinical practice. This will guide fellow researchers in conducting the most important and necessary follow-up studies and expediting the translation of this novel approach to the clinical setting.

Secondly, could the authors explain in the text why the analyses were performed on USF cells and not the tissue biopsies obtained from patients? Testing on USF-containing tissue with an

intact microenvironment would have yielded more representative results. It would benefit the work significantly if analyses could be repeated on cultured tissue material.

Thirdly, why were other mediators of fibrosis (see e.g., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2946622/) not assessed at genetic but preferably protein level? Also, oxidative stress in the graft (e.g., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3926896/) may have an effect on fibrosis in native tissue. Why did the authors discount the effect of redox stress?

Finally, USF needs to be written out at first mention in the Introduction.

Author's rebuttal

25-Jun-2019

We appreciate the time and efforts by the editor and referees in reviewing this manuscript. We have addressed all issues indicated in the review report, and believe that the revised version can meet the journal publication requirements.

Response to Referee(s)' Comments:

Response to Comments from Reviewer 1

Comment 1:

Firstly, the most important information missing from the Discussion section is:

Response to comment 1

Thanks for reviewing and for the positive feedback and will try to include missing points in discussion sections. The included portion are coloured blue.

Comment 2:

1) How is the envisaged procedure reduced to practice? I presume the HAMECs are isolated from placentas, screened for infections and critical genetic mutations, and then grown onto scaffolds that are subsequently grafted into the patient's urethra.
2) Please describe this procedure in more detail and provide argumentation regarding the similarity in cell biology between a culture dish and a scaffold environment.

Response to comment 2

Yes, The HAMECs will be isolated from placentae, screened for infections and critical genetic mutations, and then grown onto scaffolds that are subsequently grafted into the

patient's urethra. We have already published in detail, Isolation, expansion and characterization and genetic stability of HAMECs (ref.11 in main manuscript). The HAMECs batches would undergo standard quality testing as per internal specifications. The cells from these batches would be loaded on scaffolds and cultured. This would be the investigational medicinal product (IMP) for transplantation.

3) Please describe this procedure in more detail and provide argumentation regarding the similarity in cell biology between a culture dish and a scaffold environment.

HAMEC's morphological characteristics, Identity (AE1/AE3) and ability to attach and growth in culture plate were similar to that of scaffold environment. (Internal data available with us);

Comment 3:

The latter should attest to the validity of using cell monolayers as an appropriate method to assess paracrine effectors of HAMECs grown on scaffolds. Please revert to literature for corroborative evidence.

Response to comment 3

The article quoted below has shown that paracrine effect of cell monolayer and scaffold were similar in down regulating the α-SMA (ref). Our preliminary data of HAMECs has shown similar trend in monolayer and scaffolds.

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0187348

Comment 4:

Furthermore, the HAMECs were grown to P1, which seems unrealistic when the methods is used in the clinical setting. The authors need to address genotypical and phenotypical changes when the HAMECs are grown beyond P1.

Response to comment 4

The average isolated yield of HAMECs is around 100 million cells from single placenta and these can be expanded to P1 with an yield of around 700 million cells, which is sufficient to create 150 urethral grafts. The HAMECs have been cultured up to P4 without any impact on genotype and phenotype [2].ref

Comment 5:

Do they, based on available literature, expect changes in secretome composition (and therefore differential effects on USFs) with increasing population number? How does cryogenic storage of HAMECs or CM affect the fibrosis-ameliorating effects of the cells and medium?

Response to comment 5

There is no available data in the literature to show the variability of secretome composition in HAMECs with increasing population number. Currently, we have not studied the cryogenic storage of HAMECs or CM across different stability time point to check the fibrosis-ameliorating effects (potency assay) of the cells. This could be taken up in the long term stability program of potency assay.

Comment 6:

Elaborate on which research and validation steps need to be undertaken to bring this concept to clinical practice. This will guide fellow researchers in conducting the most important and necessary follow-up studies and expediting the translation of this novel approach to the clinical setting.

Response to comment 6

The validation steps are already outlined by various regulatory authorities. Which should guide the translation.

Comment 7:

Secondly, could the authors explain in the text why the analyses were performed on USF cells and not the tissue biopsies obtained from patients? Testing on USF-containing tissue with an intact microenvironment would have yielded more representative results. It would benefit the work significantly if analyses could be repeated on cultured tissue material.

Response to comment 7

We do agree that the testing on biopsy tissue would be more representative than enriched cells. But in practice this would require multiple biopsies, which would vary in micro environments. On the other hand, the enriched cells would provide us reproducible environment to test various parameters.

Comment 8: Thirdly, why were other mediators of fibrosis (see e.g., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2946622/) not assessed at genetic but preferably protein level?

Response to comment 8

One of the main objective of this paper is to provide a practical potency assay for translational studies. So we have used the marker which are very well known and published widely.

Comment 9:

Also, oxidative stress in the graft (e.g., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3926896/) may have an effect on fibrosis in native tissue. Why did the authors discount the effect of redox stress?

Response to comment 9

We agree with the reviewer comments. At present, this is being explored.

Comment 10:

Finally, USF needs to be written out at first mention in the Introduction.

Response to comment 10

Defined the USF in first-in-use in abstract as well as in introduction section.

Once again, thank you for reviewing the manuscript for publication and I look forward to hear from you as soon as possible

Best Regards,

Dr. Sridhar KN

Director,
Sri Research for Tissue Engineering Pvt. Ltd,
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2nd Editorial decision

03-Jul-2019

Ref.: Ms. No. JCTRes-D-19-00006R1
The effect of human amniotic epithelial cells on urethral stricture fibroblasts
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Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Aug 01, 2019.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Dear authors,

Please note that I have implemented some of the feedback you provided to the reviewer's comments in the text, which is attached or which you may download here via a secure server (in case the attachment is too large):

https://filesender.surf.nl/?s=download&token=490b4bd6-2041-4ee0-93e2-4e54b0e58324

Please go over these changes and approve them.

Once you reupload the (modified) version that I just sent you, we can accept the manuscript for publication.

Thank you,

Michal Heger, EiC

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

3rd Editorial decision

03-Jul-2019

Ref.: Ms. No. JCTRes-D-19-00006R2
The effect of human amniotic epithelial cells on urethral stricture fibroblasts
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: