Journal of Clinical and Translational Research
Peer review process file 08.202206.004

Endothelial progenitor cell-derived small extracellular vesicles for Myocardial angiogenesis and revascularization

Maher T. Al-Omar, Mahmoud T. Alnajjar, Ziyad T. Ahmed, Faris MI Salaas, Tamim SM Alrefaei, Khawaja H Haider*

*Corresponding author
Khawaja H Haider
College of Medicine, Sulaiman Al Rajhi University, Al-Bukairyah 52726, Saudi Arabia

Handling editor:
Michal Heger
Department of Pharmaceutics, Utrecht University, the Netherlands
Department of Pharmaceutics, Jiaxing University Medical College, Zhejiang, China

Review timeline:

Received: 27 July, 2022
Editorial decision: 12 September, 2022
Revision received: 23 September, 2022
Editorial decision: 13 October, 2022
Revision received: 15 October, 2022
Editorial decision: 20 October, 2022
Published online: 31 October, 2022

1st Editorial decision
12-Sep-2022

Ref.: Ms. No. JCTRes-D-22-00107
Endothelial progenitor cells-derived exosomes for myocardial angiogenesis and revascularization
Journal of Clinical and Translational Research

Dear Prof Haider,

Reviewers have now commented on your paper. One reviewer has recommended a reject, one a reject and resubmit, and two reviewers a major revision. The nature of the reviewers' comments, which the editorial board agrees with, is serious and warrants not only rectification of proper microparticle characterization and nomenclature, but also a profound overhaul of content. Because the paper can be amended and restructured, we would like to extend a chance to the authors to modify the manuscript in line with the reviewers' comments. 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. Please understand that substantial work is required. Our decision ultimately depends on the extent to and accuracy with which the revision has been prepared.

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 Oct 12, 2022.

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 authors submitted a narrative review article in which they managed to elucidate EPCs-derived exosomes, their proteomic and genomic payload with a primary focus on pro-angiogenic miRs that
facilitate angiogenesis in the ischemic myocardium. The aim of the study is clear and concise. The manuscript has a logical structure and composes of well-balanced subsections that cover all aspect of initial hypothesis. The figures are informative and legible. The tables are clear. The authors established that EPCs participate in angiogenesis and cardiac reparation thans their secretome including EVs. Although this issue is intriguing, I would like to put forward several comments to discuss.
1. Methodology. Please, add a separate paragraph in which the principles of pooling and interpretaion of the data should be illuminated. The quality of the article enrolled to the evaluation needs to be reported. Searching databases and other sourses requires to be provided regardless of the study has not been declared as systemtic review.
2. Tables with the results of animal and clinical studies. Please, re-arrange the tables so that the data are given more concise and legible. I recommend to minimize the text part in the tables and report the only meaningful results. The description of them requires to be moved to the appropriate subsections.
3. EVs. Please, characterize the cargo in vesicles so that the difference between pro-angiogenic and pro-apoptotic vesicles become clear.
4. Add problematic issue in the section regarding clinical use of the EPCs. Please, describe the source, route, mixture of EPCs and also describe hybride approaches when relevant.
5. Please, improve the section Abstract so that it correspond the final paragraph of the article.

Reviewer #2: This paper summarized the advancement in profiling RNA and protein content of exosomes derived from EPCs and their roles in angiogenesis and revascularization. The organization and writing of this review are poor. Too many statements are inaccurate or unclear. For instance:
1. No markers can distinguish exosomes from microvesicles, so EVs or sEVs were adopted to describe these small vesicles rather than exosomes. Please refer to the following review: Latest Advances in Endothelial Progenitor Cell-Derived Extracellular Vesicles Translation to the Clinic(frontiersin.org) Frontiers | Latest Advances in Endothelial Progenitor Cell-Derived Extracellular Vesicles Translation to the Clinic (frontiersin.org)

2. The statement of pinched from The cell surface (p13, line 9) usually described the release of microvesicles rather than exosomes packaged in MVBs.
3. Regarding the Graphical abstract. The EPC-derived exosomes contain not only nucleic acid but also proteins. The keywords angiogenesis and revascularization are missing. The meaning of the arrow is unclear.

Reviewer #3: This kind of review articles are enough written in scientific literature and this paper contribution is not significant. Anyway, I found several issues as indicated below.

Major comments:

1) Latest MISEV guidelines endorses to use extracellular vesicles (EV) instead of exosomes. We recommend to use EVs throughout the manuscript.
2) On page 6, line 53-56, authors used term "angioblast" and cited outdated reference. Please use recent works and appropriate terms.
3) Page 7, line 12-16. Authors claim that "early EPCs are characterized by CD133 (monocyte marker) and CD14 (hematopoietic marker)" which is wrong characterization. ECFC is progenitor cells and never express CD14 at all.
4) The paragraph EPCs-derived exosomes for myocardial angiogenesis and repair in experimental animal models is poor written, moreover, paragraph is not emphasized to myocardial angiogenesis. Please address and re-write.

Minor comments

1) Page 11, lines 19 and 34 authors used emigration, please correct it to migration and replace all emigration to migration throughout manuscript.

Reviewer #5: i) In the part "Keywords", I found MSCs. Nevertheless, they are mentioned three times. (p. 4, 5, 21). In the text they are not mentioned at al. Nor are the "Mesenchymal stem cells".
ii) "their proteomic and genomic payload with a primary focus on pro-angiogenic miRs that facilitate angiogenesis in the ischemic heart myocardium.". Please explain how in human patients.

Authors' response

Ref.: Ms. No. JCTRes-D-22-00107
Endothelial progenitor cells-derived exosomes for myocardial angiogenesis and revascularization
Journal of Clinical and Translational Research

Dear Editor

We thank the worthy reviewers for their comments which have certainly helped in revising and refining the manuscript.

We have uploaded Manuscript (2 files: One file showing track changes and the second with track changes accepted and revised further).

Following is our itemized response to worthy reviewers' comments.

With thank you for your support.

Prof Haider

Itemized Response to the Reviewers' comments:

Reviewer #1:

The authors submitted a narrative review article in which they managed to elucidate EPCs-derived exosomes, their proteomic and genomic payload with a primary focus on pro-angiogenic miRs that facilitate angiogenesis in the ischemic myocardium. The aim of the study is clear and concise. The manuscript has a logical structure and composes of well-balanced subsections that cover all aspect of initial hypothesis. The figures are informative and legible. The tables are clear. The authors established that EPCs participate in angiogenesis and cardiac reparation thans their secretome including EVs. Although this issue is intriguing, I would like to put forward several comments to discuss.

Author's response

We thank the worthy reviewer for his encouraging comments which are helpful to enhance the quality of the manuscript.

1. Methodology. Please, add a separate paragraph in which the principles of pooling and interpretation of the data should be illuminated. The quality of the article enrolled to the evaluation needs to be reported. Searching databases and other sourses requires to be provided regardless of the study has not been declared as systemtic review.

Author's response
The required information has been included as a last paragraph in the "Introduction section."

2. Tables with the results of animal and clinical studies. Please, re-arrange the tables so that the data are given more concise and legible. I recommend to minimize the text part in the tables and report the only meaningful results. The description of them requires to be moved to the appropriate subsections.

Author's response

The suggested changes have been made in the relevant Tables.

3. EVs. Please, characterize the cargo in vesicles so that the difference between pro-angiogenic and pro-apoptotic vesicles become clear.

Author's response

Response has been included in the last part of the experimental studies anti-apoptotic studies.

4. Add problematic issue in the section regarding clinical use of the EPCs. Please, describe the source, route, mixture of EPCs and also describe hybride approaches when relevant.

Author's response

A new section supported by a new Table (Table-5) regarding Clinical studies using EVs has been included in the revised manuscript.

5. Please, improve the section Abstract so that it correspond the final paragraph of the article.

Author's response

The suggested change has been incorporated.

Reviewer #2

This paper summarized the advancement in profiling RNA and protein content of exosomes derived from EPCs and their roles in angiogenesis and revascularization. The organization and writing of this review are poor. Too many statements are inaccurate or unclear.

For instance:
1. No markers can distinguish exosomes from microvesicles, so EVs or sEVs were adopted to describe these small vesicles rather than exosomes. Please refer to the following review: Latest Advances in Endothelial Progenitor Cell-Derived Extracellular Vesicles Translation to the Clinic(frontiersin.org) Frontiers | Latest Advances in Endothelial Progenitor Cell-Derived Extracellular Vesicles Translation to the Clinic (frontiersin.org)

Author's response

We appreciate the comment of the worthy reviewer.

Please refer to the Page#12 Line #5, we have clearly written that "Exosomes are nano-sized extracellular vesicles (<150nm in diameter) produced inside the cells by fusion of the multi-vesicular bodies with the cell membrane (26)".

This is exactly the information which the worthy reviewer is asking us to include in the manuscript.

Nevertheless, it is pertinent to state that the term "exosome" is not redundant. It is still in use (please refer to scores of Clinical trials on Clinicaltrials.gov and publications appearing in PubMed wherein "exosome" is being extensively used. Even the Frontier Journals referred by the worthy reviewer are using the term "exosomes".

Hence, the use of the term "exosome" may not inaccurate. It is more a preference at the moment rather than the matter of accuracy or inaccuracy.

However, we have replaced the term “exosomes” with “sEVs” throughout the manuscript per suggestion of the worthy reviewers’. We appreciate worthy reviewers’ input for our learning and knowledge.

2. The statement of pinched from The cell surface (p13, line 9) usually described the release of microvesicles rather than exosomes packaged in MVBs.

Author’s response

The suggested change has been incorporated.

3. Regarding the Graphical abstract. The EPC-derived exosomes contain not only nucleic acid but also proteins. The keywords angiogenesis and revascularization are missing. The meaning of the arrow is unclear.

Author’s response

The suggested changes have been incorporated in the Figure and the modified Figure has been incorporated.

Reviewer #3:

This kind of review articles are enough written in scientific literature and this paper contribution is not significant. Anyway, I found several issues as indicated below.

Major comments

1) Latest MISEV guidelines endorses to use extracellular vesicles (EV) instead of exosomes. We recommend using EVs throughout the manuscript.

Author’s response

Already responded previously in response to reviewer#2 comment#1 and the necessary change has been incorporated in the manuscript.

2) On page 6, line 53-56, authors used term "angioblast" (vasoformative) and cited outdated reference. Please use recent works and appropriate terms.

Author’s response

The suggested change and a new reference has been incorporate.

3) Page 7, line 12-16. Authors claim that "early EPCs are characterized by CD133 (monocyte marker) and CD14 (hematopoietic marker)" which is wrong characterization. ECFC is progenitor cells and never express CD14 at all.

Author’s response

The necessary change has been incorporated.

4) The paragraph EPCs-derived exosomes for myocardial angiogenesis and repair in experimental animal models is poor written; moreover, paragraph is not emphasized to

myocardial angiogenesis. Please address and re-write.

Author's response

The section on myocardial angiogenesis and repair has been revisited and revised.

Minor comments

1) Page 11, lines 19 and 34 authors used emigration, please correct it to migration and replace all emigration to migration throughout manuscript.

Author's response

The suggested change has been made throughout the text.

Reviewer #5:

i) In the part "Keywords", I found MSCs. Nevertheless, they are mentioned three times. (p. 4, 5, 21). In the text they are not mentioned at al. Nor are the "Mesenchymal stem cells".

Author's response

MSCs has been removed from the keywords.

ii) "their proteomic and genomic payload with a primary focus on pro-angiogenic miRs that facilitate angiogenesis in the ischemic heart myocardium." Please explain how in human patients.

Author's response

In response to the worthy reviewer's comment, a new section supported by clinical data has been included in the revised manuscript.

2nd Editorial decision
13-Oct-2022

Ref.: Ms. No. JCTRes-D-22-00107R1
Endothelial Progenitor Cell-derived Small Extracellular Vesicles (sEVs) for Myocardial Angiogenesis and Revascularization
Journal of Clinical and Translational Research

Dear Prof Haider,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. Please take the reviewer's comments seriously as we cannot extend multiple review rounds for papers that are not modified appropriately. 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 Nov 12, 2022.

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 #2: 1. There are still many mistakes in the revised Graphical abstract.
a. Lack of description of the biogenesis of MVB-derived exosomes and microvesicles.
b. Both have similar biological functions.
c. Protein also includes growth factors. There are the same questions in the text
d. The release of soluble paracrine factors can not be illustrated as a diffusion process.

2. Some cited references are inappropriate.
For instance, Page13, lines 20-23, Exosomes are nano-sized EVs (<150nm in diameter) produced inside the cells by fusion of the multi-vesicular bodies with the cell membrane (26)
26. Haider KhH, Aramini B. "Mircrining" the injured heart with stem cell-derived exosomes: an emerging strategy of cell-free therapy. Stem Cell Res Ther. 2020;11: Article 23, 1-12.

3, Poor organization, and many parts are hard to understand. The whole paper should be thoroughly edited.
For instance, Page 20 line 11, Various research groups have elucidated the mechanism how sEVs' contribution to improved cardiacfunction, and hence, they have reported different .strategies to engineer sEVs for their payload and modulate their stability, biodistribution, sitespecific targeting and uptake by the target cells.
What is the relationship between the mentioned mechanism and the different strategies?
4. Many statements lack references.
5. There are many Spelling mistakes or redundancy. Proofreading is required.
For instance:
Page 18, line 46: into
Page 30, line 24: fetched
Page22, line 40: I vitro

Authors' response

Dear Editor

We are resubmitting our revised (REV2) manuscript (Ref.: Ms. No. JCTRes-D-22-00107R1) entitled Endothelial Progenitor Cell-derived Small Extracellular Vesicles (sEVs) for Myocardial Angiogenesis and Revascularization for publication in your esteemed journal (Journal of Clinical and Translational Research).

We have revised the manuscript in the light of worthy reviewer#2 comments. We understand that the remaining 4 reviewers are satisfied with our responses during the first round of revision (REV1).

We have also included our itemized response to the comments.

Thank you.

Prof Haider

Reviewers' comments:

Reviewer #2:

Comment #1. There are still many mistakes in the revised Graphical abstract.
a. Lack of description of the biogenesis of MVB-derived exosomes and microvesicles.
b. Both have similar biological functions.
c. Protein also includes growth factors. There are the same questions in the text
d. The release of soluble paracrine factors cannot be illustrated as a diffusion process.

Author's response

a. We agree with the worthy reviewer about lack of biogenesis description because that is certainly not the primary focus of the review nor that of the graphical abstract. Hence, this information is not included on purpose nor it is required. The primary focus here is exosomes as insoluble paracrine factors for myocardial angiogenesis.
b. We agree that both exosomes and microvesicles have similar biological functions. That is the reason we have agreed with the three of the five reviewers of this manuscript to replace the term exosomes with sEVs.
c. The necessary suggestion has been incorporated in the Figure.
d. Paracrine secretion of soluble factors is a mix of both active and passive transport mechanisms. Whereas release of soluble factors from a cell is a mix these transport mechanism, it is dominated by diffusion in the extracellular matrix from the secreting cell to the recipient cells. In response to reviewer's comments, we have added the word "Secretion" to make it more meaningful.

Comment #2.

Some cited references are inappropriate. For instance, Page13, lines 20-23, Exosomes are nano-sized EVs (<150nm in diameter) produced inside the cells by fusion of the multi-vesicular bodies with the cell membrane (26)
26. Haider KhH, Aramini B. "Mircrining" the injured heart with stem cell-derived exosomes: an emerging strategy of cell-free therapy. Stem Cell Res Ther. 2020;11: Article 23, 1-12.

Author's response

We appreciate worthy reviewer's efforts for meticulous and in-depth review. However, the reference #26 in discussion here is not inaccurate as there is a complete section in the quoted reference regarding exosome size and characteristics (Exosomes as part of paracrine activity"). We have no hesitation

remove the said reference if the worthy reviewer insists on this but we believe the reference is not irrelevant.

Comment #3, Poor organization, and many parts are hard to understand. The whole paper should be thoroughly edited.

For instance, Page 20 line 11, Various research groups have elucidated the mechanism how sEVs' contribution to improved cardiac function, and hence, they have reported different .strategies to engineer sEVs for their payload and modulate their stability, biodistribution, sitespecific targeting and uptake by the target cells.
What is the relationship between the mentioned mechanism and the different strategies?

Author's response

Please note that REV1 PDF file had three manuscript versions put together in one file: first version was the original submission, second version showed track changes made in response to reviewer comments and the third version after track changes acceptance and further refinement by the authors).

We believe that worthy reviewer (Reviewer #2) has reviewed version 2 (with track changes instead of Version 3). The example given by the reviewer in this comment is not present in the refined version 3 (without track changes) as it was taken care of at that point of revision and refinement.

The same is possibly the reason which led to Comment #5 from Reviewer #2.

Comment #4. Many statements lack references.

Author's response

We thank the worthy reviewer for bringing this up.

In fact, we have reviewed the manuscript *in toto* and find that most statements have their relevant citation. If the reviewer means that the every statement pertaining to the data of a particular reference should be cited on each line, we consider this will make it boring for the reader. We do not think that a reference should be cited on each statement.

However, if the reviewer could specify a statement or few statements in the manuscript missing citation, this could be more helpful and we would be happy to take care of this. We have adding citation to some statements per suggestion of the reviewer.

Comment #5. There are many Spelling mistakes or redundancy. Proofreading is required.
For instance：
Page 18, line 46: into
Page 30, line 24: fetched
Page22, line 40: I vitro

Author's response

We believe that worthy reviewer used the version with track changes and not the final refined version (given in the end without track changes). Anyway, we have corrected the "fetched" while the other two mistakes mentioned were already correct during refining of the text.

The manuscript has been proofread again per suggestion of the reviewer.

Journal of Clinical and Translational Research
Peer review process file 08.202206.004

3rd Editorial decision
15-Oct-2022

Ref.: Ms. No. JCTRes-D-22-00107R2
Endothelial Progenitor Cell-derived Small Extracellular Vesicles (sEVs) for Myocardial Angiogenesis and Revascularization
Journal of Clinical and Translational Research

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 Nov 14, 2022.

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,

Thank you for submitting a revised draft. We have carefully gone through the revisions in juxtaposition to the reviewers' comments and found that the latest draft is suitable for publication after linguistic sanitization. The writing is sloppy at times (e.g., no space after period and start of new sentence) and the manuscript is still replete with grammar/spelling inconsistencies.

After you have meticulously removed the errors, we can proceed to publishing the paper.

Thank you and the best of luck with the last round of edits.

Michal heger
Editor

Authors' response

Dear Editor

We are resubmitting our revised (REV3) manuscript (Ref.: Ms. No. JCTRes-D-22-00107R1) entitled Endothelial Progenitor Cell-derived Small Extracellular Vesicles (sEVs) for Myocardial Angiogenesis and Revascularization for publication in your esteemed journal (Journal of Clinical and Translational Research).

We have thoroughly revised the manuscript for typos, and grammar in the light of Editorial Comments.

Thank you.

Prof Haider

4th Editorial decision
20-Oct-2022

Ref.: Ms. No. JCTRes-D-22-00107R3
Endothelial Progenitor Cell-derived Small Extracellular Vesicles (sEVs) for Myocardial Angiogenesis and Revascularization
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: