OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jentashapir Journal of Cellular and Molecular Biology
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Peer Review Report for "Alginate-Gelatin Microspheres Protect Human Mesenchymal Stem Cells During Deep Cryopreservation"

Author(s): Shirin Saberianpour, Reza Rahbarghazi, Arezoo Rezaie Nezhad Zamani, Mahdi Ahmadi, Morteza Heidarzade, Shahroz Amin Mozaffari

Review Timeline:

Submit Date:	28 Mar 2020
Revised Date:	20 Apr 2020
Accepted Date:	20 Apr 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | Revision (0)

5 Apr 2020

Dear AE,
The methods part needs more experiment. The most immediate use of MSCs is their ability to differentiate into different tissues, and there are a handful of genes suggesting possible MSC stemness markers, the molecular basis underlying MSC stemness, especially the key transcription factor to MSC stemness. Therefore, it is important to analyse the expression of genes that complicated in stemness of MSCs after use of Alginate-gelatin microspheres for cryopreservation, RT real-time PCR should perform after cryopreservation and 2 weeks of culture. All samples should express stemness genes of MSCs (Oct4, Lin28a, Nanog, Sall4 and Plzf).
Thanks.

Vahid Bayati: Reviewer | Revision (0)

7 Apr 2020

Dear AE,
This is an interesting study conducted to examine the effect of encapsulation on freeze-thawing process of MSCs. In order to investigate that, aliginate-gelatin scaffold was used and its cryoprotectant effects were studied using MTT assay and flowcytometry. In general,

the study was designed properly, but the following comments have to be considered or responded:
1. English has to be edited in some parts, i.e: "These data show that encapsulation as a promising biological carrier for cell preservation during freezing/thawing procedure." should be changed to "These data show that encapsulation is a promising biological carrier for cell preservation during freezing/thawing procedure." or "These data show the encapsulation as a promising biological carrier for cell preservation during freezing/thawing procedure".
2. The sentence "We hypothesized that microencapsulation of MSCs could decrease the detrimental effect of freezing/thawing procedure." from the methods section has to be omitted and added to introduction section.
3. What is the difference between the present study and the reference 30 (Katsen-Globa et al). It seemed this study was similar to the present research somehow.
4. In acknowledgment, the grant number should be written.
Thanks.

REFEREE: Reviewer | **Revision** (0) 12 Apr 2020

Dear AE,
1. In different parts of the materials and methods such as:
2.1. Cell culture, 2.2. Microencapsulation, 2.3. Cell freezing and storage, 2.4. MTT assay 2.5. Analyzing of growth dynamic by cell cycle assay and 2.6. Flow cytometry analysis adding references is required.
2. Past information about cell freezing has always shown that the survival rate is less than one hundred percent following the thawing process and the viability decrease therefore needs to clarify why in Figure 1(B) the cell viability is equal to one hundred or more than two hundred percent and what is author formula for this chart?
Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (0) 12 Apr 2020

Dear EIC,
According to reviewer comments, this manuscript needs a revision. The authors should revise it from point to point and then submit their revision. Faster submission of the revised manuscript will guarantee a faster peer-review process.
Best

Seyyed Ali Mard: EIC | **Revision** (0) 12 Apr 2020

Dear Authors
According to reviewer comments your manuscript needs a revision. Please revise it point to point and then submit your revision. A faster submission of revised manuscript will guarantee a faster peer-review process.
Best

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor

Thank you for giving me the opportunity to submit a revised draft of our manuscript)Alginate-gelatin microspheres protect human mesenchymal stem cells during deep cryopreservation (.We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. We are grateful to the reviewers for their insightful comments on my paper.

Reviewers' Comments:

Reviewer 1:

1. In different parts of the materials and methods such as:

2.1. Cell culture, 2.2. Microencapsulation, 2.3. Cell freezing and storage, 2.4. MTT assay 2.5. Analyzing of growth dynamic by cell cycle assay and 2.6. Flow cytometry analysis adding references are required.

According to your comment, changes were made in the results section and were highlighted

*2. Past information about cell freezing has always shown that survival rate is less than one hundred percent following thawing process and the viability decrease therefore is need to clarify why in Figure 1(B) the cell viability is equal one hundred or more than two hundred percent and what is author formula for this chart?*as we mentioned in discuss, alginate–gelatin cryogel scaffolds could preserve MSCs stemness and viability. It seems that the modulation of mechanical strains and the ability of alginate to absorb water and prevent the formation of ice crystals could decrease cell insult during cryopreservation. Although the use of

alginate-based hydrogels has numerous advantages to protect cells during cooling/thawing process, it was shown that the addition of gelatin to alginate backbone increased the incorporation of cryoprotectants to the hydrogel. These help to reduce ice crystal formation and facilitate the equilibration of osmotic differences thereby diminish cell damage and cytotoxic effects

Reviewer 2:

This is an interesting study conducted to examine the effect of encapsulation on freeze-thawing process of MSCs. In order to investigate that, aliginate-gelatin scaffold was used and its cryoprotectant effects were studied using MTT assay and flowcytometry. In general, the study was designed properly, but the following comments have to be considered or responded:

1. English has to be edited in some parts, i.e: "These data show that encapsulation as a promising biological carrier for cell preservation during freezing/thawing procedure." should be changed to "These data show that encapsulation is a promising biological carrier for

cell preservation during freezing/thawing procedure." or "These data show the encapsulation as a promising biological carrier for cell preservation during freezing/thawing procedure".

According to your comment, changes were made in the results section and were highlighted

2. The sentence "We hypothesized that microencapsulation of MSCs could decrease the

detrimental effect of freezing/thawing procedure." from the methods section has to be omitted and added to introduction section.

We removed this sentence from the Methods section and added it to the Introduction section . The change was highlighted.

3. What is the difference between the present study and the reference 30 (Katsen-Globa et al). It seemed this study was similar to the present research somehow.
4. In acknowledgment, the grant number should be written.

In the present study(unlike the previous study) the combination of alginate with gelatin was used. As mentioned in the discussion section, adding gelatin with a certain percentage due to gelatin load and its structure can help maintain cellviability.

1)It was demonstrated that gelatin is produced by the denaturation of type I collagen contains motifs that are essential for the bioactivity and dynamic growth of cells [22, 23].

2) it was shown that the addition of gelatin to alginate backbone increased the incorporation of cryoprotectants to the hydrogel. These help to reduce ice crystal formation and facilitate the equilibration of osmotic differences thereby diminish cell damage and cytotoxic effects [35].

Reviewer 3:
The methods part needs more experiment. The most immediate use of MSCs is

their ability to differentiate into different tissues, and there are a handful of genes suggesting possible MSC stemness markers, the molecular basis underlying MSC stemness, especially the key transcription factor to MSC stemness. Therefore, it is important to analyse the expression of genes that complicated in stemness of MSCs after use of Alginate-gelatin microspheres for cryopreservation, RT real-time PCR should perform after cryopreservation and 2 weeks of culture. All samples should express stemness genes of MSCs (Oct4, Lin28a, Nanog, Sall4 and Plzf).

Thank you for your suggestion to improve the scientific value of the article
Due to Corona's situation at this time, we do not have access to more laboratory methods at this time in the universities.

With respect , if you have any other suggestions (except for doing laboratory work that are not currently available), I will look forward to changing to improve the Scientific status of the article.

Best regards,

Shirin Saberianpour

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Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Zahra Shokati: Reviewer | **Revision** (1) 15 Apr 2020

Dear AE,
In general, comments were answered properly, but the following comments have to be considered and responded again by the authors:
1. As you mentioned, alginate–gelatin cryogel scaffolds could preserve MSCs viability and diminish cell damage and cell death, however, past information about cell freezing has always shown that the survival rate is less than 100% following the thawing process, and the viability decreases. Therefore, is needed to clarify why in Figure 1(B) the cell viability in basic form (MSC) is equal 100% and in Encapsulated form is equal 200% or more. What is your formula for this chart?
2. In Method's section, the Ethics Committee Number should be written.
Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (1) 15 Apr 2020

Dear EIC,
According to reviewer comments, this manuscript needs more revision.
Thanks.

Seyyed Ali Mard: EIC | **Revision** (1) 15 Apr 2020

Dear Author
According to our final reviewer comments, there are still 2 points in your manuscript should be corrected.
Best regards

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor

Thank you for giving me the opportunity to submit a revised draft of our manuscript .We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. We are grateful to the reviewers for their insightful comments on my paper.

1-To calculate a viability assay like MTT, do the following:

First, calculate the mean of your OD for each samples. You may convert the value to % of cells viability. Example:
For control : mean OD control/mean OD control *100 =100 %
as for treatment : mean OD treatment/mean OD control *100=_%

1) make an average of a few "empty" wells that contain your MTT solution but *no* cells. This will serve as a background control (= "blank"). Because you will calculate percentages/ratios, you need to define what the baseline (= zero viability = no cells) is, otherwise your data is meaningless.
2) substract your background control from step 1 from all the measurements for this plate. Now your values have been corrected for background fluorescence, and you can proceed building ratios.
3) calculate an average for your control (=healthy cells with 100% viability).
4) now calulate a ratio of VALUE / control * 100 for each of your values (-background control, of course)
Example:
Background fluorescence: 500
Sample A: 800
Control: 600
Relative Ratio (Sample/Control): 800 / 600 = 133%

True relative ratio: (Sample-Background)/(Control-Background) : (800-500)/(600-500)=**300%**

2-This project was done in addition to my PhD dissertation work (Ethical Code: 58906). On the other hand, there was no animal work or use of human samples in this project.

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Revision (2)

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REFEREE: Reviewer | **Revision** (2) 17 Apr 2020

Dear AE,
The following comment has to be considered:
1. Please correct Figure 1B, instead of cell viability percent, you should report the result in "Fold Change" because, in scientific papers, there is no report about 100% < cell viability.
Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (2) 17 Apr 2020

Dear EIC
The reviewer(s) have recommended publication, but also suggest some minor revisions to the manuscript. Therefore, I invite the author to respond to the reviewer(s)' comments and revise his manuscript.
Kind regards,

Seyyed Ali Mard: EIC | **Revision** (2) 17 Apr 2020

Dear Author
I am pleased to inform you our reviewers are now provisionally accepted you manuscript but there is a point to be solved before acceptance. Please change your "cell viability figure" according to reviewer recommendation.
Best

OPEN PEER REVIEW

Revision (3)

Reply to Reviewers

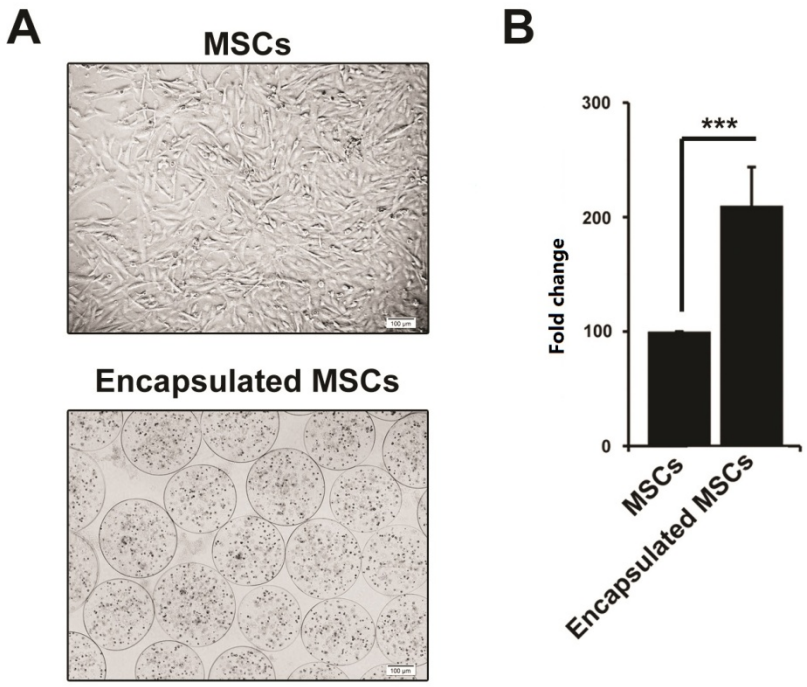
Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor

Reviewer 1:
The following comment has to be considered:

1. Please correct the Figure 1B, instead of cell viability percent, you should report the result in "Fold Change" because, in scientific papers, there is no report about 100% < cell viability.



OPEN PEER REVIEW

Revision (3)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (3) 19 Apr 2020

Dear AE,
The following comment has to be considered:
1. Please correct the Figure 1B according to "uploaded figure", instead of 100 and 200, you should report the result in "Fold Change": 0.5- 1- 1.5- 2.
2. In Method's section, the Ethics Committee Number "should be written".
Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (3) 19 Apr 2020

Dear EIC,
This article needs two more corrections.
Thanks.

Seyyed Ali Mard: EIC | **Revision** (3) 19 Apr 2020

Dear Author,
As you wrote earlier your manuscript is now provisionally accepted but there are two points that have to correct. They are
1. Please correct Figure 1B according to "uploaded figure", instead of 100 and 200, you should report the result in "Fold Change": 0.5- 1- 1.5- 2.
2. In Method's section, the Ethics Committee Number "should be written"
Thanks.

OPEN PEER REVIEW

Revision (4)

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You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor

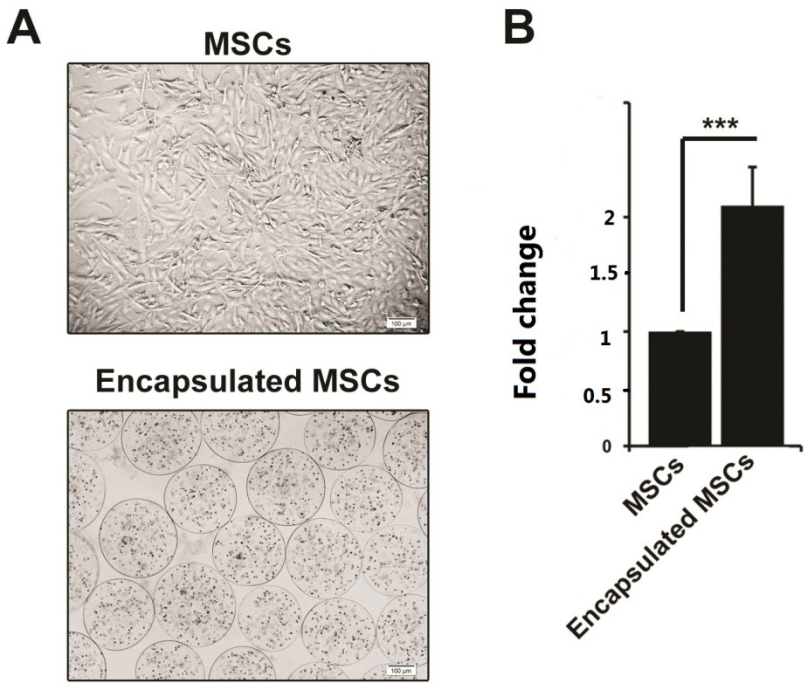
Comment 2

I added ethical code to Acknowledgment part

Comment 1

Best regards

saberianpour



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Revision (4)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Seyyed Ali Mard: EIC | **Revision** (4) 20 Apr 2020

Dear Author,

All needed corrections were done right now.

Thanks.