OPEN PEER REVIEW

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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://brief.land/briefland/knowledgebase/category/tree.html#opr.html

Gene, Cell and Tissue
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Peer Review Report for "Adipose-Derived Stem Cells Conditioned Media Promote In Vitro Osteogenic Differentiation of Hypothyroid Mesenchymal Stem Cells"

Author(s): Tayebeh Sanchooli, Mohsen Norouzian, Mahtab Teimouri, Abdolreza Ardeshirylajimi, Abbas Piryaei

Review Timeline:

Submit Date: 1 Mar 2020
Revised Date: 25 Apr 2020
Accepted Date: 27 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) 3 Mar 2020

Dear AE,
Comments for the authors:
- The application of the abbreviation must be revised in your manuscript.
- Line 83- please specify how did you concentrate the conditioned media 20-fold.
- Please update your primer table for gene names.
- Please specify that the inducted hypothyroidism by Methimazole is reversible?
- Does removing drug consumption will affect hypothyroidism?
- Please specify the percentage of your Stem cells according to the Flow cytometry result.
good job! I totally like this manuscript.
Thanks.

Nadia Sheibak: Reviewer | **Revision** (0) 5 Mar 2020

Dear AE,
Concerning the manuscript entitled “Adipose derived Stem Cells Conditioned Media promote In Vitro Osteogenic Differentiation of Hypothyroid Mesenchymal Stem Cells" for Gene, Cell and Tissue Journal.
Final evaluation:
- With respect to the authors, the article is recommended to accept after major revision. Rewriting or doing more research could improve the study.
- This manuscript suggested that ADMSC-CM may be a promising candid for bone regeneration in the hypothyroidism cases.
- Although there is a novel approach in the present study, there are several major issues with this paper:
1- It is indicated that adipose derived mesenchymal stem cells conditioned medium has many osteogenic factors, such as IGF-1, IL-6 and FGFs.
As several side effect reported in regard to using stem cells, what are advantages of using these cells instead of recombinant proteins such as rIGF-1, rIL-6 and rFGFs to stimulate osteogenesis?
2- What are the advantages of using these cells instead of other choices such as bone-conditioned medium or human marrow stromal fibroblasts?
3- Hypothyroidism induction should be described briefly.
4- The expression of surface markers of BMSCs and ADMSCs was evaluated using monoclonal antibodies against CD45, CD34, CD105, CD90 and CD73.
According to standard protocols CD31- as a marker should also be checked.
5- Authors mentioned that “As expected, the proliferation of BMSCs was significantly increased in the presence of ADMSC-CM, because some growth factors such as FGF-2 and Platelet Derived Growth Factor (PDGF), that is known to induce cell proliferation, have been detected in MSC secretion.”
There is no date that show these factors evaluated by authors to verify this hypothesis.
6- Limitations and new suggestion should be added to the manuscript.
7- Discussion section should be revised with more focus on present findings and possible hypothesis.
8- English editing is highly recommended.
Thanks

Azam Asemi Rad: Reviewer | **Revision** (0) 9 Mar 2020

Dear AE,
Concerning the manuscript entitled “Adipose derived Stem Cells Conditioned Media promote in Vitro Osteogenic Differentiation of Hypothyroid Mesenchymal Stem Cells" for Gene, Cell and Tissue Journal.
This work presents interesting results pertaining to the ADMSC-CM as a candid for bone regeneration in hypothyroidism. There is a novel approach in the present study. However, I have the following specific concerns with this particular study which should be addressed by the authors:
1. Please rewrite the introduction. The introduction must be start with explaining main concepts not with the arguments.

2. What doses of Methimazole was used to induce hypothyroidism?
3. Please add the Specifications and manufacturer detail of Methimazole.
4. How did you concentrate the conditioned media 20-fold?
5. In first paragraph of discussion you mentioned " the proliferation of BMSCs was significantly increased in the presence of ADMSC" but there is no evidence about evaluating this procedure.
6. The positive and negative CD markers must be specifying for potential readers.
7. The language quality must be improved.
Thanks.

Azam Asemi Rad: Associate Editor | **Revision (0)** 13 Mar 2020

Dear EIC,
Thanks for submitting the manuscript entitled “Adipose derived Stem Cells Conditioned Media promote in Vitro Osteogenic Differentiation of Hypothyroid Mesenchymal Stem Cells" to Gene, Cell and Tissue Journal.
The review process is now complete, and three thorough reviews are included. Although the reviewers found considerable merit in this paper, they also identified some concerns. Generally, there are some points in the introduction, material, methods, discussion, and also writing and grammatical points in this manuscript. Therefore, I invite the authors to respond to the reviewer's comments and revise the manuscript.
Best,

Zahra Heidari: EIC | **Revision (0)** 13 Mar 2020

Dear Authors,
Based on the reviewerss and AE comment, this manuscript needs major revision and a point-by-point reply to the comments.
Thanks.

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.

Reviewer 1:
Concerning the manuscript entitled "Adipose derived Stem Cells Conditioned Media promote in Vitro Osteogenic Differentiation of Hypothyroid Mesenchymal Stem Cells" for Gene, Cell and Tissue Journal. This work presents interesting results pertaining to the ADMSC-CM as a candid for bone regeneration in hypothyroidism. There is a novel approach in the present study. However, I have the following specific concerns with this particular study:

1. Please rewrite the introduction. The introduction must be start with explaining main concepts not with the arguments.

It was done. The first parts were corrected and some content was deleted.

2. What doses of Methimazole was used to induce hypothyroidism?

4mg Methimazole (Tehran, Iran hormone), dissolved in100 cc distilled water.

3. Please add the Specifications and manufacturer detail of Methimazole.

Methimazole powder (Tehran, Iran hormone)

4. How did you concentrate the conditioned media 20-fold?

the conditioned media was collected and concentrated 20 fold using Amicon Ultra Centrifugal Filter Device (Millipore) with a 3kD molecular weight cutoff.

5. In first paragraph of discussion you mentioned " the proliferation of BMSCs was significantly increased in the presence of ADMSC" but there is no evidence about evaluating this procedure.

We used MTT assay to evaluate the cell proliferation of normal BMSCs and hypothyroid BMSCs with and without ADMSC-CM. Some studies have used this test to evaluate cell proliferation. For example:

Ardeshirylajimi A, Mossahebi-Mohammadi M, Vakilian S, Langroudi L, Seyedjafari E, Atashi A, et al. Comparison of osteogenic differentiation potential of human adult stem cells loaded on bioceramic-coated electrospun poly (L-lactide) nanofibres. Cell proliferation. 2015;48(1):47-58.

6. The positive and negative CD markers must be specifying for potential readers.

The following sentence was added in the fig legend.

All stem cells were positive for CD73, CD90 and CD105, and negative for hematopoietic markers CD34, CD45.

7. The language quality must be improved.
It was done

Reviewer 2:
Concerning the manuscript entitled "Adipose derived Stem Cells Conditioned Media promote In Vitro Osteogenic Differentiation of Hypothyroid Mesenchymal Stem Cells" for Gene, Cell and Tissue Journal.

Final evaluation:
With respect to the authors, the article is recommended to accept after major revision. Rewriting or doing

more research could improve the study.

This manuscript suggested that ADMSC-CM may be a promising candid for bone regeneration in the hypothyroidism cases.
Although there is a novel approach in the present study, there are several major issues with this paper:

1- It is indicated that adipose derived mesenchymal stem cells conditioned medium has many osteogenic factors, such as IGF-1, IL-6 and FGFs.
As several side effect reported in regard to using stem cells, what are advantages of using these cells instead of recombinant proteins such as rIGF-1, rIL-6 and rFGFs to stimulate osteogenesis?

The use of growth factors such as BMP-2, which is available in the market, plays an important role in osteogenesis. But these factors are expensive and may exacerbate the inflammatory responses. In addition, the use of a single growth factor limits the ability of the osteogenesis process. Therefore, in this study, we used ADMSC-CM, which is a combination of different growth
2- What are advantages of using these cells instead of other choices such as bone-conditioned medium or human marrow stromal fibroblasts?

Since the MSC is not differentiated and has the ability to differentiate into different cells, it secretes more growth factors and cytokines compared to differentiated cells such as fibroblasts. Due to various studies that have determined the amount and type of these factors in the condition medium of MSC, we decided to use this cell.

3- Hypothyroidism induction should be described briefly.

Hypothyroidism was induced by administration of 4mg Methimazole (Tehran, Iran hormone), dissolved in

100 cc water for 60 days.

4- The expression of surface markers of BMSCs and ADMSCs was evaluated using monoclonal antibodies against CD45, CD34, CD105, CD90 and CD73.
According to standard protocols CD31- as a marker should also be checked.

You're right. But we were able to measure these markers based on available antibodies in the lab.
5- Authors mentioned that "As expected, the proliferation of BMSCs was significantly increased in the presence of ADMSC-CM, because some growth factors such as FGF-2 and Platelet Derived Growth Factor (PDGF), that is known to induce cell proliferation, have been detected in MSC secretion."
There is no date that show these factors evaluated by authors to verify this hypothesis.

Unfotunately, due to financial limitations, the level of growth factors in the ADMSC-CM was not measured, and we referred to previous studies that have evaluated these factors in CM.

6- Limitations and new suggestion should be added to the manuscript.

It was added.
7- Discussion section should be revised with more focus on present findings and possible hypothesis.

It was done.

8- English editing is highly recommended.
It was done

Reviewer 3:
Hello Dear Sir/Maam

the application of the abbreviation must be revised in your manuscript.

It was done.
line 83- please specify how did you concentrate the conditioned media 20-fold.

It was done.
please update your primer table for gene names.

It was done
please specify that the inducted hypothyroidism by Methimazole is reversible?
removing drug consumption will affect the hypothyroidism?

According to the previous studies, this is the method used to hypothyroidism induction. The disease does not appear to be reversible in the short term. Our study was conducted in vitro and the cells were cultured for a maximum of 21 days.

Refrences: Bruck R, Oren R, Shirin H, Aeed H, Papa M, Matas Z, Zaidel L, Avni Y, Halpern Z. Hypothyroidism minimizes liver damage and improves survival in rats with thioacetamide induced fulminant hepatic failure. Hepatology. 1998 Apr;27(4):1013-20
please specify the percentage of your Stem cells according to Flow cytometry result.
It was done
good job! I totally like your manuscript.

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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.

Nadia Sheibak: Reviewer | **Revision** (1) — 26 Apr 2020

Dear AE,
Thanks to the authors for point-to-point answers and corrections.
Regards

Azam Asemi Rad: Reviewer | **Revision** (1) — 26 Apr 2020

Dear AE,
Thanks for giving me the opportunity of peer review to me. I have read the manuscript entitled “Adipose derived Stem Cells Conditioned Media promote In Vitro Osteogenic Differentiation of Hypothyroid Mesenchymal Stem Cells”.
Considering that this manuscript had a peer review in the previous version, I read the manuscript and reviewed the sections modified by the author in response to the reviewers. I must point out that this version is suitable for accepting.
Sincerely,

Bita Moudi: Associate Editor | **Revision** (1) — 27 Apr 2020

Dear EIC
Based on the authors' answers to the reviewers' comments, the reviewer(s) have recommended publication.
Kind regards.

REFEREE: EIC | **Revision** (1) — 27 Apr 2020

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks.