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://brieflands.com/briefland/knowledgebase/category/tree.html#opr.html

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Peer Review Report for "TGFβ1-Pretreated Exosomes of Wharton Jelly Mesenchymal Stem Cell as a Therapeutic Strategy for Improving Liver Fibrosis"

Author(s): Samaneh Salehipour Bavarsad, Mohammad Taha Jalali, Darioush Bijan Nejad, Behnam Alypoor, Hossein Babaahmadi Rezaei, Narges Mohammadtaghvaei

Review Timeline:

Submit Date:	16 Mar 2022
Revised Date:	14 May 2022
Accepted Date:	26 May 2022

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) 13 Apr 2022

Dear E.I.C
I have now completed the article review process. Although the authors have chosen an interesting title to study, but unfortunately they have not taken into account some of the small points that I will mention briefly:
1- Due to the complexity of the work and the large number of steps, it is very necessary to generate an abstract graphic or an instead an image of the whole process in the method section.
2- It seems that one of the most important and sensitive steps of the method is missed: "how to expose the activated HSCs with extracted exosomes."
3- In the first image, the naming of the lower part is not done inside the figure
4- There is no need to mention the names of companies in abstract
5- The diagrams, especially the diagrams in Figure 2 and Figure 3, are of low quality
6- There are not many comparisons and challenges in the discussion section and this

section needs to be strengthened
7- There are some writing errors in the text that need to be reviewed the whole text, for example in the abstract: It is not stand for "Wharton jelly-derived exosomes" Or, in the other example, the structure of writing the objectivesis suggested to be modified in the following form: “To evaluate the effect of….”

REFEREE: Reviewer | **Revision (0)** 23 Apr 2022

The authors considered the effect of TGFβ1 Pretreated Exosomes originated from Wharton Jelly Mesenchymal Stem Cell in Improving Liver Fibrosis; however, the following questions should be clarified before publication:

Minor revisions
1. It would be good if you could write the type of current study (e.g. review, clinical experimental study, analytical descriptive study, double blinded clinical study, cohort study and etc.) in the both abstract and materials & methods sections.
2. Use past verbs in the results. For example, the verb “are” in “The protein of p-SMAD2/3 are significantly decreased…….” Should be changed to “were”.
3. There are some grammatical errors within the manuscript which need to be revised in right order.
4. Write the abbreviation of all words where they are mentioned for the first time within the manuscript (abstract & text body). For instance, the abbreviation of ECM should be written where it has been mentioned for the first time either in the abstract or manuscript body.
5. It was better if you wrote some of main findings (such as fold-change ratio) as quantitative or mean ±SD within the abstract.
6. Please show magnificent bar in microscopic figures.
7. Show osteogenic or adipogenic differentiation in figure-1 using some arrows.
8. Write the name of all applied apparatuses, chemicals, kits, solutions you applied for biochemical and the other examinations. You should also mention the name of all companies you purchased materials from them.
9.

Major revisions
1. How you did use WJ-MSCs-Exs for pretreatments? You have mentioned WJ-MSCs-Exs were extracted from multiple neonates. Did you evaluate each WJ-MSCs-Exs for a separate treatment or you combined all WJ-MSCs-Exs together for a treatment? It should be clarified.
2. Showing a microscopic photo presenting the fibrosis before and after treatment in rat models would strengthen the manuscript.
3. It would be well if the authors considered a wide range of oxidative stress and oxidative stress biomarkers either at gene or protein levels before and after treatments.
4. Considering the balance between MMPs/TIMPs would strengthen the quality of this work.
5. As fibrosis is a chronic disease, designing a study at chronic phase was better than acute phase. Why did you develop a study at acute phase while fibrosis is a chronic disease?

REFEREE: Associate Editor | **Revision** (0) 23 Apr 2022

Dear EIC,

The manuscript needs a major revision.

Thanks

Seyed Moayed Alavian: EIC | **Revision** (0) 24 Apr 2022

Dear Authors,

Thank you for your submission. Please follow the peer-review comments and submit a revision.

Regards,
EIC

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.

We are grateful to the *reviewers* for their insightful comments on our paper. We have been able to incorporate changes to reflect most of the suggestions provided by the *reviewers*. We have highlighted the changes within the manuscript. Here is a point-by-point *response to the reviewers*' comments and concerns.

Reviewer comment	***Response***	***Pages***
Reviewer1: The authors considered the effect of TGFβ1 Pretreated Exosomes originated from Wharton Jelly Mesenchymal Stem Cell in Improving Liver Fibrosis; however, the following questions should be clarified before publication:		
Minor revisions		
1. It would be good if you could write the type of current study (e.g. review, clinical experimental study, analytical descriptive study, double blinded clinical study, cohort study and etc.) in the both abstract and materials & methods sections.	According to reviewer comment, the type of experimental study was added. They are also highlighted in abstract and materials & methods sections.	1 & 5
2. Use past verbs in the results. For example, the verb “are” in “The protein of p-SMAD2/3 are significantly decreased…….” Should be changed to “were”.	According to reviewer comment, the grammatical errors were was modified and the changes were highlighted.	1,line 26
3. There are some grammatical errors within the manuscript which need to be revised in right order.	According to reviewer comment, the language of the paper and grammatical errors were edited and improved and highlighted in manuscript.	-
4. Write the abbreviation of all words where they are mentioned for the first time within the manuscript (abstract & text body). For instance, the abbreviation of ECM should be written where it has been mentioned for the first time either in the abstract or manuscript body.	According to reviewer comment, abbreviation of all words edited and highlighted in the abstract or manuscript body.	1
5. It was better if you wrote some of main findings (such as fold-change ratio) as quantitative or mean ±SD within the abstract.	According to reviewer comment, the fold-change ratio was included in the abstract and displayed in highlight.	
6. Please show magnificent bar in microscopic figures.	According to reviewer comment, magnificent bar in microscopic figures was placed.	16
7. Show osteogenic or adipogenic differentiation in figure-1 using some arrows.	According to reviewer comment, differentiation into osteogenic or adipogenic was shown in the figure-1 using some arrows.	15
8. Write the name of all applied apparatuses, chemicals, kits, solutions you applied for biochemical and the other examinations. You should also mention the name of all companies you purchased materials from them.	According to reviewer comment, the name of all applied apparatuses, chemicals, kits, solutions, etc. were placed in the text and was highlighted.	

Major revisions		
1. How you did use WJ-MSCs-Exs for pretreatments? You have mentioned WJ-MSCs-Exs were extracted from multiple neonates. Did you evaluate each WJ-MSCs-Exs for a separate treatment or you combined all WJ-MSCs-Exs together for a treatment? It should be clarified.	According to reviewer comment, after extracting and confirming of the WJ-MSCs, the WJ-MSCs were exposed to a lower FBS medium to extract the exosome. Every 2 days, the percentage of FBS in the media was reduced until it finally reached zero. The above protocol is the protocol proposed by Exosib Company, which does not cause stress to the WJ-MSCs by gradually reducing the FBS. Then, the WJ-MSCs were exposed to TGFβ1 at different concentrations (control, 0.1, 0.5, 1, 5 and 10 ng/ml) for 24 hours. The supernatant was then discarded and fresh FBS-free medium was added. After 72 hours, the supernatant was removed to extract the exosome (untrated and pretreated) and performed according to the manufacturer's instructions. The protein concentration of exosomes was determined by separately BCA method. Finally, the activated HSCs were exposed to 50 microgram per ml untreated and pretreated exosomes for 24 hours in 6 wells. The HSCs were then removed to real-time PCR and Western blot analysis. In this study, because we needed a large number of cells, we had to use multiple umbilical cords. In the case of exosomes, the cells were mixed after extraction after confirmation, because the separation conditions were the same.	
2. Showing a microscopic photo presenting the fibrosis before and after treatment in rat models would strengthen the manuscript.	According to reviewer comment, regarding the fact that this paper is derived from university thesis and due to expensive lab tests in order to acquire mouse fibrosis model and high cost of carbon tetrachloride (CCL4), authors didn’t present animal fibrosis modeling therefore in return they decided to focus on liver cell line in presence of TGF-β factors affecting HSCs and lead to arise indicators such as collagens.	-
3. It would be well if the authors considered a wide range of oxidative stress and oxidative stress biomarkers either at gene or protein levels before and after treatments.	According to reviewer comment, In this study, due to budget limits to analyze oxidate stress pathway, with respect to high cost of utilizing enzymes such as NOXs (including NOX, NOX2 and NOX4) and ROS, it was not possible to do that. On the other hand, this pathway occurs through two pathways of the carboxy domain and the linker of the transcription factor of SMAD, which is a complex pathway. In fibrosis circumstances, authors preferred to conduct this analytic perspective in separate study to complete the project and cover the costs, therefore the results are going to be published in near future paper.	
4. Considering the balance between MMPs/TIMPs would strengthen the quality of this work.	According to reviewer comment, the subject of MMPs/TIMPs especially MMP2, MMP9 and TIMP1 plays important role in cellular migrations, cancer	

	pathways and angiogenesis. The main goal of this paper has been the study of ways to cure activated HSCs by getting HSCs treated with exosome mediators in order to be able to decrease some markers such as collagen & a-SMA which have been increased by fibrosis circumstances and therefore enable us to relive fibrosis condition. It would be possible to mention HSCs apoptosis discussion alongside with MMPs in a separate study that isn't among this paper objectives and could be achieved in separate study.	
5. As fibrosis is a chronic disease, designing a study at chronic phase was better than acute phase. Why did you develop a study at acute phase while fibrosis is a chronic disease?	According to reviewer comment, in liver fibrosis, after extensive damage to the liver, the chronic disease eventually activates HSCs. Our goal in this study is to design a model, the condition is similar to when fibrosis occurs in the body, that HSCs are activated. In this study, the activation of HSCs was performed in the presence of TGFB1, which our desired model is created, and then the goal of treatment with exosomes, which was a more important goal in this project, was pursued.	
Reviewer 2: Dear E.I.C I have now completed the article review process. Although the authors have chosen an interesting title to study, but unfortunately they have not taken into account some of the small points that I will mention briefly:		
1- Due to the complexity of the work and the large number of steps, it is very necessary to generate an abstract graphic or an instead an image of the whole process in the method section.	According to reviewer comment, the whole process in the method section is schematically placed in Figure 1 and highlighted in the text.	
2- It seems that one of the most important and sensitive steps of the method is missed: "how to expose the activated HSCs with extracted exosomes."	According to reviewer comment, after extracting and confirming of the WJ-MSCs, the WJ-MSCs were exposed to a lower FBS medium to extract the exosome. Every 2 days, the percentage of FBS in the media was reduced until it finally reached zero. The above protocol is the protocol proposed by Exosib Company, which does not cause stress to the WJ-MSCs by gradually reducing the FBS. Then, the WJ-MSCs were exposed to TGFβ1 at different concentrations (control, 0.1, 0.5, 1, 5 and 10 ng/ml) for 24 hours. The supernatant was then discarded and fresh FBS-free medium was added. After 72 hours, the supernatant was removed to extract the exosome (untrated and pretreated) and performed according to the manufacturer's instructions. The protein concentration of exosomes was determined by separately BCA method. Finally, the activated HSCs were exposed to 50 microgram per ml untreated and pretreated exosomes for 24 hours in 6	

	wells. The HSCs were then removed to real-time PCR and Western blot analysis.	
3- In the first image, the naming of the lower part is not done inside the figure	According to reviewer comment, the naming of the lower part was corrected.	
4- There is no need to mention the names of companies in abstract	According to reviewer comment, the abstract section was modified.	
5- The diagrams, especially the diagrams in Figure 2 and Figure 3, are of low quality	According to reviewer comment, high quality diagram was placed.	
6- There are not many comparisons and challenges in the discussion section and this section needs to be strengthened	According to reviewer comment, the discussion section was updated and some references were also included. Moreover, related articles were reviewed and used, where applicable and as far as possible. All changes were highlighted.	
7- There are some writing errors in the text that need to be reviewed the whole text, for example in the abstract: It is not stand for "Wharton jelly-derived exosomes" Or, in the other example, the structure of writing the objectivesis suggested to be modified in the following form: “To evaluate the effect of….”	According to reviewer comment, the whole text was checked to correct grammatical and writing errors. The suggested sentence was replaced. The changes were displayed in highlight.	

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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.

REFEREE: Associate Editor | **Revision** (1) 25 May 2022

Dear EIC,

The revised manuscript is accepted for publication.

Thanks

Seyed Moayed Alavian: EIC | **Revision** (1) 26 May 2022

Dear Authors,

I am delighted to inform that your manuscript is accepted for publication in Hepatitis Monthly.

Regards,
EIC