

## PEER-REVIEW REPORT

**Name of journal:** World Journal of Stem Cells

**Manuscript NO:** 67776

**Title:** Exosomes derived from inflammatory myoblasts promote M1 polarization and break the balance of myoblast proliferation/differentiation

**Reviewer's code:** 05906390

**Position:** Peer Reviewer

**Academic degree:** MD

**Professional title:** Doctor

**Reviewer's Country/Territory:** China

**Author's Country/Territory:** China

**Manuscript submission date:** 2021-05-01

**Reviewer chosen by:** AI Technique

**Reviewer accepted review:** 2021-05-01 12:15

**Reviewer performed review:** 2021-05-01 12:28

**Review time:** 1 Hour

<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input checked="" type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input checked="" type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Re-review</b>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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#### **SPECIFIC COMMENTS TO AUTHORS**

The article is well designed, original, and I would love to recommend it for publication.

## PEER-REVIEW REPORT

**Name of journal:** World Journal of Stem Cells

**Manuscript NO:** 67776

**Title:** Exosomes derived from inflammatory myoblasts promote M1 polarization and break the balance of myoblast proliferation/differentiation

**Reviewer's code:** 05115904

**Position:** Peer Reviewer

**Academic degree:** PhD

**Professional title:** Assistant Professor

**Reviewer's Country/Territory:** India

**Author's Country/Territory:** China

**Manuscript submission date:** 2021-05-01

**Reviewer chosen by:** AI Technique

**Reviewer accepted review:** 2021-05-02 10:32

**Reviewer performed review:** 2021-05-08 09:29

**Review time:** 5 Days and 22 Hours

<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input checked="" type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Re-review</b>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

## **SPECIFIC COMMENTS TO AUTHORS**

Reviewer Comments Name of Journal: World Journal of Stem Cells Manuscript NO:  
Manuscript Type: ORIGINAL ARTICLE Basic Study Exosomes derived from inflammatory myoblasts promote M1 polarization and break the balance of myoblast proliferation/differentiation Zhiwen Luo, Yaying Sun, Jinrong Lin, Beijie Qi, and Jiwu Chen Summary: The start with the premise that excessive inflammation leads to fibrosis, but this fibrosis could be controlled by reducing macrophage polarization using exosomes generated from muscle cells (C2C12). The C2C12 Exos induce M1 macrophage which is proinflammatory and this inflammatory environment leads to suppression of myoblast differentiation, but increases proliferation thereby leading to muscle regeneration and prevent fibrosis. It seems paradoxical that inflammation that started the process itself helps in muscle regeneration and when its been know that prolonged inflammation leads to fibrosis. Major concerns: • The first thing authors wish to recreate, is the inflammatory environment that muscle cells experience during injury. To achieve this authors, used LPS, but its not shown whether LPS concentration has induced changes in the C2C12 cells. LPS addition ideally inhibit differentiation of C2C12, however this has not been demonstrated, So, its is not clear if LPS treated C2C12 cells are inflamed and are now ready for harvesting exosomes. • The authors using immunolocalization showed how Exos affect myofibroblast differentiation but I would advise the authors to perform western blotting to quantitate these changes between different exosome doses. Although authors in Figure 9 show western blot data, but even 24hrs seems sufficient to induce same changes that 2 and 4 days have given. It seems surprising that these changes can occur after 24 hrs. And authors should address this in the discussion. • The giemsa stained myotubes has been shown after 2 days and 4 days of IF C2C12 Exos. But there appears to be no difference between day 2 and day4

treatment. There is no explanation offered by the authors. Minor comments: • In the Figure 2E, it is not indicated what the 4 lanes are? Are they duplicates? • Scale bars not shown for all images. Scale bars should be shown for every IF, geimsa stained and brighfield image. • It is not clear how the conditioned media for C2C12 was collected. Especially since author mention “ The C2C12 conditioned mediums (C2C12-CM) were collected after 24h and the exosomes from C2C12 myoblast (C2C12-Exos) were extracted by following steps. RAW 264.7 cells (mouse leukemia cells of monocyte-macrophage), purchased from the American Type Culture Collection, were maintained in DMEM with 10% FBS and 0.5 ml of penicillin/streptomycin solution in a humidified incubator at 37°C and 5% CO<sub>2</sub> atmosphere. I request the authors to clear this procedure. There are some jarring words, which do not seem appropriate (highlighted in bold), for example •

The Lipopolysaccharide, LPS, with a concentration of 1000ng/ml was used to induce the inflammatory environment for C2C12. Then, washed the medium three times to abandon LPS and a fresh exosome-depleted medium was added. • The procedure of SEM referred to the previous study. • Generally, freeze 100 µl of the exosome suspension overnight in the refrigerator and transferred it to a vacuum dryer for lyophilization. • Then, the sample was coated with gold by an ion sputterer and observed in the microscope. • Detailly, the decline of MyoD, MyoG, and MYHC protein levels[25,26,40] suggested that IF-C2C12-Exos evidently reduced the myogenic differentiation ability of myoblast. I would request the authors to address these issues.

## PEER-REVIEW REPORT

**Name of journal:** World Journal of Stem Cells

**Manuscript NO:** 67776

**Title:** Exosomes derived from inflammatory myoblasts promote M1 polarization and break the balance of myoblast proliferation/differentiation

**Reviewer's code:** 05818012

**Position:** Peer Reviewer

**Academic degree:** MD

**Professional title:** Doctor

**Reviewer's Country/Territory:** China

**Author's Country/Territory:** China

**Manuscript submission date:** 2021-05-01

**Reviewer chosen by:** AI Technique

**Reviewer accepted review:** 2021-05-03 00:29

**Reviewer performed review:** 2021-05-13 16:57

**Review time:** 10 Days and 16 Hours

<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input checked="" type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input checked="" type="checkbox"/> Rejection
<b>Re-review</b>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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#### **SPECIFIC COMMENTS TO AUTHORS**

In this paper, the authors evaluated the effects of exosomes from inflammatory C2C12 myoblast on macrophage polarization and myoblast proliferation/differentiation. However, several experimental methods were not described in detail in this paper, and the contents of mesenchymal stem cells unrelated to this paper appeared in many parts. Please review your paper carefully and submit it after revision.

## RE-REVIEW REPORT OF REVISED MANUSCRIPT

**Name of journal:** World Journal of Stem Cells

**Manuscript NO:** 67776

**Title:** Exosomes derived from inflammatory myoblasts promote M1 polarization and break the balance of myoblast proliferation/differentiation

**Reviewer's code:** 05115904

**Position:** Peer Reviewer

**Academic degree:** PhD

**Professional title:** Assistant Professor

**Reviewer's Country/Territory:** India

**Author's Country/Territory:** China

**Manuscript submission date:** 2021-05-01

**Reviewer chosen by:** Jia-Ru Fan

**Reviewer accepted review:** 2021-08-02 08:21

**Reviewer performed review:** 2021-08-02 09:13

**Review time:** 1 Hour

<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input checked="" type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

## SPECIFIC COMMENTS TO AUTHORS





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The authors have answered most of my queries effectively. However, there are some revisions necessary. The authors have claimed that scale bars have been added to all images as per my suggestion. But if I see the powerpoint file uploaded by the authors the scale bars are only sporadically added. Also, in material and methods section authors use 'uL' to denote micro litres, but this is not correct convention. The authors must use greek alphabet micro instead of english u. Also, the authors haven't highlighted the changes done in the revised main text, so it's difficult to verify the claims made by authors.