

RE: Manuscript NO: 72997

Title: Physical Energy-Ultrasound shifts M1 macrophage differentiation towards M2 state

Science Editor comments:

Dear Authors, the letter to the editor entitled "Physical Energy-Ultrasound shifts M1 macrophage differentiation towards M2 state" contains valuable information regarding how the sound wave can potentially shift the macrophage phenotype and finally adopt the anti-inflammatory phenotype M2. I do not have anything to comment regarding this letter of editor. Both reviewers have performed key point comments. Please perform the corrections as the reviewers have proposed.

Response to Science Editor:

Thank you so much for your recognition of our article. We have answered the reviewer's comments carefully and step by step as follows. At the same time, the paper has carried on the thorough screening and modification to the language error. Revised portions are marked in **blue** throughout the paper, and changes to the manuscript are also highlighted with **red** text. We have also sent our article to a professional English language editing service to improve its readability. These changes have substantially improved our manuscript while preserving the content and general framework.

Company editor-in-chief comments:

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Stem Cells, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file.

Response to Company editor-in-chief:

Thank you very much for your time on our article and constructive suggestions. According to your suggestion, we have added all the pictures into one PowerPoint file and classified them.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author:

The authors use this letter to the editor to present preliminary results of energy from ultrasound and its effect on macrophage differentiation. The findings are consistent with previous results in literature (ref 11, 12) and calls for more research into this.

Thank you for your affirmation of our experimental results and direction, which is of great significance to us. In our following experiments, we will further explore the specific mechanism of macrophage polarization by therapeutic ultrasound. At the same time, we will further explore whether ultrasound has the same effect on other stem cells. As a noninvasive and simple treatment, ultrasound therapy has a wide range of applications.

Reviewer(s)' Comments to Author:

Reviewer: 2

Comments to the Author:

I would like to congratulate the authors for this article. It is interesting and can bring new perspective. I have some comments: Regarding the key word: stem cells, it would be more informative if the authors can discuss more about ultrasound physical energy or LIPUS (low-intensity pulsed ultrasound) use on stem cells and its effects from other previous studies. Figure legends: In Figure 1, please add (A) and (B) respectively in the sentence.

Thank you for your kind suggestions. We have made detailed modifications for the deficiencies you mentioned.

Q1:

Regarding the key word: stem cells, it would be more informative if the authors can discuss more about ultrasound physical energy or LIPUS (low-intensity pulsed ultrasound) use on stem cells and its effects from other previous studies.

Response: Thank you for giving us the correct guidance. More articles about LIPUS acting on stem cells are included. Here's what we added:

Tan et al. [7] listed studies of LIPUS acting on various neural stem cells in recent years and concluded that LIPUS can stimulate stem cells *in vitro*; promote stem cell proliferation, differentiation, and migration; maintain stem cell activity. In the central nervous system, Wu et al.'s findings [8] implied that LIPUS regulates the Notch signaling system, causing neural stem cells proliferation and differentiation.

7 Tan Y, Guo Y, Reed-Maldonado AB, Li Z, Lin G, Xia SJ and Lue TF. Low-intensity pulsed ultrasound stimulates proliferation of stem/progenitor cells: what we need to know to translate basic science research into clinical applications. *Asian J Androl* 2021; 23: 602-610 [PMID: 33818526 DOI: 10.4103/aja.aja_25_21

8 Wu Y, Gao Q, Zhu S, Wu Q, Zhu R, Zhong H, Xing C, Qu H, Wang D, Li B, Ning G and Feng S. Low-intensity pulsed ultrasound regulates proliferation and differentiation of neural stem cells through notch signaling pathway. *Biochem Biophys Res Commun* 2020; 526: 793-798 [PMID: 32268957 DOI: 10.1016/j.bbrc.2020.03.142

Q2:

Figure legends: In Figure 1, please add (A) and (B) respectively in the sentence.

Response: According to the actual situation of our experiment, we respectively introduce the detailed information that the Figure1 wants to express. The following are the additions:

“(A)24h before lipopolysaccharide (LPS) was added to simulate the inflammatory environment, macrophages at the M1 stage were uniformly subcultured into the six-well plate. Then, the first ultrasound treatment was performed 24 hours after the inflammatory environment was maintained. At 48 h, ultrasound was performed on the group requiring a second treatment. The supernatant of culture medium was separated after 24 days of culture (that is, 3 days after LPS was added). (B) In order to easily adjust the ultrasonic probe to fit the culture holes on the bottom of the six-well plate, we fixed the ultrasonic probe on a sponge pad. Additionally, a box of the same height is used to support the six-hole plate to prevent it from tilting.”