

# World Journal of *Stem Cells*

*World J Stem Cells* 2022 February 26; 14(2): 146-218



## Contents

Monthly Volume 14 Number 2 February 26, 2022

## REVIEW

- 146 Abnormal lipid synthesis as a therapeutic target for cancer stem cells  
*Wang SY, Hu QC, Wu T, Xia J, Tao XA, Cheng B*

## ORIGINAL ARTICLE

## Basic Study

- 163 Transcription regulators differentiate mesenchymal stem cells into chondroprogenitors, and their *in vivo* implantation regenerated the intervertebral disc degeneration  
*Khalid S, Ekram S, Salim A, Chaudhry GR, Khan I*
- 183 Extracellular vesicles from hypoxia-preconditioned mesenchymal stem cells alleviates myocardial injury by targeting thioredoxin-interacting protein-mediated hypoxia-inducible factor-1 $\alpha$  pathway  
*Mao CY, Zhang TT, Li DJ, Zhou E, Fan YQ, He Q, Wang CQ, Zhang JF*

## SYSTEMATIC REVIEWS

- 200 Anti-fibrotic effect of adipose-derived stem cells on fibrotic scars  
*Vanderstichele S, Vranckx JJ*

## LETTER TO THE EDITOR

- 214 Physical energy-based ultrasound shifts M1 macrophage differentiation towards M2 state  
*Qin HC, Luo ZW, Zhu YL*

**ABOUT COVER**

Editorial Board Member of *World Journal of Stem Cells*, Mohammed Grawish, PhD, Professor, Department of Oral Biology, Faculty of Dentistry, Mansoura University, Mansoura 740005, Egypt. grawish2005@yahoo.com

**AIMS AND SCOPE**

The primary aim of *World Journal of Stem Cells (WJSC, World J Stem Cells)* is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, etc.

**INDEXING/ABSTRACTING**

The *WJSC* is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, BIOSIS Previews, Scopus, PubMed, and PubMed Central. The 2021 Edition of Journal Citation Reports® cites the 2020 impact factor (IF) for *WJSC* as 5.326; IF without journal self cites: 5.035; 5-year IF: 4.956; Journal Citation Indicator: 0.55; Ranking: 14 among 29 journals in cell and tissue engineering; Quartile category: Q2; Ranking: 72 among 195 journals in cell biology; and Quartile category: Q2. The *WJSC*'s CiteScore for 2020 is 3.1 and Scopus CiteScore rank 2020: Histology is 31/60; Genetics is 205/325; Genetics (clinical) is 64/87; Molecular Biology is 285/382; Cell Biology is 208/279.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Production Editor: Yan-Liang Zhang; Production Department Director: Xu Guo; Editorial Office Director: Ze-Mao Gong.

**NAME OF JOURNAL**

*World Journal of Stem Cells*

**ISSN**

ISSN 1948-0210 (online)

**LAUNCH DATE**

December 31, 2009

**FREQUENCY**

Monthly

**EDITORS-IN-CHIEF**

Shengwen Calvin Li, Carlo Ventura

**EDITORIAL BOARD MEMBERS**

<https://www.wjnet.com/1948-0210/editorialboard.htm>

**PUBLICATION DATE**

February 26, 2022

**COPYRIGHT**

© 2022 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjnet.com/bpg/gerinfo/240>

**PUBLICATION ETHICS**

<https://www.wjnet.com/bpg/GerInfo/288>

**PUBLICATION MISCONDUCT**

<https://www.wjnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>



## Physical energy-based ultrasound shifts M1 macrophage differentiation towards M2 state

Hao-Cheng Qin, Zhi-Wen Luo, Yu-Lian Zhu

**Specialty type:** Cell and tissue engineering

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0  
Grade B (Very good): B, B  
Grade C (Good): 0  
Grade D (Fair): 0  
Grade E (Poor): 0

**P-Reviewer:** Ankrah AO, Prasetyo EP

**Received:** November 5, 2021

**Peer-review started:** November 5, 2021

**First decision:** December 4, 2021

**Revised:** December 12, 2021

**Accepted:** February 16, 2022

**Article in press:** February 16, 2022

**Published online:** February 26, 2022



**Hao-Cheng Qin, Yu-Lian Zhu,** Department of Rehabilitation Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China

**Zhi-Wen Luo,** Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China

**Corresponding author:** Yu-Lian Zhu, MD, PhD, Chief Doctor, Doctor, Professor, Department of Rehabilitation Medicine, Huashan Hospital, Fudan University, No. 12 Wulumuqi Road, Shanghai 200040, China. [zyljully@163.com](mailto:zyljully@163.com)

### Abstract

Recently, we read with interest the article entitled “Unveiling the Morphogenetic Code: A New Path at the Intersection of Physical Energies and Chemical Signaling”. In this paper, the investigation into the systematic and comprehensive bio-effects of physical energies prompted us to reflect on our research. We believe that ultrasound, which possesses a special physical energy, also has a certain positive regulatory effect on macrophages, and we have already obtained some preliminary research results that support our hypothesis.

**Key Words:** Ultrasound; Macrophages; Stem cells; Physical energies; Inflammation

©The Author(s) 2022. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** Because physical energies can contribute to the recovery of tissue damage in multiple aspects, it is widely used in clinical practice. The unique insights of the article “Unveiling the Morphogenetic Code: A New Path at the Intersection of Physical Energies and Chemical Signaling” inspired the direction of our experiments concerning the impact of physical energies on stem cells. In the future, we will conduct experiments and analytical techniques to reveal the mechanism of the regulatory effects behind ultrasound.

**Citation:** Qin HC, Luo ZW, Zhu YL. Physical energy-based ultrasound shifts M1 macrophage differentiation towards M2 state. *World J Stem Cells* 2022; 14(2): 214-218

**URL:** <https://www.wjgnet.com/1948-0210/full/v14/i2/214.htm>

**DOI:** <https://dx.doi.org/10.4252/wjsc.v14.i2.214>

## TO THE EDITOR

Recently, the article named “Unveiling the Morphogenetic Code: A New Path at the Intersection of Physical Energies and Chemical Signaling” contributed by the Editor-in-Chief Carlo Ventura[1] motivated us to reconsider the biological roles of physical energy. In that article, the authors provided a detailed summary of the developmental history of physical energy, especially bioelectricity, as well as its applications and prospects in stem cell research. We strongly support the proposition about the high efficacy of physical energy in tissue repair. An article recently released in “Science Translational Medicine” described how massage, which is also a physical stimulus, regulates muscle repair[2]. The systematic and accurate explanation of physical energies by the editor echoes our dedicated research, which is the effect of therapeutic ultrasound on macrophages.

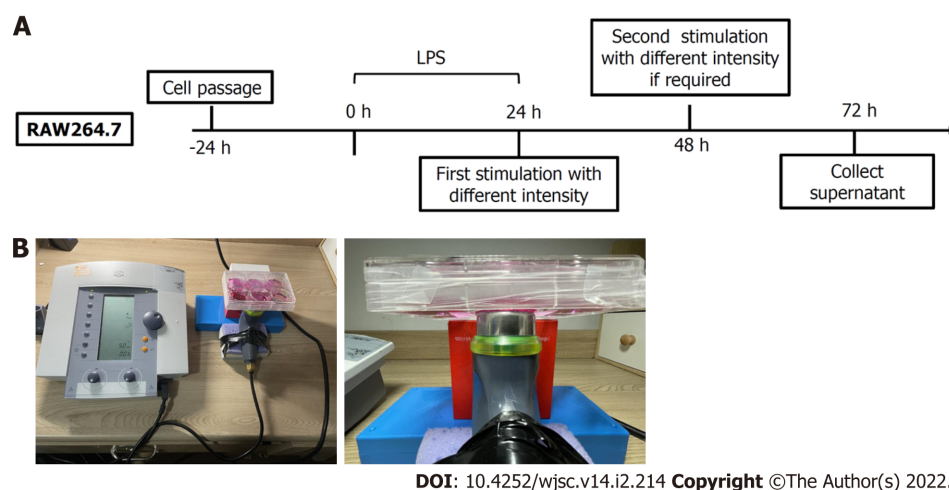
As early as the 1920s, Wood and Loomis began to investigate ultrasound as a therapeutic intervention [3]. In recent years, one particular type of ultrasound, low-intensity pulsed ultrasound (LIPUS), has gained much attention. This is due to its non-thermal effects, such as acoustic cavitation and “cellular massage”, which produce a range of biological effects[4]. Clinically, LIPUS can be used as a non-invasive adjunctive therapy for a variety of diseases, such as fractures, muscle injury, osteoarthritis, as well as nerve injury[4]. Besides, LIPUS has received considerable attention in the discipline of stem cell research. Salgarella *et al*[5] proved the bio-effect of ultrasound therapy on the proliferation and differentiation of C2C12 myoblasts *in vitro*, while the research of Wang *et al*[6] indicated that LIPUS promotes the production of mesenchymal stem cells (MSC) derived mainly from bone marrow differentiation. Both of these studies verified the positive bio-effects of this therapeutic technique. Tan *et al*[7] listed recent studies regarding the action of LIPUS on various neural stem cells and concluded that LIPUS can stimulate stem cells *in vitro*, promote stem cell proliferation, differentiation, and migration, and maintain stem cell activity. The findings of Wu *et al*[8] suggested that LIPUS regulates the Notch signalling pathway in the central nervous system, causing neural stem cell proliferation and differentiation. Additionally, LIPUS can also accelerate tissue repair[9] and promote the dissipation of inflammation[10], angiopoiesis[11], *etc.*

Our research focused on discovering how sound waves, a kind of physical energy, exert potential effects on macrophage polarisation, which is of great significance during the inflammation stage. To ensure accurate muscle regeneration, a balance between M1 and M2 activity (pro- and anti-inflammatory, respectively) shifting over time is required[12]. Previous studies have found that ultrasound may regulate macrophages in the spinal fusion model of male Sprague Dawley (SD) rats[13]. However, very few studies have explored the direct effect of ultrasound on macrophages *in vitro*. We induced macrophages into M1 macrophages using lipopolysaccharide to mimic the inflammatory microenvironment *in vivo*. Then, we collected and analysed the secretion composition and gene expression following ultrasound therapy. Details of the experiments are presented in Figure 1.

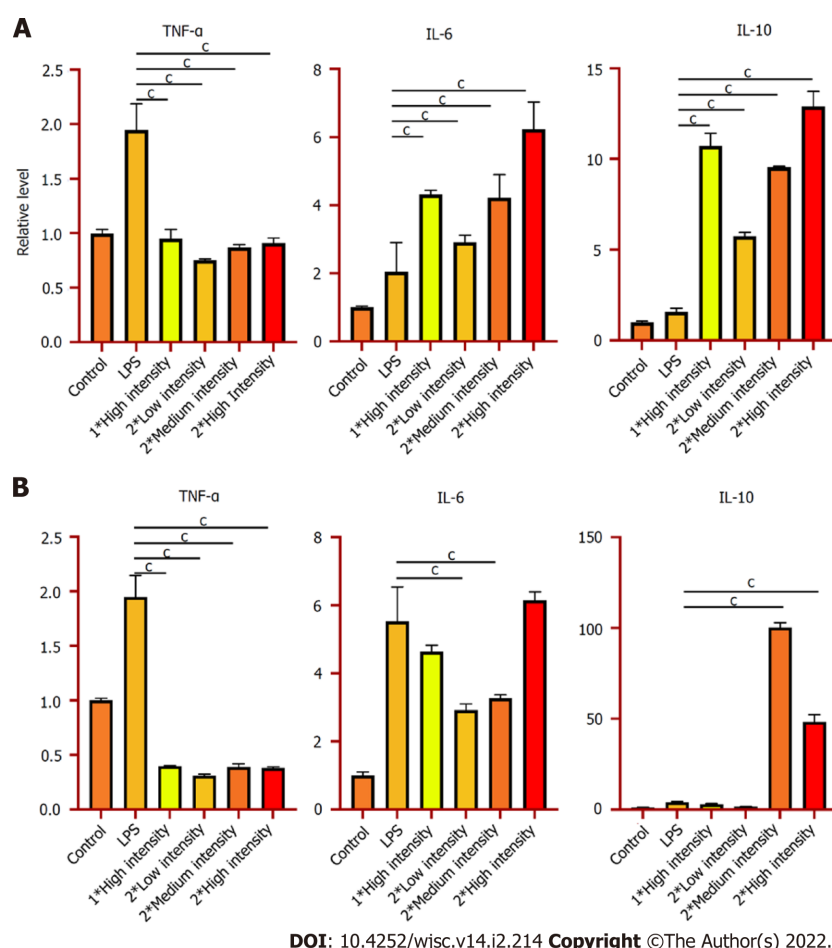
So far, we have already obtained some preliminary results. As Figure 2 indicates, after LIPUS treatment, the secretion of anti-inflammatory cytokine interleukin (IL)-10 significantly increased, while quantities of pro-inflammatory cytokines tumor necrosis factor- $\alpha$  and IL-6 fell substantially at genetic and secreted proteins levels. Besides, we determined that the phenotypes of the macrophages are polarised into M2 after ultrasound stimulation (Figure 3). According to the above experimental data, we can conclude that ultrasound facilitates the transition of macrophages from the M1 to M2 phenotype *in vitro*, which is consistent with da Silva Junior's discovery[14]. For subsequent research, we will further investigate the underlying mechanism of macrophage polarisation caused by LIPUS, and the potentially affected molecular pathways. Moreover, we will conduct both *in vivo* (skeletal muscle contusion model) [15] and *in vitro* experiments to verify the mechanism and ascertain how LIPUS exerts a series of downstream bio-effects.

### Conclusion and perspective

Various studies have established that physical energies can modulate inflammation and further promote tissue repair. As a conventional form of physical energy, ultrasound has extensive application prospects due to its non-thermal mechanical effect. Accordingly, it warrants further investigation to elucidate its influence on cell signalling. We predict that subsequent research can be extended from the aspect of monotypic cell regulation to the integral tissue, by employing single-cell transcriptomic analysis and spatial transcriptomic analysis[16]. Thus, we will gain an increasingly comprehensive insight into the role of ultrasound in tissue repair.

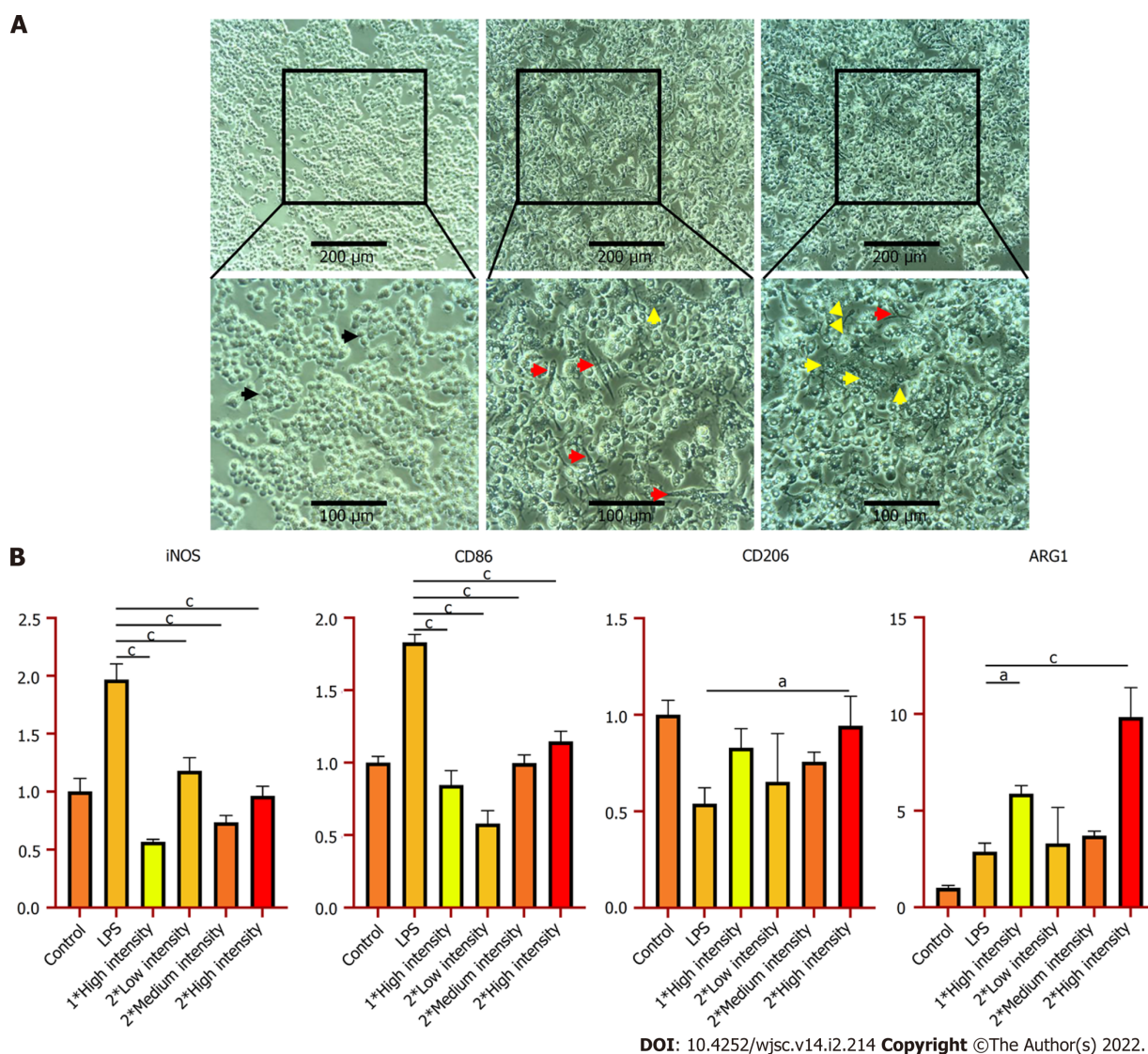


**Figure 1 Experimental design for this study *in vitro* and the method of treating cells.** A: At 24 h before lipopolysaccharide (LPS) was added to simulate the inflammatory environment, macrophages at the M1 stage were uniformly subcultured into the six-well plate. Then, The first ultrasound treatment was performed 24 h 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 d of culture (3 d 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.



**Figure 2 Low-Intensity pulsed ultrasound significantly increases the expression of anti-inflammatory cytokine and decreases the expression of pro-inflammatory cytokine.** A: Real-time quantitative polymerase chain reaction (qPCR) was used to detect the gene expression of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, and IL-10 after being treated by low-intensity pulsed ultrasound (LIPUS); B: ELISA was used to analyze the protein expression of TNF- $\alpha$ , IL-6, and IL-10 after being treated with LIPUS. Data are expressed as the mean  $\pm$  standard error of the mean. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ . Low intensity = 0.25 W/cm<sup>2</sup>, medium intensity = 0.5 W/cm<sup>2</sup>, and high intensity = 0.75 W/cm<sup>2</sup>. TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL: Interleukin.





**Figure 3 Low-intensity pulsed ultrasound shifts M1 macrophages towards M2 state.** A: The morphology of macrophages was observed by microscopy. Black, red, and yellow arrows represent M0, M1, and M2 macrophages, respectively; B: Quantitative polymerase chain reaction was used to detect the gene expression of cell phenotypes maker iNOS, CD86, CD206, and ARG1 after being treated by low-intensity pulsed ultrasound. Data are expressed as the mean  $\pm$  standard error of the mean. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ . Low intensity = 0.25 W/cm<sup>2</sup>, medium intensity = 0.5 W/cm<sup>2</sup>, and high intensity = 0.75 W/cm<sup>2</sup>.

## FOOTNOTES

**Author contributions:** Qin HC and Luo ZW conducted the original search, wrote the first draft of the paper, and contributed to subsequent revisions of the manuscript; Zhu YL generated the original idea of this study and provided suggestions; Qin HC and Luo ZW made equal contributions to the work.

**Conflict-of-interest statement:** The authors declare no conflict of interest for this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <https://creativecommons.org/licenses/by-nc/4.0/>

**Country/Territory of origin:** China

**ORCID number:** Hao-Cheng Qin 0000-0003-2723-4299; Zhi-Wen Luo 0000-0002-0524-9951; Yu-Lian Zhu 0000-0001-5530-144X.

**S-Editor:** Fan JR

**L-Editor:** Wang TQ

**P-Editor:** Zhang YL

## REFERENCES

- 1 **Tassinari R**, Cavallini C, Olivi E, Taglioli V, Zannini C, Ventura C. Unveiling the morphogenetic code: A new path at the intersection of physical energies and chemical signaling. *World J Stem Cells* 2021; **13**: 1382-1393 [PMID: [34786150](#) DOI: [10.4252/wjsc.v13.i10.1382](#)]
- 2 **Seo BR**, Payne CJ, McNamara SL, Freedman BR, Kwee BJ, Nam S, de Lázaro I, Darnell M, Alvarez JT, Dellacherie MO, Vandenburg HH, Walsh CJ, Mooney DJ. Skeletal muscle regeneration with robotic actuation-mediated clearance of neutrophils. *Sci Transl Med* 2021; **13**: eabe8868 [PMID: [34613813](#) DOI: [10.1126/scitranslmed.abe8868](#)]
- 3 **Jiang X**, Savchenko O, Li Y, Qi S, Yang T, Zhang W, Chen J. A Review of Low-Intensity Pulsed Ultrasound for Therapeutic Applications. *IEEE Trans Biomed Eng* 2019; **66**: 2704-2718 [PMID: [30596564](#) DOI: [10.1109/TBME.2018.2889669](#)]
- 4 **Lin G**, Reed-Maldonado AB, Lin M, Xin Z, Lue TF. Effects and Mechanisms of Low-Intensity Pulsed Ultrasound for Chronic Prostatitis and Chronic Pelvic Pain Syndrome. *Int J Mol Sci* 2016; **17** [PMID: [27376284](#) DOI: [10.3390/ijms17071057](#)]
- 5 **Salgarella AR**, Cafarelli A, Ricotti L, Capineri L, Dario P, Menciassi A. Optimal Ultrasound Exposure Conditions for Maximizing C2C12 Muscle Cell Proliferation and Differentiation. *Ultrasound Med Biol* 2017; **43**: 1452-1465 [PMID: [28433437](#) DOI: [10.1016/j.ultrasmedbio.2017.03.003](#)]
- 6 **Wang X**, Lin Q, Zhang T, Wang X, Cheng K, Gao M, Xia P, Li X. Low-intensity pulsed ultrasound promotes chondrogenesis of mesenchymal stem cells *via* regulation of autophagy. *Stem Cell Res Ther* 2019; **10**: 41 [PMID: [30670079](#) DOI: [10.1186/s13287-019-1142-z](#)]
- 7 **Tan Y**, Guo Y, Reed-Maldonado AB, Li Z, Lin G, Xia SJ, 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, 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](#)]
- 9 **Yang B**, Li M, Lei H, Xu Y, Li H, Gao Z, Guan R, Xin Z. Low Intensity Pulsed Ultrasound Influences the Myogenic Differentiation of Muscle Satellite Cells in a Stress Urinary Incontinence Rat Model. *Urology* 2019; **123**: 297.e1-297.e8 [PMID: [30273612](#) DOI: [10.1016/j.urology.2018.09.020](#)]
- 10 **Zheng C**, Wu SM, Lian H, Lin YZ, Zhuang R, Thapa S, Chen QZ, Chen YF, Lin JF. Low-intensity pulsed ultrasound attenuates cardiac inflammation of CVB3-induced viral myocarditis *via* regulation of caveolin-1 and MAPK pathways. *J Cell Mol Med* 2019; **23**: 1963-1975 [PMID: [30592150](#) DOI: [10.1111/jcmm.14098](#)]
- 11 **Li J**, Guo W, Yu F, Liu L, Wang X, Li L, Fang B, Xia L. Low-intensity pulsed ultrasound promotes angiogenesis *via* the AKT pathway and DNA methylation in human umbilical vein endothelial cells. *Ultrasonics* 2022; **118**: 106561 [PMID: [34500338](#) DOI: [10.1016/j.ultras.2021.106561](#)]
- 12 **De Santa F**, Vitiello L, Torcinaro A, Ferraro E. The Role of Metabolic Remodeling in Macrophage Polarization and Its Effect on Skeletal Muscle Regeneration. *Antioxid Redox Signal* 2019; **30**: 1553-1598 [PMID: [30070144](#) DOI: [10.1089/ars.2017.7420](#)]
- 13 **Zhang ZC**, Yang YL, Li B, Hu XC, Xu S, Wang F, Li M, Zhou XY, Wei XZ. Low-intensity pulsed ultrasound promotes spinal fusion by regulating macrophage polarization. *Biomed Pharmacother* 2019; **120**: 109499 [PMID: [31707028](#) DOI: [10.1016/j.biopha.2019.109499](#)]
- 14 **da Silva Junior EM**, Mesquita-Ferrari RA, França CM, Andreo L, Bussadori SK, Fernandes KPS. Modulating effect of low intensity pulsed ultrasound on the phenotype of inflammatory cells. *Biomed Pharmacother* 2017; **96**: 1147-1153 [PMID: [29191696](#) DOI: [10.1016/j.biopha.2017.11.108](#)]
- 15 **Luo Z**, Lin J, Sun Y, Wang C, Chen J. Bone Marrow Stromal Cell-Derived Exosomes Promote Muscle Healing Following Contusion Through Macrophage Polarization. *Stem Cells Dev* 2021; **30**: 135-148 [PMID: [33323007](#) DOI: [10.1089/scd.2020.0167](#)]
- 16 **van den Brink SC**, Alemany A, van Batenburg V, Moris N, Blotenburg M, Vivie J, Baillie-Johnson P, Nichols J, Sonnen KF, Martinez Arias A, van Oudenaarden A. Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids. *Nature* 2020; **582**: 405-409 [PMID: [32076263](#) DOI: [10.1038/s41586-020-2024-3](#)]





Published by **Baishideng Publishing Group Inc**  
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

**Help Desk:** <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

