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LETTER TO THE EDITOR

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

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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

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.

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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 energies. 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-α 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.

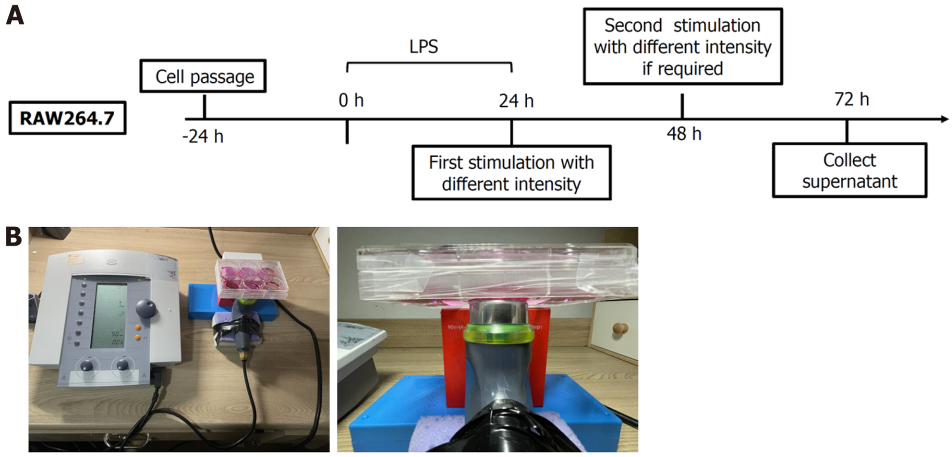

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Figure 1 Experimental design for this study *in vitro* and the method of treating cells. A: At 24 h before lipopolysaccharide (LPS) was added to simulate the inflammatory environment, macrophages at the M1 stage were uniformly subcultured into the 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.

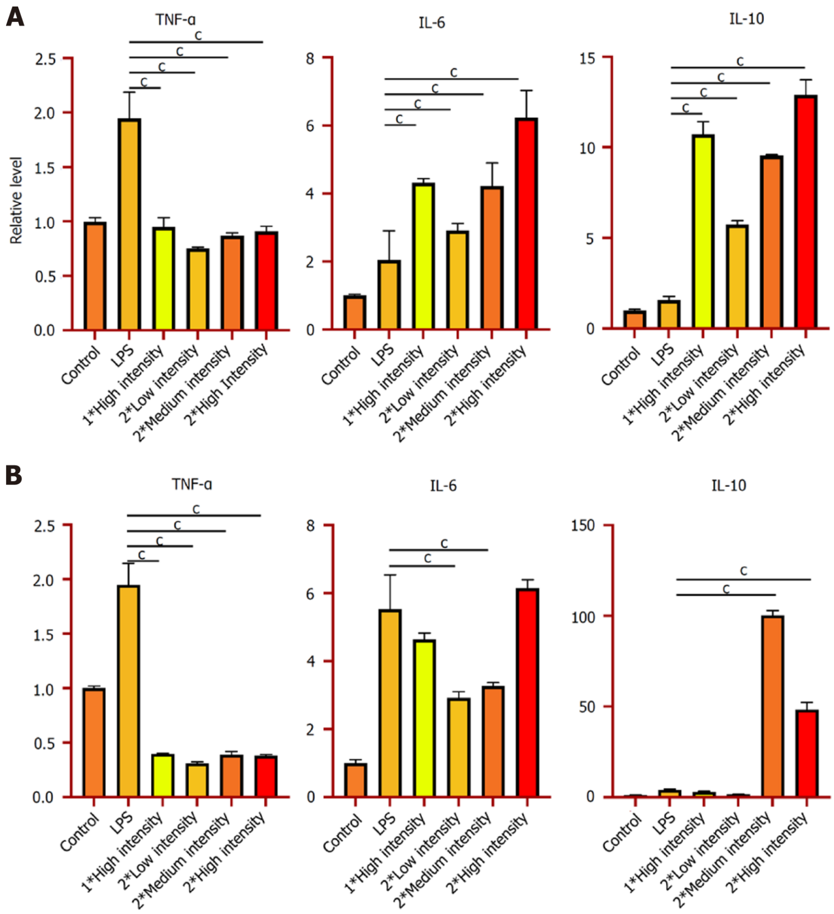

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Figure 2 Low-Intensity pulsed ultrasound significantly increases the expression of anti-inflammatory cytokine and decreases the expression of pro-inflammatory cytokine. A: Real-time quantitative polymerase chain reaction (qPCR) was used to detect the gene expression of tumor necrosis factor (TNF)-α, 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-α, IL-6, and IL-10 after being treated with LIPUS. Data are expressed as the mean ± standard error of the mean. $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.001$. Low intensity = 0.25 W/cm^2, medium intensity = 0.5 W/cm^2, and high intensity = 0.75 W/cm^2. TNF-α: Tumor necrosis factor-α; IL: Interleukin.

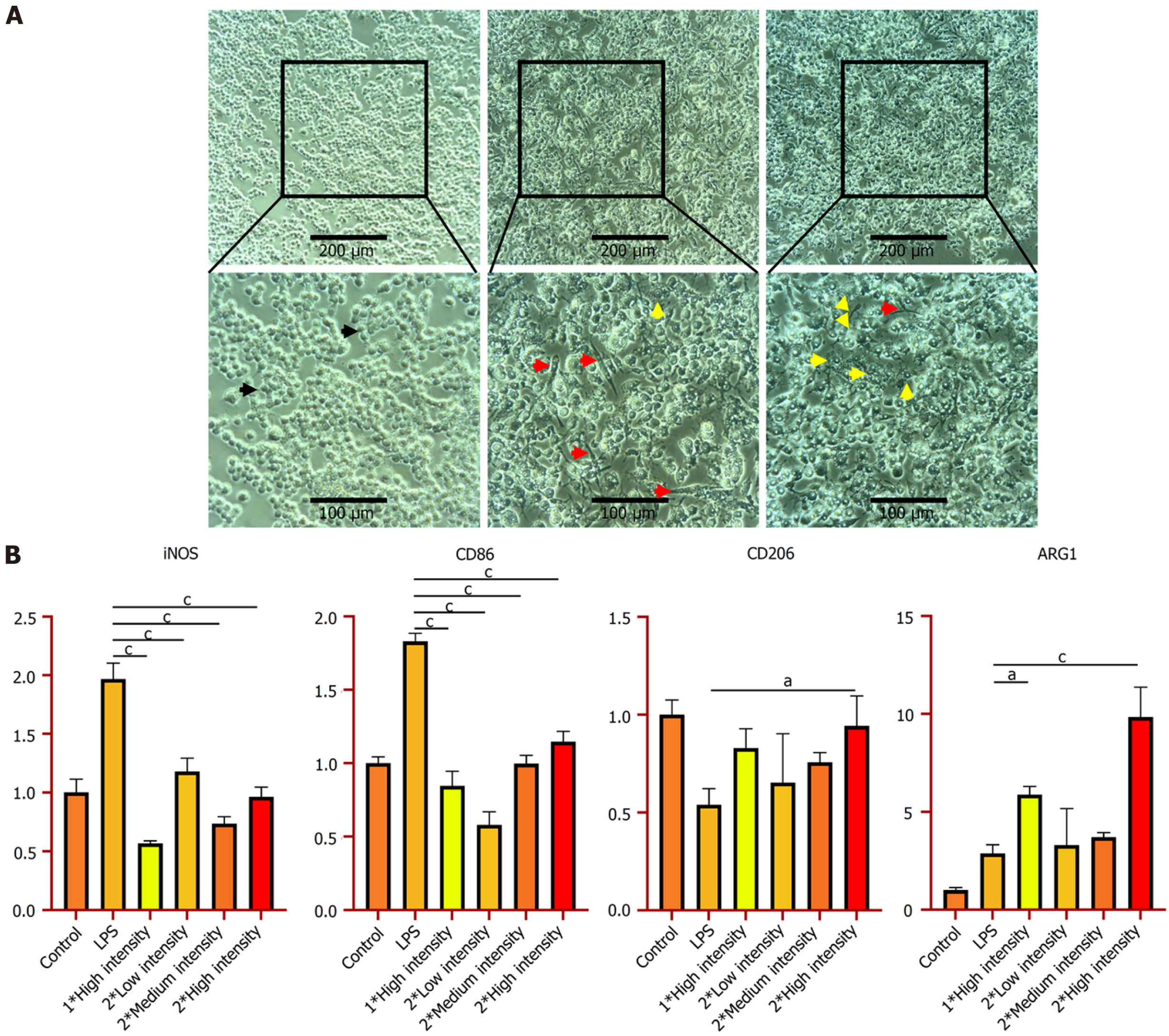

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Figure 3 Low-intensity pulsed ultrasound shifts M1 macrophages towards M2 state. A: The morphology of macrophages was observed by microscopy. Black, red, and yellow arrows represent M0, M1, and M2 macrophages, respectively; B: Quantitative polymerase 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 ± standard error of the mean. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. Low intensity = 0.25 W/cm^2, medium intensity = 0.5 W/cm^2, and high intensity = 0.75 W/cm^2.

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.

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