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RESEARCH ARTICLE

Audible Sound-Induced MicroVibrations Enhance Wound Healing in Human Dermal Fibroblasts

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ABSTRACT: Mechanical micro-vibrations caused by audible sound waves have the potential to alter cellular behavior. While some studies suggest a role for sound-induced stimulation in tissue repair, the overall effectiveness remains unclear, and the specific effects of audible sound waves on wound healing are largely understudied. This paper examined how the audible sound of different frequencies influenced fibroblast migration and wound healing undertaken in an *in vitro* scratch assay. Human dermal fibroblasts from Detroit 551 were subjected to sound at frequencies of 100 Hz, 480 Hz, 1,000 Hz, 10,000 Hz, and 20,000 Hz for one hour daily over four days. The amplitude of vibrations was measured quantitatively using a 650 nm laser module. Interestingly, the 100 Hz and 480 Hz groups showed a significantly greater improvement in wound healing compared to the control ($p < 0.0001$), with the 480 Hz group exhibiting the most significant effect. A negative correlation between frequency of sound and amplitude of vibration was observed, whereby the highest values occur at lower frequencies. These findings suggest that the audible sound of low-frequency stimulation stimulates the migration and wound healing of fibroblasts by inducing mechanically engineered micro-vibrations. This study provides evidence that non-invasive sound-based treatment can be an affordable method for enhancing tissue regeneration, particularly in cases of slow-healing or chronic wounds.

KEYWORDS: Biomedical and Health Sciences, Pathophysiology, Microvibration, Fibroblast Migration, Audible Sound Therapy.

Introduction

This study examined how different audible sound frequencies affect skin cell migration by analyzing the relationship between vibration amplitude—measured with a 650 nm laser—and wound healing outcomes. Wound healing is a highly coordinated biological process involving cell migration, proliferation, and tissue remodeling. Various physical stimuli have been recently studied to promote this process. Electrical, magnetic, and mechanical stimuli are representative, and among them, sound stimulation is receiving attention because it is noninvasive and easy to apply. However, most prior studies have focused on ultrasound (>20 kHz) or infrasound (<20 Hz), with minimal exploration of the audible frequency range (20 Hz–20 kHz), despite its non-invasive and accessible nature.[1-3] Of the 62 studies reviewed in 2023 on acoustic stimulation and wound healing, fewer than 5% investigated frequencies between 20 Hz and 20 kHz—the range humans can hear—revealing an important research gap.[1] Some benefits have been observed at specific audible frequencies, such as enhanced fibroblast migration near 100 Hz and keratinocyte activation at 10–20 kHz. However, detailed frequency-resolved mechanistic studies remain limited, hindering a comprehensive understanding of how cells respond to audible sound stimulation.[1] Though audible sound is usually seen as an auditory stimulus, it is also a physical wave capable of generating micro-vibrations in solids and liquids. These vibrations can be transferred to cells through the culture dish or extracellular matrix and activate mechanotransduction pathways, thereby affecting cell migration, shape, and regeneration.[4,5] Despite encouraging results, the direct mechanical effect of audible frequency vibrations on human fibroblast healing remains experimentally unproven.

Recent research has drawn attention to the therapeutic potential of acoustic wave-based therapy as a noninvasive and cost-effective approach to enhancing wound healing. Previous *in vitro* studies show that low-frequency stimulation—especially below 100 Hz—directly increases fibroblast migration rates and leads to measurable remodeling of actin networks.[3] In addition, both preclinical and clinical research on chronic wounds, including diabetic foot ulcers, report that such vibrations result in greater granulation tissue formation and improved blood flow, directly correlating with accelerated recovery.[6,7] Beyond these low-frequency approaches, higher-frequency and ultrasonic-band acoustic technologies have also been employed. Wearable ultrasound devices operating at 20–100 kHz have achieved wound healing rates up to 60% faster.[8]

Meanwhile, surface acoustic waves (SAWs) in the MHz range have been utilized to monitor and promote cell migration and adhesion without thermal or fluidic side effects.[9,10] Notably, SAW-induced vibrations have been reported to increase wound closure rates by up to 135%, underscoring the role of physical vibration as a key regenerative stimulus.[11] Although audible sound (20 Hz–20 kHz) remains relatively underexplored, initial studies using 111 Hz exposure in animal models have suggested its potential to promote wound healing, albeit without statistical significance.[12] Importantly, few prior investigations have quantitatively assessed the amplitude of audible-frequency vibrations or directly correlated them with

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human fibroblast recovery. Distinct from these prior works, this study systematically applies sound waves spanning the entire audible spectrum to human fibroblasts, directly measures induced microvibrational effects using laser-based detection, and quantitatively correlates these with wound closure outcomes in a scratch assay model. Thus, our work provides the first comprehensive assessment of the relationship between sound-induced microvibration amplitude and wound closure in fibroblasts.

Preliminary results showed a nonlinear relationship between exposure time and skin cell recovery after 7 days of acoustic stimulation. When low-frequency sounds of 100 Hz and 480 Hz were applied for 1 hour daily for four consecutive days, fibroblast migration increased by about 25% and 55%, respectively.

This study was designed around the hypothesis that the mechanical aspects of sound—especially vibration amplitude—may influence how skin cells respond to external stimuli. Because lower-frequency acoustic signals are known to generate stronger vibrations in both liquid and solid environments, we speculated that such physical oscillations might act as cues to support tissue repair. This study investigated the impact of different audible frequencies on skin cell migration by correlating vibration amplitude, measured using a 650 nm laser, with healing outcomes.

Methods

Human skin cell culture:

The Detroit 551 cell line, derived from human skin cells, was purchased from the Korea Cell Line Bank. Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin–streptomycin (100 U/mL penicillin, 100 µg/mL streptomycin) to prevent microbial contamination. Cells were incubated at standard culture conditions (37°C, 5% CO_2). Cells were maintained in a healthy condition by changing to fresh media every three to four days.

Scratched wound healing assay:

After the skin cells were attached to the surface of the culture plate, a pipette tip was used to make a cross-shaped wound. Following scratch creation, the spent medium was aspirated to remove detached cells, and fresh culture medium was added to ensure consistent recovery conditions. Then, the fresh media was added. Bright-field images of the scratch area were captured immediately after wound creation (Day 0) to document the initial wound width. For each experimental condition, additional bright-field images were acquired after 4 days or 7 days of recovery to quantify cell migration and wound closure over time. All images were taken using identical microscope settings—including magnification, exposure, and field of view—to ensure consistent comparison across conditions.

Soundwave treatment:

Various frequencies were applied to the cultured cells using the soundwave generator: 100 Hz, 480 Hz, 1,000 Hz, 10,000 Hz, and 20,000 Hz. Cells were exposed to sound waves for one hour per day over four consecutive days. Sound waves were applied from above the culture plate to generate mechanical microvibrations.

Analyzing sound wave vibration and amplitude:

To measure the amplitude of the vibration caused by each frequency of sound, a 650 nm red laser module was utilized, along with a reflective surface placed at the bottom of the culture plate. The laser beam was incident at an oblique angle (approximately 45 degrees) to the surface of the culture plate. It was reflected by a small, lightweight reflective tape or mirror attached to the underside of the plate. The reflected beam was projected onto a white screen at approximately 1 meter, allowing for visualization of the beam displacement caused by micro-vibrations of the culture plate surface. When sound waves of different frequencies were applied above the culture plate, the reflected laser point vibrated measurably due to the induced vibrations. A high-resolution video camera was used to record the displacements at a rate of 60 frames per second. The peak-to-peak displacement of the reflected laser point was measured frame by frame using motion analysis software (e.g., ImageJ with the Manual Tracking plugin). The amplitude of vibration (in millimeters) was calculated as the total amplitude using the angular geometry of the arrangement and the distance between the reflective surface and the screen, obtained through trigonometric conversion of the observed displacement.

Results

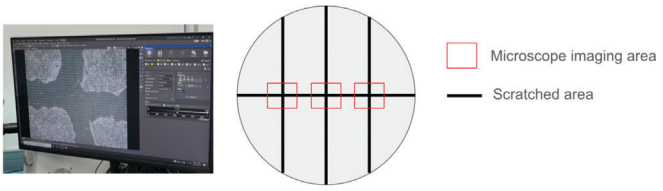

Figure 1: Schematic of Scratch Assay and Imaging Setup. The experimental setup used to evaluate wound closure in human dermal fibroblasts is shown. This figure illustrates how wound healing was assessed using a scratch assay. A uniform "wound" was created by dragging a pipette tip across a confluent monolayer of Detroit 551 fibroblasts, producing a gap where cells had been removed. Three fixed imaging sites along the scratch (red rectangles) were selected to ensure consistent measurements across time. Bright-field images were taken at Day 0 (immediately after scratching) and at later time points to track how quickly cells migrated into the wound area. This standardized setup allowed reliable comparisons of wound closure between control and sound-stimulated groups.

Figure 1 illustrates the experimental setup used to evaluate wound closure in cultured human dermal fibroblasts following scratch induction and soundwave treatment. The left panel depicts a live-cell imaging system in which confluent monolayers of Detroit 551 fibroblasts were scratched with a pipette tip to simulate wound gaps. The right panel shows a schematic of the culture dish, including the scratch regions (black vertical lines) and the three standardized imaging sites (red rectangles) selected along each scratch line. These positions were monitored using time-lapse microscopy to enable consistent and quantitative assessment of cell migration and wound healing

in response to microvibrations induced by audible sound stimulation.

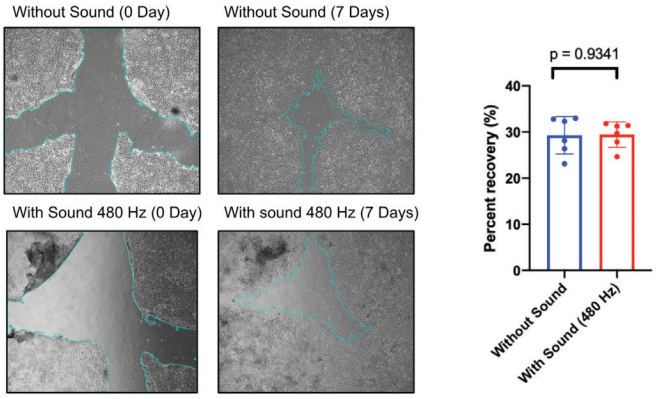

Figure 2: Effect of 480 Hz audible sound stimulation on wound healing over 7 days. Representative images of fibroblast scratch assays in control and 480 Hz sound-treated groups are shown at Day 0 and Day 7. After 7 days of daily 1-hour stimulation, the 480 Hz group showed a mean wound closure of 30.8% ± 4.1%, which was statistically indistinguishable from the control group (29.6% ± 3.7%). A two-tailed unpaired t-test confirmed that the difference between the two groups was not significant (p = 0.9341), indicating that prolonged 480 Hz exposure did not improve fibroblast migration or wound healing under the tested conditions (n = 6).

The impact of 480 Hz audible sound treatment on *in vitro* wound healing for 7 days is presented in Figure 2. As shown in the first row, control samples that were not sound-stimulated display clear boundaries of the scratch at Day 0, with partial wound closure apparent by Day 7. In comparison, the lower row displays the experimental group, which was exposed to 480 Hz sound for 1 hour per day. Clear wound boundaries are also visible on Day 0, and similar wound closure is observed at Day 7. To measure healing, the edges of the cells were highlighted in cyan, and the wound area was measured over time. The right bar graph indicates the percentage recovery of both conditions after 7 days. The average wound closure rate in the control (without sound) and the experimental (480 Hz) groups was about 30 percent. There was no significant difference between the two groups. These results indicate that audible sound stimulation at 480 Hz had no significant effect on wound healing in this model under the specified conditions. Thus, the two groups exhibited comparable levels of cell migration and wound closure over the 7 days.

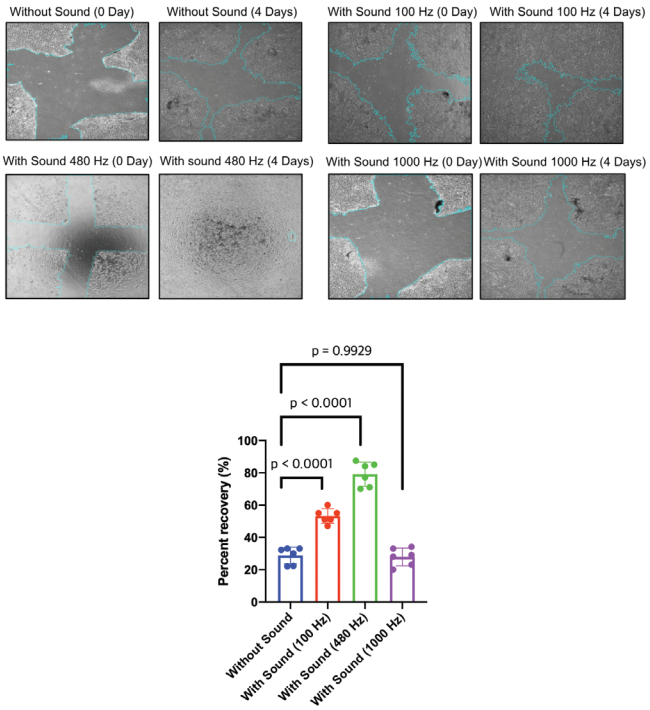

Figure 3: Frequency-specific effects of audible sound (100 Hz, 480 Hz, and 1,000 Hz) on wound healing over 4 days. Fibroblast scratch assays were analyzed after exposure to different frequencies for 1 hour daily over four days. These results demonstrate that wound-healing responses are highly frequency-dependent, with low-frequency audible sound—particularly 100 Hz and 480 Hz—producing the most robust enhancement of fibroblast migration (n = 6).

Figure 3 shows how different sound frequencies—specifically 100 Hz, 480 Hz, and 1,000 Hz—affected wound healing in human fibroblast cultures over four days. The images compare the initial scratch (day 0) and the state of healing after four days. Cells exposed to 100 Hz and 480 Hz demonstrated markedly greater wound closure compared to the untreated control, indicating a frequency-dependent enhancement of fibroblast migration. Cyan lines were drawn on each image to mark the wound edges and track changes over time. On the fourth day, the cultures treated with 100 Hz and 480 Hz sound showed noticeably better healing than the control group ($p<0.0001$), especially at 480 Hz. The 1,000 Hz group, on the other hand, looked very similar to the control ($p = 0.9929$). This indicates that the healing response may depend on the frequency of the sound, with 480 Hz being the most effective among the three tested.

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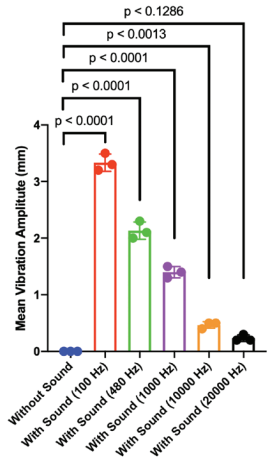

Figure 4: Frequency-dependent vibration amplitude induced by audible sound. Vibration amplitudes produced by sound frequencies ranging from 100 Hz to 20,000 Hz were measured using a 650 nm laser displacement tracking system. The strongest vibrations occurred at 100 Hz (3.4 mm) and 480 Hz (2.8 mm), both significantly higher than the control ($p < 0.0001$) ($n = 6$). A small reflective surface was attached under the culture plate, and a laser pointer was aimed at it at a shallow angle. As sound waves caused the plate to vibrate, the reflected laser dot moved on a screen positioned ~1 meter away. High-speed video recordings captured this movement, and peak-to-peak displacement was quantified using image analysis software. The bar graph shows that low-frequency sounds (100 Hz and 480 Hz) produced the largest vibration amplitudes (3.4 mm and 2.8 mm), while higher frequencies generated progressively smaller displacements. These mechanical differences help explain why only the low-frequency groups showed enhanced wound healing.

Figure 4 illustrates the vibration amplitudes generated by different audible sound frequencies (100 Hz to 20,000 Hz), as measured using a laser-based tracking setup with a wavelength of 650 nm. In the absence of sound, vibration was virtually undetectable, confirming that no external mechanical stimulation was applied to the control group—offering a reliable baseline. The most pronounced vibration occurred at 100 Hz (3.4 mm), followed by 480 Hz (2.8 mm), both of which were significantly stronger than the control and reflect the system's high sensitivity to low-frequency auditory input. Above 480 Hz, the amplitude gradually declined: approximately 1.6 mm at 1,000 Hz, 0.5 mm at 10,000 Hz, and just 0.2 mm at 20,000 Hz. Statistical analysis confirmed that vibration amplitudes at 100 Hz and 480 Hz were significantly higher than the control ($p < 0.0001$), and all other frequencies—except 20,000 Hz—also demonstrated statistically significant increases compared to the control. The amplitude at 20,000 Hz, however, was not statistically different from the control. Above 480 Hz, vibration intensity consistently declined as frequency increased. Interestingly, the most noticeable improvements in cell migration occurred at 100 Hz and 480 Hz—both frequencies that produced the strongest microvibrations—pointing to a possible link between vibration strength and the wound-healing effects of sound. Taken together, these findings indicate that low-frequency audible sound induces stronger mechanical stimulation, which in turn plays a key role in promoting fibroblast migration and wound repair.

Discussion

Fibroblasts exposed to 480 Hz audible sound for one hour daily over 4 days exhibited approximately 55% greater wound closure, indicating a frequency-specific enhancement of healing. Previous results showed that continuous sound exposure for seven days does not enhance healing and, in some cases, such as at 480 Hz, suppresses recovery. Thus, precise control of amplitude and exposure is needed.

To address this, we used brief daily hour sound exposures for four days and measured vibration amplitude with a 650 nm laser. The goal was to determine how sound-induced vibrational forces affect cellular behavior, particularly in the context of migration and repair.[7]

The selection of 100 Hz and 480 Hz as key test frequencies was based on both theoretical and empirical considerations from prior acoustic-biology research. Low-frequency audible sound (<500 Hz) is known to generate stronger mechanical displacements in solids and liquids due to reduced damping, making this range ideal for producing measurable microvibrations that cells can detect.[1–3] Previous studies also reported enhanced fibroblast migration around ~100 Hz and improved cytoskeletal remodeling under low-frequency mechanical cues, suggesting a biologically responsive window in this range.[3,14] In addition, preliminary pilot tests in our system showed that vibration amplitudes at 100–500 Hz were markedly higher than those at higher frequencies, providing a practical justification for selecting these points for deeper investigation. The inclusion of 480 Hz specifically allowed us to test a mid-low frequency that still produced substantial mechanical stimulation but had not been widely studied in wound-healing literature, enabling a comparative analysis across different parts of the audible spectrum. Frequencies above 1,000 Hz and into the ultrasonic range were included primarily as mechanistic controls to examine how diminishing vibration amplitude corresponds to reduced biological effects. Overall, this strategy enabled a frequency-resolved evaluation across the audible range while focusing on low-frequency candidates most likely to generate strong mechanobiological responses.

Building on these observations, we next evaluated how 100 Hz and 480 Hz sound stimulation influenced fibroblast migration and wound closure within our scratch assay model. The group exposed to 480 Hz showed about 55% more healing than the control group ($p < 0.0001$), suggesting that this frequency might help cells move more effectively. In contrast, higher frequencies, such as 1,000 Hz, showed no statistically significant benefit, corroborating previous observations that regenerative responses are more prominent at lower frequency ranges, potentially due to the sensitivity of the actin cytoskeleton.[1,2]

These findings indicate that cellular responses are closely linked to the magnitude of mechanically induced microvibrations, suggesting that vibration amplitude is a key determinant of the observed migratory enhancement. By better understanding this relationship, we may be able to enhance the use of sound in treatments that support tissue repair and regeneration.

While most past research has used ultrasound or very low-frequency sounds, our method employed regular sound in the air, eliminating the need for special tools or gels.[7-16] These

characteristics suggest that the method could be broadly applicable as a low-cost, non-invasive therapeutic approach—especially in underserved communities and among vulnerable populations such as older adults or diabetic patients.[13]

Although the findings are encouraging, one important limitation is that molecular-level changes were not directly assessed in this study. However, earlier research indicates that acoustic vibrations can stimulate core mechanotransduction cascades, in which physical forces from sound are translated into biochemical signals within the cell. In particular, the YAP/TAZ pathway is a well-established mechanism that detects microvibrations through cell structures and responds by moving these regulators into the nucleus, ultimately promoting the expression of genes related to migration, proliferation, and extracellular matrix remodeling—key processes in wound healing.[12] Fibroblast responses to mechanical signals often involve ERK and PI3K/Akt pathways, which also mediate cytoskeletal changes and cell movement.[17] This suggests exposure to 480 Hz sound may activate such mechanotransduction pathways through specific vibrational cues. Our study employed a simple scratch test and examined only one cell type; additional cell types and direct analysis of cell signaling remain unaddressed.

A key limitation of this study is the exclusive use of a single human dermal fibroblast line (Detroit 551), which restricts the generalizability of the findings to other skin-relevant cell types, such as keratinocytes, endothelial cells, or immune cells that also contribute to wound repair. Because fibroblasts represent only one component of the wound-healing environment, the observed benefits of 100 Hz and 480 Hz microvibrations may not fully reflect how entire tissues respond to audible sound stimulation. Additionally, all experiments were conducted *in vitro*, where the mechanical properties of culture plastics differ substantially from those of living tissue, and thus, *in vivo* confirmation is essential to determine whether similar vibration amplitudes can be safely and effectively delivered in real wounds. Longer-term safety data are also lacking: although brief, daily hour exposures were well tolerated *in vitro*, prolonged or repeated vibrational stimulation in living organisms may pose risks such as inflammation, tissue fatigue, or unwanted mechanotransduction effects. Consequently, future studies should incorporate multiple human cell types, 3D or organoid skin models, animal wound-healing studies, and systematic biocompatibility assessments to evaluate both efficacy and safety before translation to clinical applications.

Although we did not examine the cells in this study, earlier research indicates that sound-induced vibrations may trigger key pathways, such as YAP/TAZ, ERK, or PI3K/Akt. These are involved in cell movement and growth, which could explain the improved healing seen with certain frequencies. Future experiments—like western blotting, gene expression studies, or immunofluorescence—could help identify which signals are active. Animal models would also help test how well this works in real tissues and whether it's safe for longer-term use.

In this context, the measured vibration amplitudes help explain how audible sound may couple into specific mechanotransduction pathways. The 100 Hz and 480 Hz conditions generated the largest plate displacements (3.4 mm and 2.8 mm, respectively), indicating that fibroblasts in these groups experienced greater substrate strain than at higher frequencies. Such cyclic micro-deformations are known to be sensed primarily through integrins and focal adhesion complexes, which transmit forces to the actin cytoskeleton and upstream regulators of YAP/TAZ, ERK, and PI3K/Akt signaling.[6,8,10,15–17] When the deformation is large enough—yet still within a non-damaging range—these pathways are activated, driving nuclear translocation of YAP/TAZ and phosphorylation of ERK/Akt, which in turn upregulate genes involved in cytoskeletal remodeling, migration, and extracellular matrix production. Conversely, the smaller amplitudes observed at ≥1,000 Hz likely generate sub-threshold mechanical cues that are insufficient to robustly engage these force-sensitive signaling networks, consistent with the minimal change in wound closure at 1,000 Hz and 20,000 Hz. Taken together, our data support a model in which vibration amplitude, rather than frequency per se, is the critical physical parameter that determines whether audible sound activates mechanotransduction cascades that promote fibroblast migration and wound repair.

Conclusion

The study shows that low-frequency sound (100 Hz and 480 Hz) causes critical microvibrations in the experimental system. This also suggests a high possibility of promoting mechanotransduction in cellular conditions that facilitate wound healing. Conversely, increasing frequency above 1,000 Hz yielded successively smaller vibration amplitudes, and little mechanical response was recorded at ultrasonic frequencies (10,000 to 20,000 Hz). Our study results confirm that low-frequency sound induces a more powerful mechanical stimulus, significantly improving fibroblast migration and wound closure. This suggests the potential of audible sound as a noninvasive and accessible tool in regenerative medicine. The inverse correlation between frequency and vibration amplitude observed above 480 Hz confirms the hypothesis that audible sound can be used as a non-invasive mechanical stimulus to induce positive cellular effects, including migration and proliferation. These results suggest that low-frequency audible sound may serve as a practical, noninvasive mechanical stimulus capable of enhancing fibroblast migration, highlighting its potential utility in regenerative medicine applications. Going forward, studies should investigate how vibration-induced effects relate to cellular changes—such as cytoskeletal dynamics, molecular signaling, and gene activity—to better understand which sound frequencies are most effective and why.

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Cheng Min Sun is a student researcher with a strong interest in physics and cellular biomechanics. This project reflects Cheng Min's broader commitment to exploring innovative, non-invasive methods for enhancing cellular responses and advancing regenerative medicine.