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Effect of Harmonic Sound Frequencies on the Regeneration of Connective Tissue Fibroblasts

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ABSTRACT

Background: Sound-based interventions are attracting growing scientific interest as potential complementary approaches to promoting health and well-being. Information Medicine provides harmonic sound frequencies that can be delivered through its streaming platform. In the present study, the sound files were played through a conventional external speaker, and only the effects of sound exposure were investigated. The aim of this study was to use animal-free cell biological test methods to investigate the effects of appropriate harmonic sound frequencies on the regeneration of cultured connective tissue fibroblasts.

Experimental: The Information Medicine streaming platform with an individual premium account (www.informationmedicine.org) was used in a preliminary experiment to apply the sound files to the fibroblasts at the beginning of the regeneration process for 1 to 3 × 30 min. Two sound files were used simultaneously: (1) “Connective Tissue – Fascia” and (2) “Regeneration.” The two sound files were applied to the fibroblasts using a 2-way speaker system with 28 watts RMS power and separate controls for treble and bass levels (GigaWorks T20 Series II; Creative), which was connected to a personal computer and the premium information software. Since the application of 3 × 30 min had the strongest effect, this exposure duration was used for the five subsequent experiments. Statistical analysis was performed using the two-tailed Wilcoxon-Mann-Whitney rank-sum test.

Results: The preliminary experiment examining the time-dependent response of connective tissue fibroblasts to the harmonic sound files demonstrated a statistically significant improvement in regeneration at 2 × 30 min and 3 × 30 min ($p \leq 0.01$). Based on the preliminary experiment, the 3 × 30 min harmonic sound file treatment was used in the subsequent five independent experiments, which confirmed improved regeneration of connective tissue fibroblasts. Taken together, the overall improvement in connective tissue fibroblast regeneration across all five independent experiments was 26.8 ± 7.4% (mean value ± standard deviation, n = 5) compared with untreated controls. This demonstrates a statistically significant improvement ($p \leq 0.01$) associated with the harmonic frequencies of the sound files used here at the cellular level.

Conclusions: The present findings demonstrate that exposure to the selected harmonic sound files significantly enhanced fibroblast gap closure under the experimental conditions used in this study. As fibroblast migration and proliferation are fundamental processes in connective tissue repair, these findings point to a biologically relevant interaction between harmonic sound exposure and cellular regenerative activity. These findings support the potential biological relevance of harmonic sound frequencies as used within the Information Medicine approach.

Keywords

Information Medicine
Sound frequencies
Health effects
Regeneration
Connective tissue fibroblasts
L-929
Cell culture

INTRODUCTION

Sound-based interventions are attracting growing scientific interest as potential complementary approaches to promoting health and well-being. Evidence suggests that exposure to specific auditory stimuli and frequencies may reduce perceived stress, anxiety, and negative environmental input, while in some contexts also supporting physiological recovery and relaxation [1-5]. These effects are generally attributed to modulation of autonomic nervous system activity, attentional processes, and emotional regulation rather than to any broad, universal healing-frequency mechanism [6-8]. Most health effects from sound are driven by sound level and duration rather than by frequency alone. As a result, in conventional mainstream medicine, sound is currently regarded more as a complementary than a primary therapeutic modality, with further research needed to clarify its mechanisms and clinical applicability.

According to the Information Medicine streaming platform, the harmonic sound frequencies “support the body’s natural resilience and innate intelligence. The harmonic frequencies ‘inform’ the body with electromagnetic and sound frequencies. The water in our body ... plays the leading role in this process. The cells resonate with these body-attuned vibrations, which can support subtle communication, alignment, and harmony within the system. The aim is to encourage a state of inner coherence across all layers of body, mind, and spirit. To be most effective, it is important that the information is transmitted as purely and naturally as possible.”

We used connective tissue fibroblasts to examine whether the sound files might improve regeneration/wound healing, an essential feature of cellular structural restoration and tissue functional recovery after injury [9].

MATERIALS AND METHODS

Cell culture

The investigations were conducted with connective tissue fibroblasts (cell line L-929; ACC 2; Leibniz Institute – DSMZ, Braunschweig, Germany). Cells were routinely grown in RPMI 1640 culture medium supplemented with 10% growth mixture and standard amounts of antibiotics and were regularly subcultured in fresh culture medium by trypsinization once a week. Cell cultivation was performed in a gassed CO_2 incubator at 37 °C in a humid atmosphere of 5% CO_2 and 95% air. Cells for the experiments were taken from 80 to 90% confluent mass cultures over a period of three months, using different cell batches at different passages.

Sound files and cell treatment

The premium information software was unlocked for an individual account for the duration of the experiments on the Information Medicine streaming platform (www.informationmedicine.org). This individual premium account allows two sound files to be played simultaneously. For this study, we selected the sound files “Connective Tissue – Fascia” and “Regeneration” (Figure 1). The two sound files were applied to the fibroblasts using a 2-way speaker system

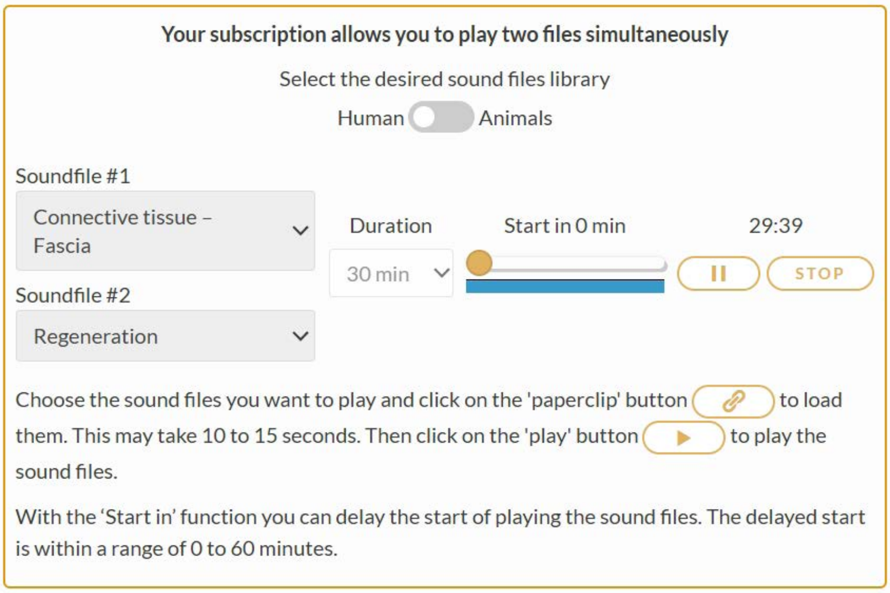

Figure 1: The surface of the Information Medicine streaming platform with the two sound files used in this study and the time settings for a single 30-min run.

with 28 watts RMS power and separate controls for treble and bass levels (GigaWorks T20 Series II; Creative), which was connected to a personal computer and the premium information software.

In a preliminary experiment, we examined the time-dependent effect of the sound files on the cells by treating the cells for 1 × 30 min, 2 × 30 min, and 3 × 30 min at a distance of 20 cm from the speakers, with a sound level ranging from 55 to 75 dB(A) at the position of the cells. In the main experiments, we used the same setup, but the cells were always treated for 3 × 30 min.

Examination of cell regeneration

Connective tissue fibroblasts were seeded at a density of 200,000 cells/mL into the four individual compartments of silicone 4-well culture inserts (ibidi, Gräfelfing, Germany). The individual compartments are separated from each other by 500 µm thick silicone bars. Due to the special adhesive surface of the silicone frames, they adhere firmly to the bottom of a culture dish, thus forming a cell-free area that the cells can colonize by migration and proliferation after removal of the silicone frames. The test system simulates the proliferative and migratory stages of the wound healing/regeneration process [10].

Immediately after removal of the silicone frames, the culture medium was exchanged for a mixture of Leibowitz L-15 and RPMI 1640 (2:1) supplemented with 2.5% growth mixture, 20 mM HEPES buffer, and standard amounts of antibiotics. This culture medium maintains a neutral pH under normal atmospheric conditions outside the gassed incubator. The fibroblasts were then treated with the sound files as described above and were allowed to migrate and proliferate for a period of 14 to 16 hours in an external mini-incubator at 37 °C without gas supply. Cell samples were either photographed directly using phase-contrast microscopy or fixed with methanol, stained with Giemsa's azure eosin methylene blue solution (Sigma-Aldrich, Deisenhofen, Germany), and air-dried. Micrographs documenting the residual cell-free area and the cell front lines were taken at different locations for each sample. A total of four measurements were performed for each sample. IKOSA AI software with artificial intelligence (Kolaido, Altenrhein, Switzerland) was used to examine and calculate the residual cell-free area and the front lines of the cell cultures. Five independent experiments ($n = 5$) with biological replicates were performed.

STATISTICAL ANALYSIS

Data are given as mean values ± standard deviations. For final statistical analysis, the mean values of the individual experiments with biological replicates were averaged. Statistical analysis was performed using the non-parametric, two-tailed Wilcoxon-Mann-Whitney rank-sum test, and significance was determined at the 5% ($p \leq 0.05$) or 1% ($p \leq 0.01$) level.

RESULTS

The results of the preliminary experiment examining the time-dependent response of the cells to the harmonic sound files are documented micrographically in Figure 2. Treatment for 1 × 30 min did not result in a visually detectable improvement in the regeneration of connective tissue fibroblasts, whereas 2 × 30 min and, especially, 3 × 30 min resulted in improved regeneration with a smaller cell-free area. Quantification of the remaining cell-free areas at the end of the experiment showed a statistically significant improvement in regeneration at 2 × 30 min and 3 × 30 min ($p \leq 0.01$) for the mean values of the biological replicates of each individual experiment (Figure 3).

Based on the preliminary experiment, the sound file treatment with harmonic frequencies for 3 × 30 min was used in the subsequent five independent experiments, which confirmed improved regeneration of connective tissue fibroblasts. A representative visualization of the micrographic results is depicted in Figure 4, and the quantitative results for the improvement in regeneration in each individual experiment are shown in Figure 5.

Taken together, the overall improvement in connective tissue fibroblast regeneration across all five independent experiments was 26.8 ± 7.4% (mean value ± standard deviation) compared with untreated controls. This demonstrates a statistically significant improvement ($p \leq 0.01$) associated with the harmonic frequencies of the sound files used here at the cellular level.

DISCUSSION

In this study, we investigated the effects of the harmonic frequencies of sound files from the Information Medicine streaming platform on the regeneration of cultured connective tissue fibroblasts. This cell type was chosen because fibroblasts are key mesenchymal cells responsible for maintaining connective tissue architecture and regulating extracellular matrix synthesis and turnover. After tissue injury, they are activated and contribute to repair through proliferation, migration, and increased production of matrix components [11,12]. In a well-regulated healing process, fibroblasts multiply, migrate into the injured area, and begin to build new tissue there. Their regenerative activity is influenced by biochemical and biomechanical factors [13]. During wound

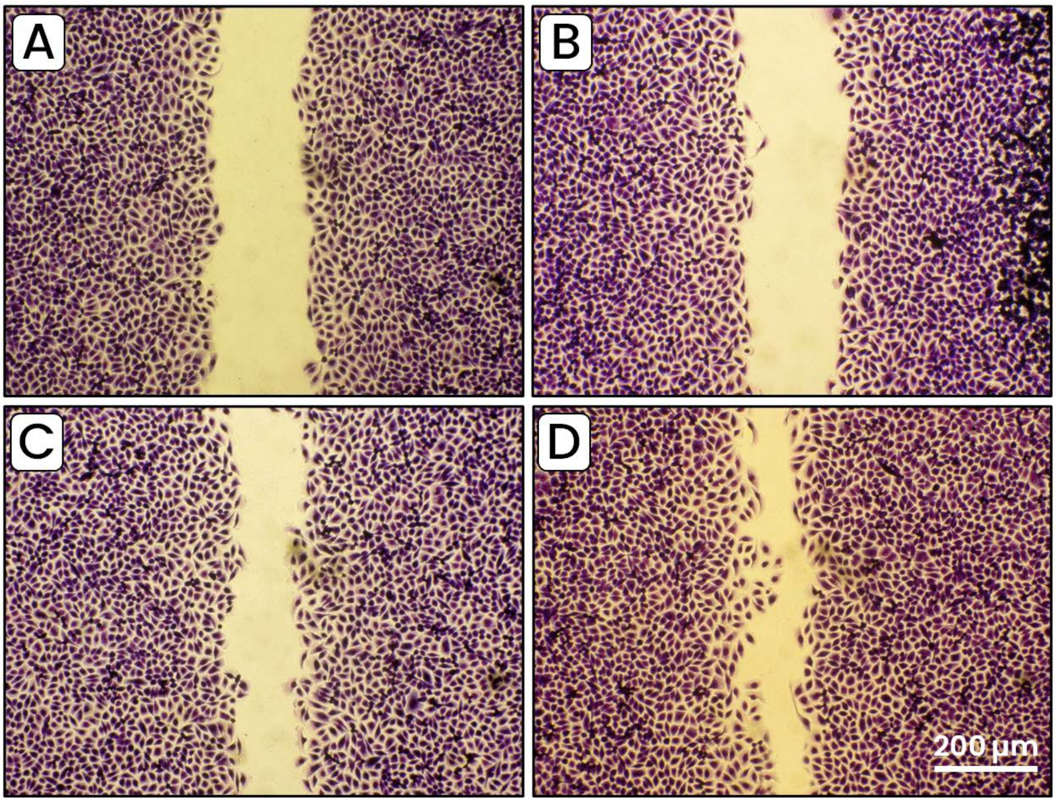

Figure 2: Micrographic documentation of the time-dependent results of sound file treatment after 14 hours of connective tissue fibroblast regeneration. (A) Untreated control; (B) treatment for 1 × 30 min; (C) treatment for 2 × 30 min; (D) treatment for 3 × 30 min. Note the treatment time-dependent improvement in regeneration, as visualized by the smaller residual cell-free area at the end of the experiment. At the beginning of the experiment, the width of the cell-free area was 500 µm. Olympus IX 50 with a 10× Planachromat lens and an Olympus E-20 digital camera at 5-megapixel resolution. Stained samples at bright-field illumination.

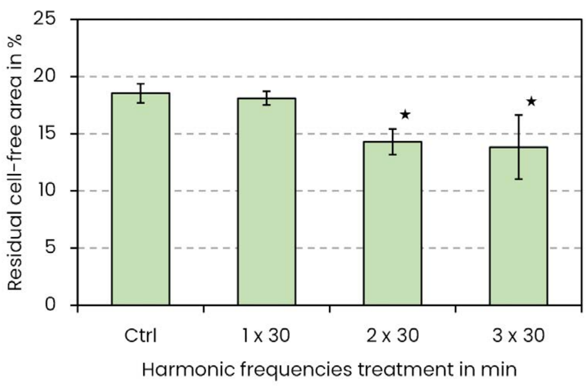

Figure 3: Quantitative measurement data for the residual cell-free area from the preliminary experiment. Data show the time-dependent improvement in connective tissue fibroblast regeneration 14 hours after sound file treatment. Data represent mean values ± standard deviations of the biological replicates for each individual experiment. (*) Statistical significance at $p \leq 0.01$ according to the non-parametric, two-tailed Wilcoxon-Mann-Whitney rank-sum test.

healing, fibroblasts support granulation tissue formation and extracellular matrix remodeling, both of which are essential for structural restoration [9]. A better understanding of the mechanisms governing fibroblast regeneration is therefore important for improving tissue repair and advancing regenerative medicine.

The harmonic sound files used here for the regeneration studies are especially related to fascia, which forms a continuous connective tissue network that surrounds and interconnects organs, muscles, nerves, and cells throughout the body. It forms a framework that helps to hold muscles, organs, nerves, and vessels in position; creates compartments between muscle groups and other tissues; helps to distribute mechanical forces through the body rather than through individual muscles alone; reduces friction between layers and allows tissues to glide more smoothly during movement; and can help protect structures from stress and strain [14,15]. This collagen-rich matrix has viscoelastic, polymorphic, and piezoelectric properties, meaning that mechanical

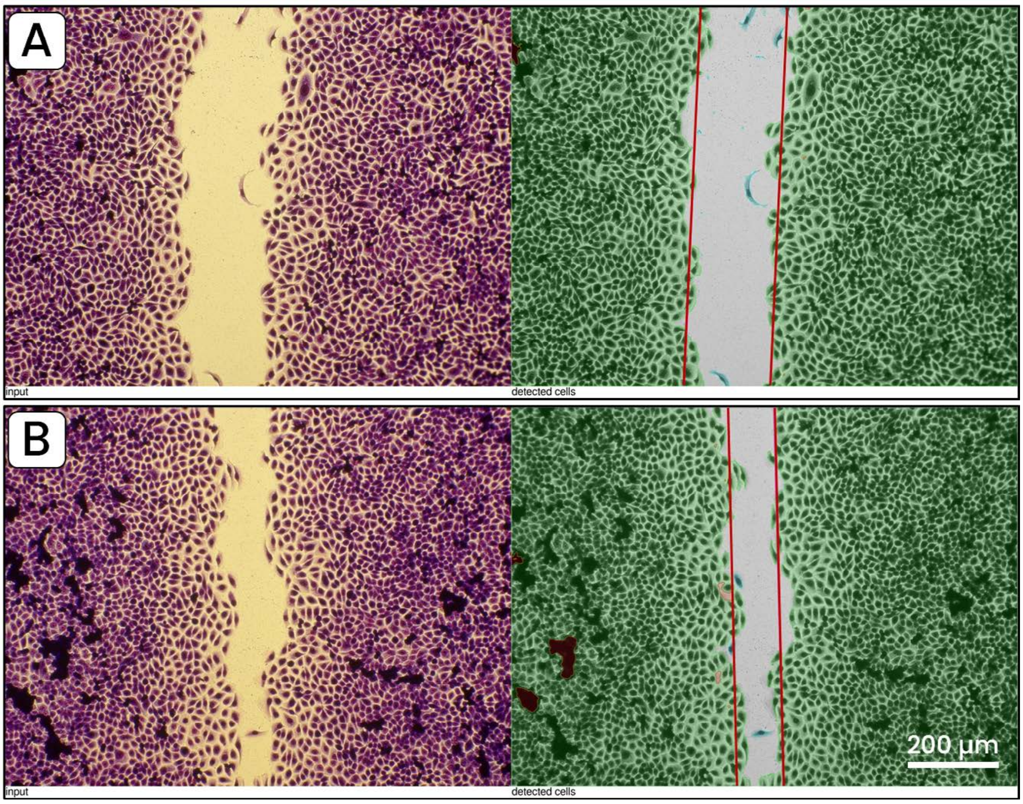

Figure 4: Micrographic documentation of the regeneration of connective tissue fibroblasts after 16 hours. (A) Untreated control cells; (B) cells after harmonic sound file treatment for 3 × 30 min. The left side shows the original micrographs, and the right side shows the results evaluated by the IKOSA software. Detected cells are marked in green, and the front lines of cell migration and proliferation from both sides into the cell-free area are marked by red lines. At the beginning of the experiment, the width of the cell-free area was 500 µm. Olympus IX 50 with a 10× Planachromat lens and an E-20 digital camera at 5-megapixel resolution. Stained samples under bright-field illumination.

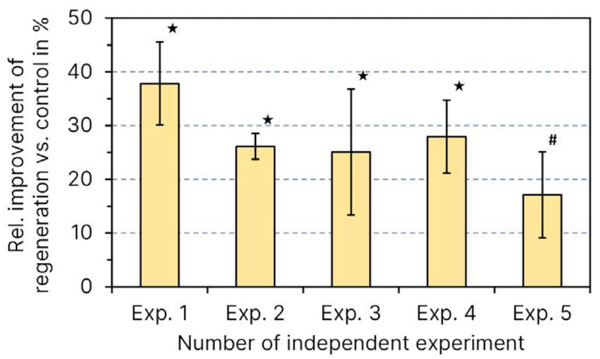

Figure 5: Presentation of the relative improvement in connective tissue fibroblast regeneration for each of the five independent experiments in comparison with untreated controls. Data show the improvement in connective tissue fibroblast regeneration 16 hours after harmonic sound file treatment for 3 × 30 min. Data represent mean values ± standard deviations of the biological replicates for each individual experiment. Statistical significance according to the non-parametric, two-tailed Wilcoxon-Mann-Whitney rank-sum test is marked as #$p \leq 0.05$ and *$p \leq 0.01$.

deformation can produce electrical charge [16,30]. Fascia treatment has gained increasing attention due to its potential benefits in pain management, neuromuscular function, and tissue repair [17,18].

As demonstrated in this study, the appropriate sound files of the Information Medicine platform significantly improved the regeneration process of connective tissue fibroblasts in a time-dependent manner, with the strongest effect observed after 3 × 30 min of application. These findings demonstrate that harmonic sound exposure can influence fibroblast behaviour at the cellular level. Given the central role of fibroblasts in the maintenance and repair of fascia and other connective tissues, the observed response provides a compelling biological link between acoustic stimulation and connective tissue dynamics.

Our findings are consistent with those previously reported by Mohammed et al. [19]. They used an acoustic vibration system and examined fibroblasts, including the L-929 cell line used in our study. Interestingly, they found that acoustic vibration affected fibroblast migration in a frequency-dependent manner, with 100 Hz enhancing migration and higher frequencies reducing it. Moreover, they observed that mechanical stimulation by sound could affect cell morphology and F-actin (stress fibre) organisation, with acoustic vibration increasing the formation of lamellipodia and filopodia. Cell morphology and the highly dynamic structures of the cytoskeleton, such as F-actin, microtubules, and intermediate filaments, are known to play an essential role in the migratory activity of cells [20,21]. Both features can be examined in our laboratory, so future work in this respect might lead to a closer understanding of the underlying mechanisms.

Nevertheless, the precise mechanisms underlying the observed response to harmonic sound exposure remain an area for further exploration. The fascial network has been described as a communication system capable of transmitting mechanical, electrical, and biochemical signals throughout the body. It may contribute to the coordination of physiological processes by facilitating the propagation of vibrational and electrical information across tissues [22-24].

The fascial network is also highly hydrated. The extracellular matrix surrounding the collagen fibres contains large amounts of water, and recent experimental work has demonstrated heterogeneous, spatially organised water populations within hydrated collagen microfibrils [25,26]. From this perspective, water is not merely a transport medium in living biological systems but can also influence the functioning of biomolecules, communication between cells, and the balance of complex biological systems [25-28].

Since sound frequencies, as pressure waves, can penetrate biological tissues, they can interact with water and the fascial network and trigger microdeformations within this collagen-rich structure. Due to the piezoelectric properties of collagen, such mechanical changes can generate electrical potentials; direct experimental measurements have demonstrated a piezoelectric response in collagen-based materials [16,29].

One possible mechanism is that acoustic pressure waves interact with the highly hydrated, collagen-rich extracellular matrix through mechanical deformation and mechanotransductive processes. Given the intimate interaction between collagen and interfacial water, changes within this collagen-water matrix could potentially contribute to the propagation and biological effects of acoustic stimulation. This possible mechanism was not directly investigated in the present study.

Finally, the results of this study can be considered alongside previous user-reported observations collected between 2021 and 2023 from more than 2,000 participants using the Information Medicine platform, with more than 80% reporting a subjective improvement in their health situation (unpublished results). Further investigations are needed to determine whether there is a clear relationship between these user-reported observations and the experimental cellular data presented here.

CONCLUSIONS

The present findings demonstrate that exposure to the selected harmonic sound files significantly enhanced fibroblast gap closure under the experimental conditions used in this study. As fibroblast migration and proliferation are fundamental processes in connective tissue repair, these findings point to a biologically relevant interaction between harmonic sound exposure and cellular regenerative activity. These findings support the potential biological relevance of harmonic sound frequencies as used within the Information Medicine approach.

CONFLICT OF INTEREST

The present study was funded by Information Medicine B.V., NL – 3732 HW De Bilt, The Netherlands. The company made the Information Medicine account on its streaming platform and both sound files, “Connective Tissue – Fascia” and “Regeneration,” available for the experiments.

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