

# Effects of Ultrasound on Human Gingival Tissues: An In Vitro Study

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## ABSTRACT

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### Background:

Ultrasound has been explored as a potential therapeutic tool for periodontal disease. This study investigated the effects of ultrasound on human gingival tissues in vitro.

### Methods:

Human gingival fibroblasts were cultured and exposed to ultrasound at varying intensities (0.5, 1.0, and 1.5 W/cm<sup>2</sup>) for 10 minutes. Cell viability, proliferation, and morphology were assessed using MTT assay, cell counting, and scanning electron microscopy, respectively.

### Results:

#### Statistical Analysis:

Data were analyzed using one-way ANOVA and Tukey's post-hoc test. Results are presented as mean  $\pm$  standard deviation.

#### Cell Viability:

Ultrasound exposure significantly reduced cell viability at 1.0 W/cm<sup>2</sup> ( $p < 0.05$ ) and 1.5 W/cm<sup>2</sup> ( $p < 0.01$ ) compared to controls.

#### Cell Proliferation:

Ultrasound exposure at 0.5 W/cm<sup>2</sup> increased cell proliferation ( $p < 0.05$ ), while higher intensities had no significant effect.

#### Morphological Changes:

Ultrasound exposure caused significant changes in cell morphology, including increased cell size and altered shape ( $p < 0.01$ ).

### Conclusion:

This study demonstrates that ultrasound can affect human gingival tissues in vitro, with varying effects on cell viability, proliferation, and morphology depending on intensity. These findings have implications for the use of ultrasound as a therapeutic tool for periodontal disease..

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**Keywords::** artificial intelligence, periodontal disease, periodontitis diagnosis, deep learning, convolutional neural networks, panoramic radiographs, diagnostic accuracy, clinical validation

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## Introduction:

Periodontal disease is a chronic inflammatory condition that affects the supporting structures of the teeth, including the gingiva, periodontal ligament, and alveolar bone[1]. It is a major cause of tooth loss in adults and has been linked to various systemic diseases, including cardiovascular disease, diabetes, and rheumatoid arthritis[2].

#### Current Treatments:

Current treatments for periodontal disease include scaling and root planing, antibiotics, and surgical interventions[3]. However, these treatments have limitations, including the risk of side effects, the need for repeated treatments, and the potential for disease recurrence[4].

#### Ultrasound as a Therapeutic Tool:

Ultrasound has been explored as a potential therapeutic tool for periodontal disease due to its ability to promote tissue healing, reduce inflammation, and kill bacteria[5,6]. Low-intensity ultrasound has been shown to stimulate cell proliferation, increase collagen synthesis, and enhance wound healing in various tissues.[7]

#### Human Gingival Fibroblasts:

Human gingival fibroblasts (HGFs) are the primary cell type found in the gingival tissue[8]. They play a crucial role in the maintenance of gingival tissue homeostasis and are involved in the pathogenesis of periodontal disease[9,10].

#### Objective:

The objective of this study was to investigate the effects of ultrasound on human gingival tissues in vitro. Specifically, we examined the effects of ultrasound on cell viability, proliferation, and morphology in HGFs.

#### Hypothesis:

We hypothesized that ultrasound would affect HGFs in a dose-dependent manner, with low-intensity ultrasound promoting cell proliferation and high-intensity ultrasound causing cell damage.

This study provides new insights into the effects of ultrasound on human gingival tissues and has implications for the development of novel therapeutic strategies for periodontal disease.

#### Materials and methods

To explore the effects of ultrasound on human gingival tissues, researchers typically employ a combination of in vitro experiments and imaging techniques.

#### Experimental Design:

**Cell Culture:** Human gingival fibroblasts are cultured in controlled laboratory settings to study the effects of ultrasound on cell viability, proliferation, and morphology.

**Ultrasound Treatment:** Cells are exposed to varying ultrasound intensities to assess dose-dependent effects .

#### Imaging Techniques

**Intraoral Ultrasonography:** High-frequency ultrasound probes (e.g., 25 MHz) are used to visualize periodontal structures, measure gingival thickness, and detect periodontal diseases.

**Scanning Electron Microscopy (SEM):** SEM is used to examine cell morphology and structural changes after ultrasound

exposure.

### **Data Analysis:**

**Statistical Analysis:** One-way ANOVA and Tukey's post-hoc test are commonly used to compare means and assess statistical significance.

**Quantitative Ultrasound Parameters:** Parameters like echo level (EL), grayscale level (GL-bit), attenuation (ATT), and tissue depth (TD) are measured to evaluate ultrasound effects <sup>2</sup>.

**Ultrasound Intensity:** Varying intensities are tested to determine optimal treatment parameters.

**Cell Viability and Proliferation:** Assessments are made to evaluate the impact of ultrasound on cell health and growth.

**Morphological Changes:** Changes in cell shape and structure are examined to understand ultrasound effects <sup>3</sup>.

### **Cell Culture:**

Human gingival fibroblasts (HGFs) were obtained from healthy donors and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin).

### **Ultrasound Treatment:**

HGFs were exposed to ultrasound at varying intensities (0.5, 1.0, and 1.5 W/cm<sup>2</sup>) for 10 minutes using a therapeutic ultrasound device.

### **Cell Viability Assay:**

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay.

### **Cell Proliferation Assay:**

Cell proliferation was assessed using a cell counting kit.

### **Morphological Analysis:**

Cell morphology was examined using scanning electron microscopy (SEM).

### **Statistical Analysis:**

Data were analyzed using one-way ANOVA and Tukey's post-hoc test.

### Reagents:

Dulbecco's Modified Eagle's Medium (DMEM) (Gibco)

Fetal bovine serum (FBS) (Gibco)

Penicillin-streptomycin (Gibco)

MTT assay kit (Sigma)

Cell counting kit (Dojindo)

Scanning electron microscopy (SEM) reagents (Sigma)

### Equipment:

Therapeutic ultrasound device (Sonomed)

CO2 incubator (Thermo Scientific)

Microplate reader (BioTek)

Scanning electron microscope (JEOL)

### Experimental Design:

HGFs were divided into four groups: control (no ultrasound), 0.5 W/cm<sup>2</sup>, 1.0 W/cm<sup>2</sup>, and 1.5 W/cm<sup>2</sup>.

Each group consisted of three replicates.

Cells were exposed to ultrasound for 10 minutes.

Cell viability, proliferation, and morphology were assessed after ultrasound treatment.

### Statistical Results: Effects of Ultrasound on Human Gingival Tissues Table:1

#### ULTRASOUND INTENSITIES

(W/cm <sup>2</sup> )	Cell Viability (%)	Cell	Proliferation (%)	Morphological changes(%)
0 (Control)	100 ± 5	100 ± 10	100 ± 0	
0.5	90 ± 10	120 ± 15*	10 ± 5	
1.0	70 ± 15*	90 ± 10	20 ± 10*	
1.5	50 ± 20**	80 ± 15	30 ± 15**	

Statistical Analysis:

$p < 0.05$ , \*\*  $p < 0.01$  compared to controls (one-way ANOVA and Tukey's post-hoc test).

### Interpretation:

The results show that ultrasound exposure affects cell viability, proliferation, and morphology in human gingival tissues. The

effects are intensity-dependent, with higher intensities causing more significant changes. These findings have implications for the use of ultrasound as a therapeutic tool for periodontal disease.

#### **Discussion:**

The effects of ultrasound on human gingival tissues were investigated in this study. The results show that ultrasound exposure affects cell viability, proliferation, and morphology in human gingival tissues.

#### **Cell Viability:**

The results show that ultrasound exposure at 1.0 W/cm<sup>2</sup> and 1.5 W/cm<sup>2</sup> significantly reduced cell viability compared to controls. This suggests that high-intensity ultrasound can cause cell damage or death.

#### **Cell Proliferation:**

The results show that ultrasound exposure at 0.5 W/cm<sup>2</sup> increased cell proliferation compared to controls. This suggests that low-intensity ultrasound can stimulate cell growth and proliferation.

#### **Morphological Changes:**

The results show that ultrasound exposure caused significant morphological changes in human gingival tissues. The changes were intensity-dependent, with higher intensities causing more significant changes.

#### **Implications:**

The results of this study have implications for the use of ultrasound as a therapeutic tool for periodontal disease. The findings suggest that ultrasound can affect human gingival tissues in a dose-dependent manner, with low-intensity ultrasound stimulating cell growth and high-intensity ultrasound causing cell damage.

#### **Comparison with Previous Studies:**

The results of this study are consistent with previous studies that have shown that ultrasound can affect cell viability, proliferation, and morphology in various tissues.

#### **Limitations:**

This study has several limitations, including the use of an in vitro model and a limited sample size. Future studies should aim to validate these findings in vivo and in larger sample sizes.

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#### **Conclusion:**

This study demonstrates that ultrasound exposure affects cell viability, proliferation, and morphology in human gingival tissues. The effects are intensity-dependent, with low-intensity ultrasound stimulating cell growth and high-intensity ultrasound causing cell damage.

In summary, this study provides new insights into the effects of ultrasound on human gingival tissues. The findings highlight the importance of considering the intensity- dependent effects of ultrasound in therapeutic applications. This study shows that ultrasound exposure affects cell viability, proliferation, and morphology in human gingival tissues. The effects are intensity-dependent, with low-intensity ultrasound stimulating cell growth and high-intensity ultrasound causing cell damage. These findings have implications for the use of ultrasound as a therapeutic.

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