

Synergistic effects of high-frequency vibration and orthodontic force on osteoclast numbers and root resorption in a rat model

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ABSTRACT

Objective: To evaluate how high-frequency vibration (125 Hz) combined with light or optimal orthodontic forces affects osteoclast numbers and root volume during tooth movement in Wistar rats.

Materials and Methods: Using a split-mouth design, 96 sites in male Wistar rats were randomly assigned to six groups: control, high-frequency vibration (HFV), light force (LF, 5g), light force with vibration (LF/HFV), optimal force (OF, 10g), and optimal force with vibration (OF/HFV). First maxillary molars were moved mesially using nickel-titanium (NiTi) closed coil springs. Root volume and osteoclast numbers were measured using Micro-CT and histomorphometry at Days 1, 7, 14, and 21.

Results: After 21 days, osteoclast numbers increased significantly in HFV (5.25 ± 0.48 , $P = .002$), LF/HFV (10.00 ± 0.41 , $P < .0001$), OF (13.75 ± 0.48 , $P < .0001$), and OF/HFV (15.25 ± 0.85 , $P < .0001$) groups. Root volume decreased significantly in LF/HFV ($7.75 \pm 0.18 \text{ mm}^3$), OF ($6.68 \pm 0.24 \text{ mm}^3$), and OF/HFV ($6.28 \pm 0.14 \text{ mm}^3$) groups compared to control (all $P < .0001$). HFV alone increased osteoclast numbers but did not affect root volume. The OF/HFV group showed the highest osteoclast numbers and root volume reduction. Three-way analysis of variance revealed that time, vibration, and force significantly reduced root volume ($P < .0001$). Notably, the interaction effects on osteoclast numbers were significant in LF group ($P < .0001$), but not OF group ($P = .338$).

Conclusions: Combined high-frequency vibration and orthodontic forces increased osteoclast numbers and root resorption. Light forces with high-frequency vibration promoted osteoclast formation while minimizing root resorption compared to optimal forces. Additionally, the duration of this combined treatment significantly affected the extent of root resorption. (*Angle Orthod.* 2025;95:647–655.)

KEY WORDS: Mechanical vibration; Micro-CT; Root volume

INTRODUCTION

High-frequency vibration (HFV) devices operating at frequencies of ≥ 90 Hz have been used intraorally as a supplementary method in orthodontics to accelerate tooth movement.^{1–7} This approach enhanced cellular

activity in the periodontal ligament and alveolar bone by regulating osteoclast and osteoblast activity. Animal studies suggested that vibration combined with orthodontic forces accelerates tooth movement,^{2,3,8,9} although concerns about increased osteoclast numbers

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and root resorption exist. Clinical trials showed controversial results,¹⁰ with some reporting reduced treatment time and others finding no significant effect due to varying vibration parameters and individual differences. Based on previous research, vibration frequencies around 120–125 Hz effectively stimulate osteoblast and osteoclast activity,¹¹ which is crucial for bone resorption and formation. Recent studies demonstrated that frequencies within the range of 60–125 Hz enhanced tooth movement by optimizing the transfer of mechanical energy to cellular components.^{2,9} High-frequency vibration of 125 Hz has been widely used in *in vivo*³ and clinical trials^{1,4} due to its ability to optimize orthodontic tooth movement while ensuring safety and patient comfort.

Root resorption, an irreversible complication of orthodontic treatment, occurs at locations where the periodontal ligament (PDL) is severely compressed. Heavy forces can cause necrotic tissue, recruiting macrophage-like cells that destroy the cementoid, leading to root resorption. This process differs from bone remodeling due to the absence of an unmineralized cementoid layer and the presence of immature PDL collagen.^{12,13}

Light forces, while potentially moving teeth more slowly, typically do not create complications such as root resorption.^{3,14} Modern orthodontics continually scrutinizes the effectiveness, safety, and impact of accelerated treatment procedures on patient satisfaction, classifying methods into biological, surgical, and physical approaches.^{15,16}

A previous study demonstrated that, while combining light force with HFV resulted in slower tooth movement compared to using optimal force with HFV, this light force with HFV nonetheless achieved faster tooth movement with less root resorption compared to using optimal force alone.³ HFV stimulates inflammatory cytokines, subsequently increasing osteoclast cells.^{1,8,9,17–19} Light force, by not causing PDL necrosis, can preserve the cementoid layer covering the root surface,^{12,13} potentially explaining why light force with HFV accelerated greater tooth movement with less root resorption area than optimal force alone. This study aimed to investigate the combined effects of minimally invasive, high-frequency vibration (125 Hz) with light and optimal force during orthodontic tooth movement, on osteoclast numbers and root volume in a rat model.

MATERIAL AND METHODS

Experimental Animals

The study was approved by the Animal Ethics Committee of Prince of Songkla University (AR031/2024),

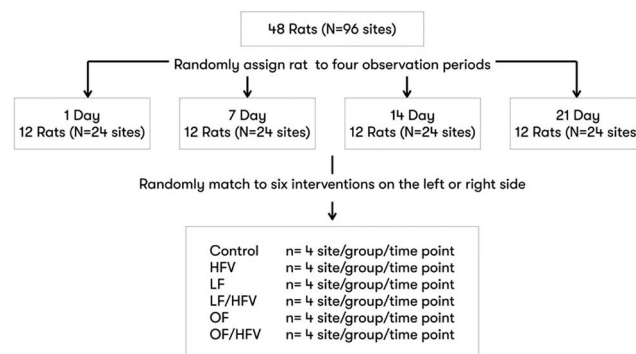


Figure 1. Schematic of split-mouth design and group allocation, four timepoints: Day 1, 7, 14, and 21.

following laboratory animal care guidelines.²⁰ Forty-eight adult male Wistar rats (10–12 weeks, 300–400 grams) were used in this split-mouth experimental design. After a 7-day acclimation period, animals were randomly assigned to four observation points: Day 1, 7, 14, and 21 (Figure 1). A priori power analysis was conducted using G*Power 3.1.9.4. The assumed mean and standard deviation were based on root resorption lacunae from a previous study.³ With an effect size of 0.66, an alpha level of 0.05, and a desired power (1- β) of 0.80.

The maxillary left and right first molars were randomly assigned to six groups, with four samples per group:

- HFV: high-frequency vibration (125 Hz, 5 min/d)
- LF: light force (5 grams)
- LF/HFV: Combined light force and high-frequency vibration
- OF: Optimal force (10 grams)
- OF/HFV: Combined optimal force and high-frequency vibration
- Control: no treatment

Orthodontic Tooth Movement

Animals were sedated using intraperitoneal injection (90 mg/kg ketamine (Calypsol, Gedeon Richter Ltd., Budapest, Hungary), 10 mg/kg xylazine (Xylavet, Thai Meiji Pharmaceutical Ltd., Bangkok, Thailand)). The toe pinch technique was utilized to evaluate the degree of sedation. Ultra-light NiTi closed coil springs (Dentos Inc., Daegu, South Korea) were used for tooth movement, applying 5 g force for LF and 10 g for OF groups.³ Rats were monitored until recovery from anesthesia.

Mechanical High-frequency Vibration Application

Isoflurane (Isoflurane-USP, Piramal Critical Care, Inc., Bethlehem, USA) was administered during vibration application. An electric toothbrush (D12 Pro

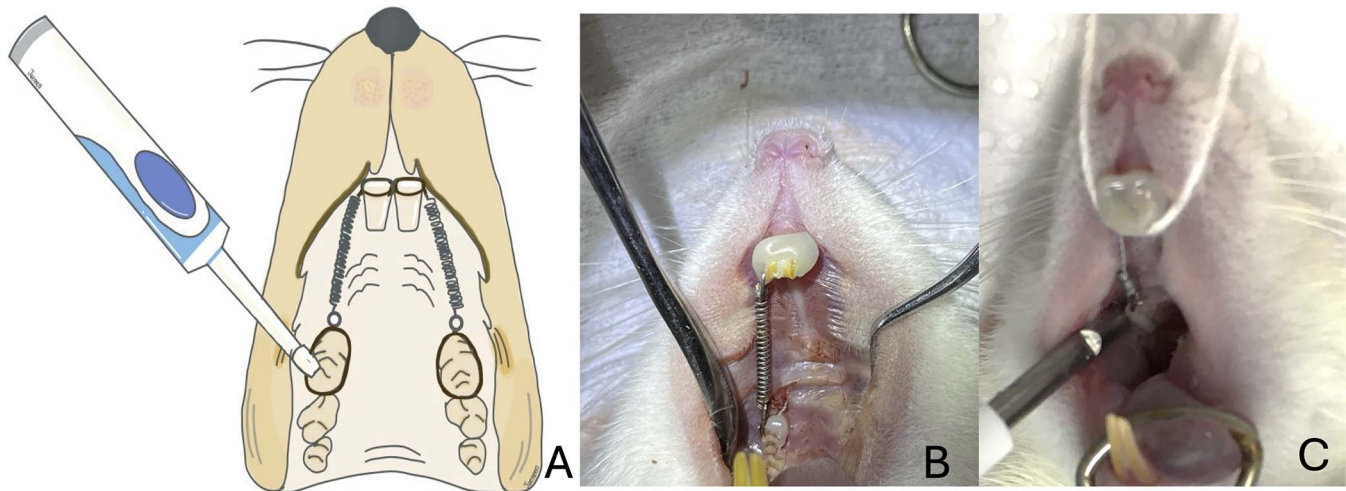


Figure 2. Illustration of the experiment. (A) schematic of the orthodontic appliances (ultra-light NiTi closed coil springs) and vibration applicator; (B) image of the ultra-light NiTi closed coil springs attached at the maxillary first molar; (C) HFV applied to the occlusal surface (distolingual surface) of the maxillary first molar. NiTi indicates nickel-titanium; HFV, high-frequency vibration.

White, Oral-B®, USA) with removed bristle head was used for 5 minutes daily in HFV, LF/HFV, and OF/HFV groups. The tip was placed perpendicular to the distolingual surface of the maxillary first molar, with a self-cured acrylic reference landmark ensuring consistent placement (Figure 2).

Animals were euthanized with pentobarbital sodium (800 mg/kg) at the end of Day 1, 7, 14, and 21. Maxillae were removed within 10 minutes and fixed in 4% paraformaldehyde.

Immunohistomorphometry Analysis

The maxilla bone specimens were decalcified for histologic processing. Briefly, the specimens were demineralized for 6 weeks in 10% ethylene diamine tetra-acetate at 4°C. Then, the specimens were subjected to a dehydration sequence using graded ethanol series and, finally, embedded in paraffin for histological investigation. Five- μ m horizontal sections were performed parallel to the first molar long axis (Anglia Scientific 0325 sliding microtome). These sections were then mounted on a surface coated with 3-aminopropyltriethoxysilane.

The osteoclast numbers of TRAP-positive, multinucleated (≥ 3 nuclei) cells visualized on the mesial root of the maxillary first molar were manually counted under a light microscope.²¹ The TRAP staining method was conducted following the protocol described in a previous study.²² Briefly, the slides were first deparaffinized and rehydrated through graded ethanol to distilled water. The slides were then incubated in a TRAP staining solution mix (Takara Bio, Shiga, Japan) at 37°C for 4 hours and subsequently rinsed in distilled water. Next, the slides were counter-stained with 0.08% Fast

Green for 10 minutes. The specificity of the staining was confirmed using a negative control medium. The number of TRAP-stained cells was identified based on their presence on the bone surface or their location within Howship's lacunae on the root at high magnification ($\times 100$) (Figure 3).

Micro-Computed Tomography

The samples were scanned using a Scanco μ CT 35 (Scanco Medical, Bassersdorf, Switzerland) with settings of 70 kVp and 114 μ A. The image voxel size was 10 μ m, and the exposure duration was 256 ms. Scanning was performed with the orientation parallel to the occlusal surface of the maxillary second and third molars. The maxillary first molar root samples were contoured in serial horizontal sections, beginning from the

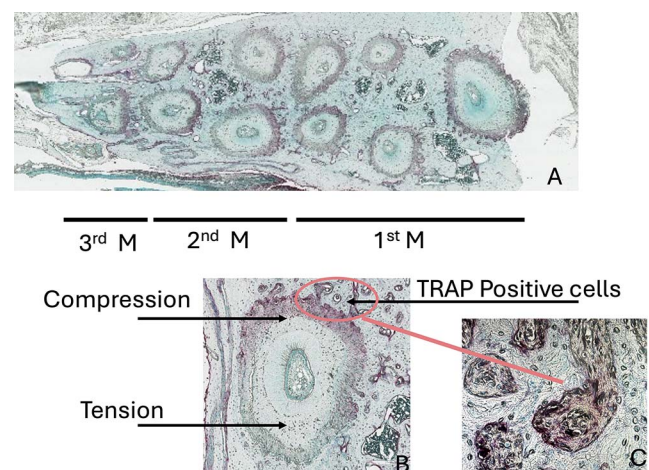


Figure 3. TRAP Positive cell, (A) axial view of the maxillary molar; (B) mesial root of the maxillary first molar; (C) TRAP positive cell.

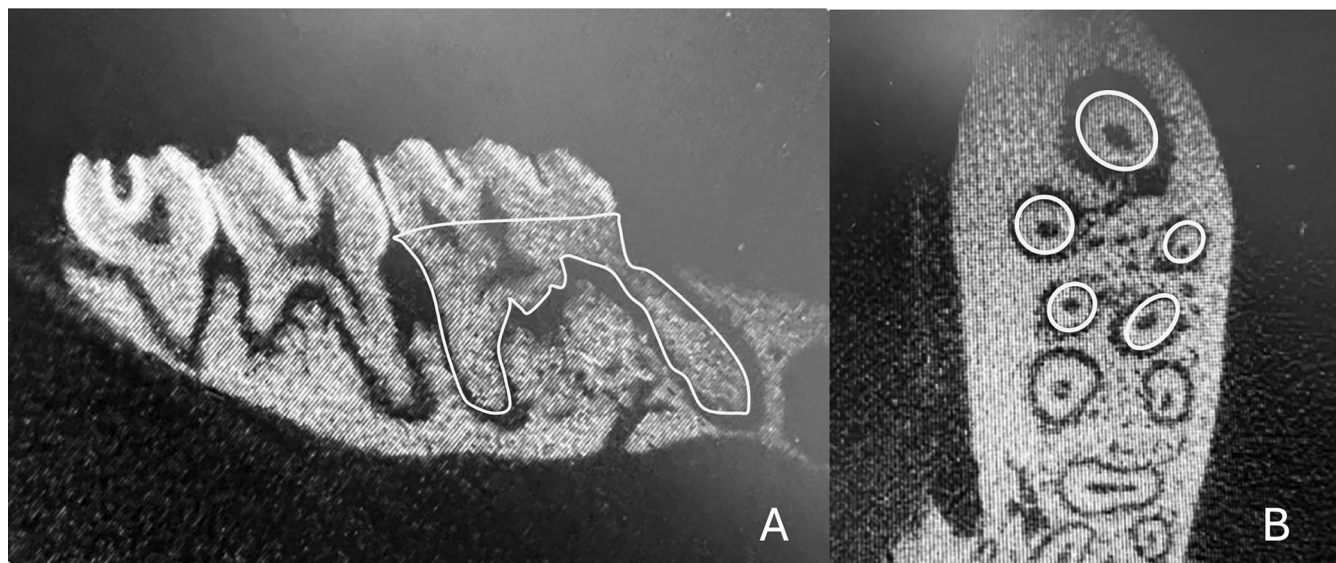


Figure 4. Micro-CT images of the area of the upper first molar of the rat. (A) sagittal view of the tooth, yellow line indicates the area of interest; (B) axial (top-down) view of the visible and the root areas marked with yellow circles. CT indicates computed tomography.

cementoenamel junction (CEJ) to the apex of each root. The parameter generated for analysis was root volume (RV; mm^3), which represented the total volume of the five roots of the maxillary first molar (Figure. 4).

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 29 (SPSS, Chicago, IL, USA). Data normality was assessed with the Shapiro-Wilk test, and results were expressed as mean and standard deviation (SD). The significance of the differences among the six groups at each time point was tested by one-way analysis of variance (ANOVA) and post-hoc Tukey tests at $P < .05$. A three-way ANOVA and post-hoc Bonferroni test were used to evaluate interaction effects of time, force and vibration on osteoclast numbers and root resorption. The significance level was adjusted to $P < .017$ to assess multiple comparisons and prevent Type I error associated with the Bonferroni correction. Reliability of the quantification was assessed by randomly selecting 30% of the samples and remeasuring after a 1-month interval. Intra-observer reliability showed excellent agreement with intraclass coefficient values of 0.88 for osteoclast numbers and 0.86 for root volume.

RESULTS

Rat body weight was monitored after orthodontic appliance placement. Slight weight loss was observed in the initial days; however, weight subsequently increased throughout the study, with no significant differences among the groups. This indicated that the appliances did not negatively affect feeding behavior.

All appliances performed as intended, delivering consistent forces throughout the study.

Osteoclast Numbers

The TRAP-positive multinucleated cells observed on the mesial root of the first maxillary molar were identified as osteoclasts (Figure 3). Osteoclast numbers increased over time in all experimental groups, except for the control group. The OF/HFV group consistently exhibited the highest osteoclast numbers at all time points, followed by the OF, LF/HFV, LF, and control groups. On day 21, osteoclast numbers peaked in the OF/HFV group (15.25 ± 0.85), followed by the OF group (13.75 ± 0.48) and the LF/HFV group (10.00 ± 0.41). Although the LF/HFV group demonstrated osteoclast activity, it consistently induced lower osteoclast numbers compared to the OF group across all time points ($P < .0001$), as shown in Figure 5 and Table 1.

However, the three-way interaction approached, but did not show significance on osteoclast numbers ($F = 3.857$, $P = .061$).

Root Volume (RV)

The root volume of all roots of the first maxillary molar were analyzed using Micro-CT (Figure 6 and Table 2). Root volume decreased over time in all experimental groups, except for the control and HFV groups, which showed no significant changes across time points. On day 1, there were no significant differences in root volume among all groups ($P = .800$). On day 7, significant reductions in root volume were observed in the OF/HFV ($8.22 \pm 0.24 \text{ mm}^3$), OF ($8.36 \pm$

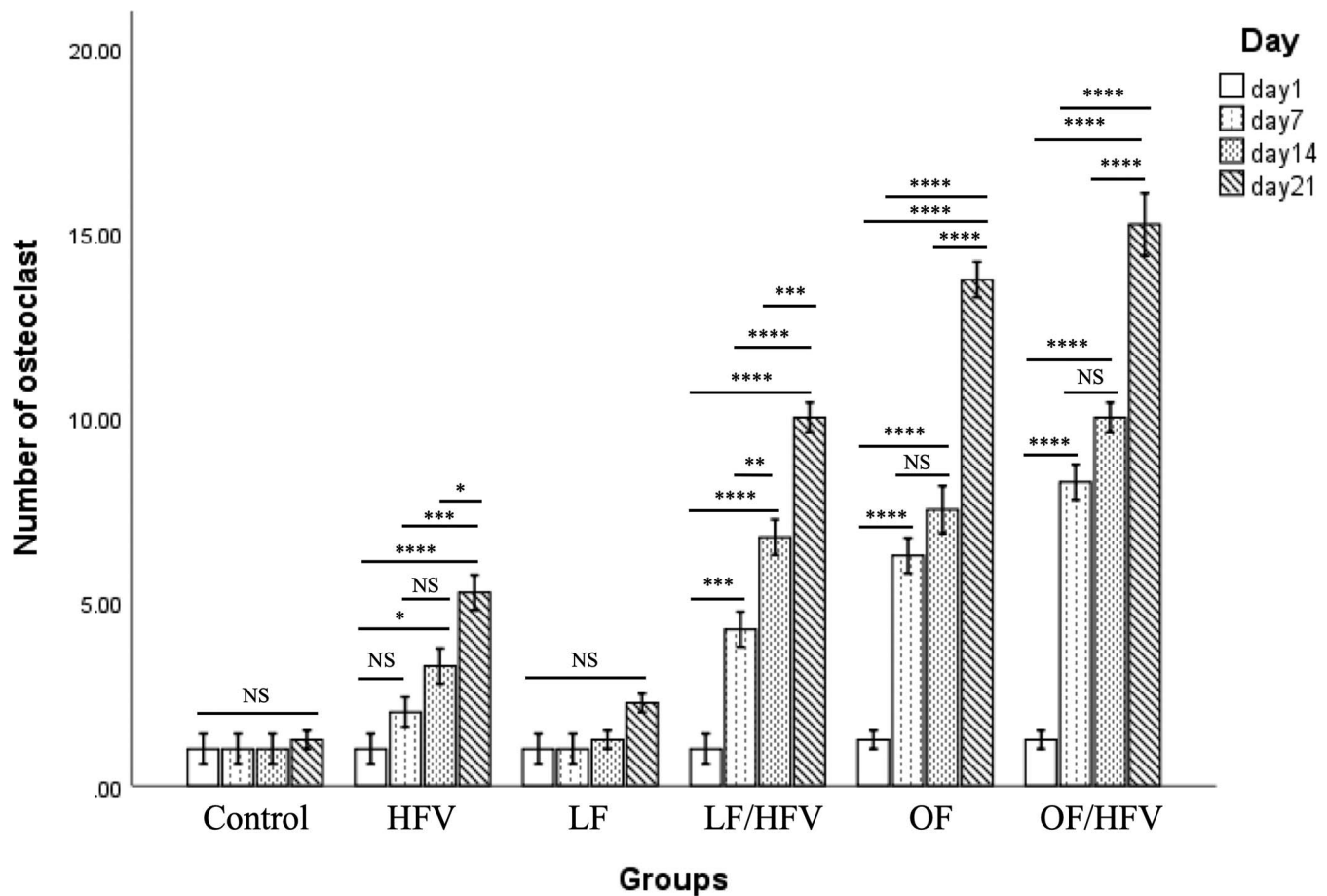


Figure 5. The mean $\pm$ SD of osteoclast numbers in the intervention groups on Day 1, 7, 14, and 21; comparison within groups using one-way ANOVA (* $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$). SD indicates standard deviation; ANOVA, analysis of variance.

0.07 mm³), and LF/HFV (8.63 $\pm$ 0.09 mm³) groups compared to the Control, HFV, and LF groups ($P < .0001$). On day 14, root volume reduction became more pronounced in the OF/HFV group (6.28 $\pm$ 0.14 mm³), followed by the OF group (6.68 $\pm$ 0.24 mm³) and the LF/HFV group (7.75 $\pm$ 0.18 mm³). Significant differences were observed between these

groups and the Control, HFV, and LF groups ($P < .0001$). Importantly, the LF/HFV group consistently exhibited less root volume reduction than the OF group on both Day 7 and Day 14.

A three-way ANOVA indicated a significant interaction between time and force, as well as time and vibration, on osteoclast numbers was not significant (Table 3).

Table 1. Osteoclast Numbers on Day 1, 7, 14, and 21, With Statistical Significance Determined by ANOVA and Tukey's Post Hoc Test ($P < .05$)

Group/Day	Osteoclast Cell Per mm ²				P Value
	Day 1	Day 7	Day 14	Day 21	
Control	1.00 $\pm$ 0.82	1.00 $\pm$ 0.41†,Ⓞ,¶	1.00 $\pm$ 0.58‡,†,Ⓞ,¶	1.25 $\pm$ 0.48‡,†,Ⓞ,¶	.919
HFV	1.00 $\pm$ 0.82	2.00 $\pm$ 0.41†,Ⓞ,¶	3.25 $\pm$ 0.48Ⓞ,†,Ⓞ,¶	5.25 $\pm$ 0.48Ⓞ,†,Ⓞ,¶	.002
LF	1.00 $\pm$ 0.00	1.00 $\pm$ 0.41†,Ⓞ,¶	1.25 $\pm$ 0.25†,Ⓞ,¶	2.25 $\pm$ 0.25‡,†,Ⓞ,¶	.044
LF/HFV	1.00 $\pm$ 0.82	4.25 $\pm$ 0.48Ⓞ,‡,Ⓞ,¶	6.75 $\pm$ 0.48Ⓞ,‡,Ⓞ,¶	10.00 $\pm$ 0.41Ⓞ,‡,Ⓞ,¶	<.0001
OF	1.00 $\pm$ 1.15	6.25 $\pm$ 0.48Ⓞ,‡,Ⓞ,¶	7.50 $\pm$ 0.65Ⓞ,‡,Ⓞ,¶	13.75 $\pm$ 0.48Ⓞ,‡,Ⓞ,¶	<.0001
OF/HFV	1.00 $\pm$ 0.82	8.25 $\pm$ 0.48Ⓞ,‡,Ⓞ,¶	10.00 $\pm$ 0.41Ⓞ,‡,Ⓞ,¶	15.25 $\pm$ 0.85Ⓞ,‡,Ⓞ,¶	<.0001
P value	1.00	<.0001	<.0001	<.0001	

^a ANOVA indicates analysis of variance; HFV, high-frequency vibration; LF, light force; OF, optimal force.

^b Ⓞ significant difference between groups compared to the control group; ‡ significant difference between groups compared to the HFV group; Ⓞ significant differences between groups compared to the LF group; † significant difference between groups compared to the LF/HFV group; Ⓞ significant difference between groups compared to the OF group; ¶ significant difference between groups compared to the OF/HFV group.

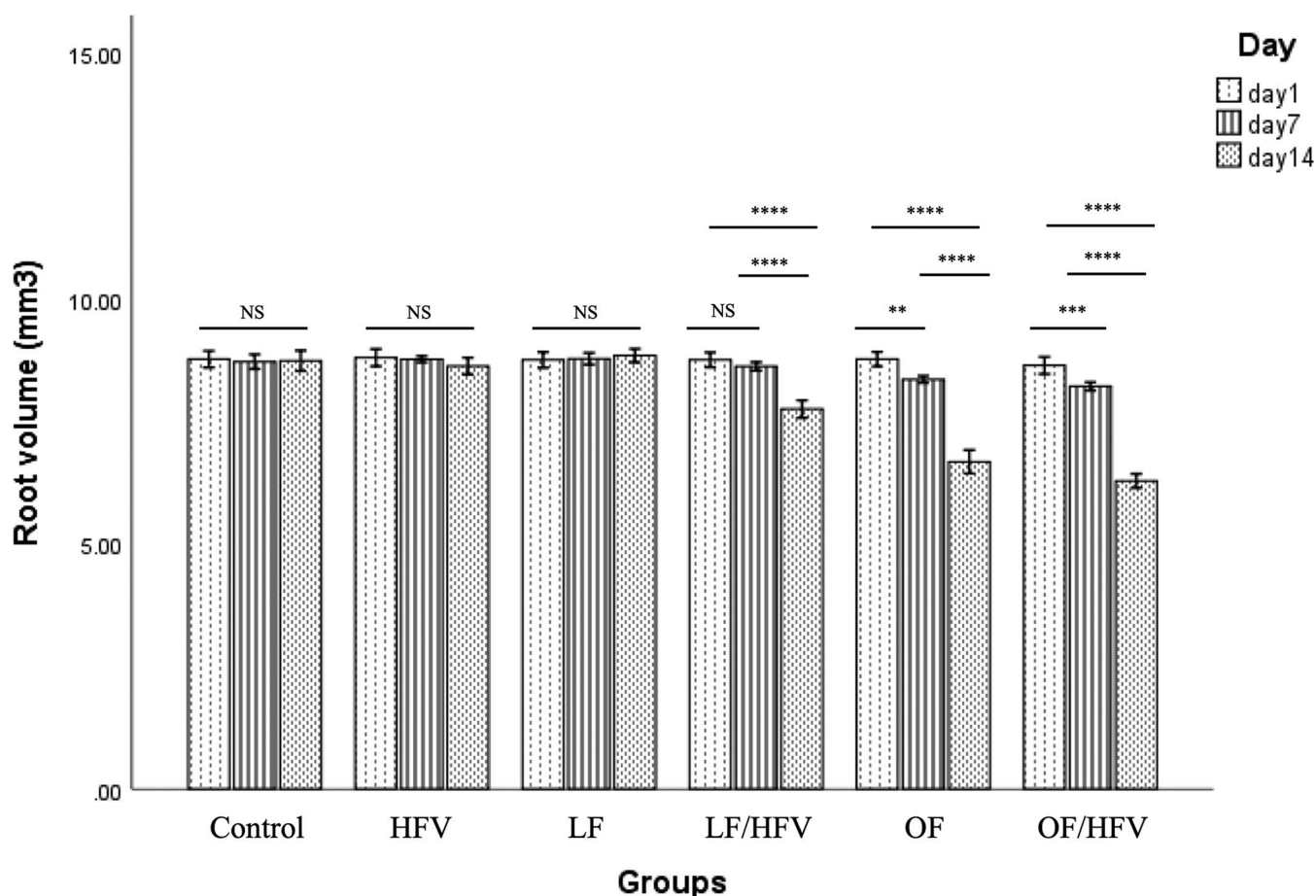


Figure 6. The mean $\pm$ SD of root volume (mm^3) in the intervention groups on Day 1, 7, and 14; comparison within groups using one-way ANOVA (* $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$).

However, these interactions significantly impacted reduction of root volume (Table 4).

DISCUSSION

Alveolar bone surrounding the tooth root undergoes resorption and formation during the orthodontic tooth

movement. On the compression side, mechanical force induces a period of hyalinization, during which aseptic necrosis occurs.^{22,23} Osteoclasts are subsequently formed to resorb the bone, facilitating tooth movement. However, this process can lead to external root resorption, which arises from reduction of the hyaline zone, and increased activity of osteoclasts and

Table 2. Root Volume Measurements on Day 1, 7, and 14, With Statistical Significance Determined by ANOVA and Tukey's Post Hoc Test ($P < .05$)

Group/Day	Root Volume (mm^3)			P Value
	Day 1	Day 7	Day 14	
Control	8.77 $\pm$ 0.17	8.72 $\pm$ 0.15 ϕ , $\parallel$	8.74 $\pm$ 0.21 $\dagger$, ϕ , $\parallel$	.916
HFV	8.80 $\pm$ 0.18	8.77 $\pm$ 0.09 ϕ , $\parallel$	8.63 $\pm$ 0.17 $\dagger$, ϕ , $\parallel$	.262
LF	8.76 $\pm$ 0.16	8.78 $\pm$ 0.12 ϕ , $\parallel$	8.84 $\pm$ 0.14 $\dagger$, ϕ , $\parallel$	.670
LF/HFV	8.76 $\pm$ 0.14	8.63 $\pm$ 0.09 ϕ , $\parallel$	7.75 $\pm$ 0.18 ϕ , Δ , ω , ϕ , $\parallel$	<.0001
OF	8.77 $\pm$ 0.15	8.36 $\pm$ 0.07 ϕ , Δ , ω , $\dagger$	6.68 $\pm$ 0.24 ϕ , Δ , ω , $\dagger$	<.0001
OF/HFV	8.64 $\pm$ 0.18	8.22 $\pm$ 0.24 ϕ , Δ , ω , $\dagger$	6.28 $\pm$ 0.14 ϕ , Δ , ω , $\dagger$	<.0001
P-value	.800	<.0001	<.0001	

^a ANOVA indicates analysis of variance; HFV, high-frequency vibration; LF, light force; OF, optimal force.

^b ϕ significant difference between groups compared to the control group; Δ significant difference between groups compared to the HFV group; ω significant differences between groups compared to the LF group; $\dagger$ significant difference between groups compared to the LF/HFV group; ϕ significant difference between groups compared to the OF group; $\parallel$ significant difference between groups compared to the OF/HFV group.

Table 3. Results of Three-Way ANOVA Summarizing the Main and Interaction Effects of Time, Vibration, and Force on Osteoclast Numbers (at a Significance Level of $*P = .017$)

Source	df	Mean Square	F Value	P Value
Corrected Model	12	60.643	207.918	.000*
Intercept	1	1200.500	14116.000	.000*
Time: day 1, 7, 14, 21	3	32.000	409.714	.000*
Vibration: yes or no	1	120.125	411.857	.000*
Force: light or optimal	1	242.000	829.714	.000*
Time * Vibration	3	6.125	71.000	.000*
Time * Force	3	2.000	76.857	.015*
Vibrate * Force	1	21.125	72.429	.000*
Time * Vibration * Force	3	1.125	3.857	.061
Error	48	.292		
Total	60			

cementoclasts. A recent study³ proposed the use of light force combined with HFV to achieve orthodontic tooth movement comparable to that achieved using optimal force. However, the cellular response of osteoclasts to the root is still unclear. The current animal study evaluated the effect of combining light force and optimal force with HFV on osteoclast numbers and root volume. The results demonstrated a significant increase in osteoclast numbers in the HFV and combination groups, suggesting that HFV enhanced osteoclast activity. However, root volume analysis showed no significant difference between the HFV and LF groups compared to the control group. This indicated that neither HFV alone nor light force significantly contributed to root resorption.³ This finding suggested that, although these conditions do not cause root damage, they may also be insufficient to accelerate tooth movement effectively in orthodontic treatment.

Osteoclast numbers significantly increased in the HFV group, demonstrating that HFV alone can stimulate osteoclast activity, even in the absence of orthodontic force. This indicated the potential role of HFV in preparing the cellular environment for enhanced osteoclast numbers.^{8,9} A combination of HFV and orthodontic force, in the LF/HFV and OF/HFV groups, exhibited significantly higher osteoclast numbers compared to their respective nonvibration groups (LF and OF groups); this demonstrated the effect of HFV in enhancing osteoclast activity when combined with orthodontic force (Table 3). Although previous studies showed that various frequencies of vibration could enhance the efficiency of tooth movement,^{3,5-9} some studies indicated that lower frequency vibration did not accelerate tooth movement.^{24,25} The current findings suggested that HFV alone can stimulate osteoclast activity,²⁶ potentially creating the cellular environment for accelerated tooth movement.

Table 4. Results of Three-Way ANOVA Summarizing the Main and Interaction Effects of Time, Vibration, and Force on Root Volume (at a Significance Level of $*P = .017$)

Source	df	Mean Square	F Value	P Value
Corrected Model	11	3.010	136.691	.000*
Intercept	1	3232.506	146811.428	.000*
Time: Day 1, 7, 14	2	8.204	372.611	.000*
Vibration: yes or no	1	1.217	55.285	.000*
Force: light or optimal	1	6.928	314.661	.000*
Time * Force	2	3.477	157.927	.000*
Time * Vibrate	2	0.544	24.726	.000*
Vibration * Force	1	0.114	5.164	.029
Time * Vibration * Force	2	0.198	8.981	.001*
Error	36	0.022		
Total	48			

Relative to force magnitude, osteoclast numbers were highest in the OF/HFV group, followed by the OF group, which showed the greater impact of optimal force on osteoclast stimulation. However, the LF/HFV group showed significantly increased osteoclast numbers compared to the LF group, indicating that HFV could effectively enhance osteoclast activity even under light force conditions.

For the treatment period, osteoclast numbers increased progressively over time (Day 7, 14, to Day 21), with the highest numbers observed in groups combining HFV and orthodontic forces (LF/HFV and OF/HFV). The interaction effect of time and frequency on osteoclast numbers was significant (Table 3). This suggested a time-dependent enhancement of osteoclast numbers by HFV. These findings emphasized the role of HFV in amplifying osteoclast numbers, particularly when combined with orthodontic forces, which facilitate efficient bone remodeling and tooth movement during orthodontic treatment.⁵

Root volume analysis revealed no significant difference between the HFV and LF groups compared to the control, suggesting that neither HFV alone nor LF cause root resorption. In contrast, significant root volume reduction was observed in the OF and OF/HFV groups, with the greatest reduction seen in the OF/HFV group. Three-way ANOVA confirmed that the combination of vibration and force further contributed to root volume reduction, with a significant interaction between time and force. The significant difference in osteoclast numbers and reduced root volume loss in the LF/HFV group suggested that HFV improves light force efficiency in orthodontic treatment, offering effective tooth movement with less root resorption compared to the OF group.³ This highlighted the potential of combining light forces with HFV to optimize treatment, while minimizing root damage.

The specific advantages of this study were the assessment of cellular-level changes and histologic

analysis of tissue responses that could not be processed in clinical human studies.^{27,28} However, a limitation of this study was that it was focused on short-term effects. The interaction effect of vibration, force, and time on osteoclast numbers was remarkable only for its effect on root volume loss. Future studies should include clinical trials to validate these findings in human subjects and optimize these techniques for safe and effective orthodontic treatment. Additionally, investigating the long-term effects of combined orthodontic force and vibration on tooth movement and root resorption would provide valuable insights for clinical application.

CONCLUSIONS

This study highlighted the potential of HFV to enhance osteoclast numbers and improve the efficiency of orthodontic forces, particularly when combined with light force. By balancing effective tooth movement with minimized root resorption, HFV may serve as a valuable adjunct in orthodontic treatment, paving the way for safer and more efficient clinical practices.

- High-frequency vibration increased osteoclast numbers without causing root volume loss.
- The combination of orthodontic forces and HFV significantly increased osteoclast numbers, especially with light force.
- HFV combined with optimal forces resulted in greater root volume loss compared to its combination with light force.
- The use of light force combined with HFV warrants consideration for its potential to minimize root volume loss while still promoting increased osteoclast activity, contributing to safer orthodontic treatment outcomes.

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