

Light orthodontic force with high-frequency vibration accelerates tooth movement with minimal root resorption in rats

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Abstract

Objectives

To determine and compare the effects of high-frequency mechanical vibration (HFV) with light force and optimal force on the tooth movement and root resorption in rat model.

Materials and Methods

Seventy-two sites in 36 male Wistar rats were randomly assigned using a split-mouth design to control (no force/no vibration) or experimental groups: HFV (125 Hz), light force (5 g), optimal force (10 g), light force with HFV, and optimal force with HFV for 14 and 21 days. The amount of tooth movement, three-dimensional root volume and root resorption area were assessed by micro-computed tomography and histomorphometric analysis.

Results

Adjunction of HFV with light force significantly increased the amount of tooth movement by 1.8-fold ($p = 0.01$), 2.0-fold ($p = 0.01$) at days 14 and 21 respectively. The HFV combine with optimal force significantly increased the amount of tooth movement by 2.1-fold ($p = 0.01$), 2.2-fold ($p = 0.01$) at days 14 and 21 respectively. The root volume in control (distobuccal root (DB): $0.60 \pm 0.19 \text{ mm}^3$, distopalatal root (DPa): $0.60 \pm 0.07 \text{ mm}^3$) and HFV (DB: $0.60 \pm 0.08 \text{ mm}^3$, DPa: $0.59 \pm 0.11 \text{ mm}^3$) were not different from the other experimental group (range from $0.44 \pm 0.05 \text{ mm}^3$ to $0.60 \pm 0.1 \text{ mm}^3$) with the lowest volume in optimal force group.

Conclusions

Adjunction of HFV with orthodontic force significantly increased tooth movement without causing root resorption

Clinical Relevance

Using light force with HFV could help to identify alternative treatment option to reduce the risk of root resorption.

Introduction

Conventional orthodontic treatment normally takes two to three years. Prolonged orthodontic treatment impairs patient compliance and increases the risk of gingival inflammation, dental caries, pulpal

reactions, and root resorption, all of which can have long-term, burdensome consequences [1, 2]. Several accelerated tooth movement approaches have been proposed to alleviate these issues, and can be categorized as biological, surgical, or physical [3]. The biological methods aim to modify paradental tissue remodeling by injecting various key molecules into the periodontium; however, multiple painful injections are required and this approach increases the risk of root resorption [4]. Surgical methods are based on the regional acceleratory phenomenon (RAP) theory and aim to increase tissue remodeling and decrease bone density. The drawbacks of this method are surgical complications such as pain, swelling, and loss of tooth vitality. Lastly, physical approaches include low-level laser illumination or vibration. While their efficacy is still controversial, physical approaches offer the unique advantages of being non-invasive, painless, and complication-free.

Vibration has two main effects on bone remodeling, as it can enhance both osteogenesis and osteoclastogenesis. Vibration is employed as a safe medical treatment to enhance osteogenesis in order to prevent bone loss and increase bone density in the long bones [5, 6]. However, the ability of vibration to enhance osteoclastogenesis and accelerate tooth movement during orthodontic treatment is still controversial [7]. At the cellular level, high-frequency vibration increases secretion of inflammatory cytokines and the numbers of osteoclasts and has been shown to accelerate tooth movement in animal models [8–10]. However, low-frequency vibration does not affect tooth movement in animal models [11]. Moreover, several *in vitro* studies have demonstrated that mechanical vibration promotes the expression of PGE2, RANKL, interleukin-6 (IL-6), and IL-8 in compressed human periodontal ligament cells [12–14]. In addition to, a previous clinical study demonstrated that light force with HFV can increase secretion of IL-1 β in GCF and accelerate tooth movement [15].

Root resorption is a serious problem that can occur during orthodontic treatment, especially when exerting a heavy force [16, 17]. The optimal level of clinical force for maximizing orthodontic tooth movement while avoiding iatrogenic tissue damage and patient discomfort has been extensively researched [18–21]. According to the systematic review, the inability to quantify the distribution of stress and strain in the periodontal ligament (PDL), the lack of control over the kind of tooth movement, and the considerable inter-individual variation, no ideal magnitude of force could be identified in the clinic [18]. Despite the fact that light force may lead to a slower rate of tooth movement than optimal force, previous study showed that it may help to prevent complications including hyalinization and root resorption [22, 23].

At lower force magnitudes, there is a positive correlation between the force magnitude and the rate of tooth movement. However, increasing the magnitude above the optimal force level does not further accelerate tooth movement [24]. The association between tooth movement and the force magnitude in rats was shown to be linear up to a point (10 grams), beyond which tooth movement did not further increase [25]. In addition, a range of magnitudes of light force below 10 grams (1.2, 3.6, 6.5, and 10 grams) were shown to promote bone remodeling via frontal bone resorption. However, a force magnitude of 1.2 grams did not effectively promote tooth movement, and led to significantly less tooth movement

than other magnitudes of force [23]. Thus, for this investigation, the optimal force was defined as 10 grams, while light force was defined as 5 grams.

To investigate an effective approach for orthodontic treatment with a shorter treatment duration and less complications, the high-frequency mechanical vibration (HFV) was considered as the adjunctive device for accelerated tooth movement. Then, the aim of the present study was to determine and compare the effects of high-frequency mechanical vibration (HFV) with light force and optimal force on the tooth movement and root resorption in rat model.

Materials And Methods

Experimental animals

The Animal Ethics Committee of Prince of Songkla University gave their approval for this research (2562-05-061). The animals were cared for and handled according to the guide for care and use of laboratory animals [26]. Thirty-six adults male Wistar rats aged between 10–12 weeks (weight, 300–400 g) were used in this split-mouth experiment. After seven days of acclimatization, the animals were randomly assigned to two observation periods (day 14, day 21). Then, the left and right sides of the maxilla were randomly assigned to the six interventions: control, HFV, light force, optimal force, light force with HFV, and optimal force with HFV, with six samples (six sites of the maxilla) for each intervention (Fig. 1).

Orthodontic tooth movement

A mixture of 90 mg/kg ketamine (Calypsol, Gedeon Richter Ltd., Budapest, Hungary) and 10 mg/kg xylazine (Xylavet, Thai Meiji Pharmaceutical Ltd., Bangkok, Thailand) was intraperitoneally injected before placement of the NiTi closed coil spring (Dentos Inc., Daegu, South Korea). Throughout the experimental period, the springs delivered a constant force of 5 g or 10 g for light and optimal force, respectively. The schematic of the study design is shown in Fig. 1.

Mechanical high frequency vibration

In the HFV, light force with HFV, and optimal force with HFV groups, HFV was applied on the occlusal surface of the maxillary first molar in a perpendicular direction for 5 min/day using an electrical toothbrush D12ProWhite (Oral b®, USA) without the bristle brush while the rats were under general anesthesia by inhalation of 2% isoflurane. According to the operating specifications, the D12ProWhite operates at approximately 7600 RPM, which corresponds to 125 Hz.

Micro-computed tomography

The rats were sacrificed with an overdose of pentobarbitone sodium (800 mg/kg). The maxillae were removed within 10 min of euthanasia, placed in 4% paraformaldehyde within 12 h [27], and scanned and reconstructed using a Scanco MicroCT μ CT 35 system (Scanco Medical, Bassersdorf, Switzerland). After

calibration, the samples were scanned parallel to the occlusal surface of the maxillary second and third molars at 70 kVP and 114 μ A with an exposure time of 256 milliseconds and voxel size of 10 μ m.

Measurement of tooth movement

Tooth movement was measured in the transverse dimension using the built-in software of the micro-CT scanner. The heights of the contours of the first and second molars were registered in the sagittal dimension to provide a reference plane in the vertical plane, then the distance between first and second maxillary molars was measured in the transverse dimension at the most distal point of the maxillary first molar. Representative data for the tooth movement measurements based on micro-CT in each group are shown in Fig. 2.

3D Image construction and root volume measurement

The samples of maxillary first molar root from days 21 were contoured in serial horizontal sections, beginning from the point where all roots completely split and finishing at the apex of each root. The contour components were exported as three-dimensional objects in stereolithography file format. Volumetric examination of the distobuccal (DB) and distopalatal (DPa) roots was performed to compare the six intervention groups at 21 days using Geomagic® Control X™ software (Cx Geomagic® Control X™ Luxembourg), as shown in Fig. 3.

Histological analysis of the percentage of root resorption

After the micro-CT scanning, all maxillae were demineralized for 4 weeks in 10% ethylenediaminetetraacetic acid at room temperature, then 3- μ m horizontal sections were collected at 150- μ m intervals and stained with hematoxylin and eosin for histomorphometric analysis. Six serial sections of the distobuccal (DB) and distopalatal (DPa) roots of the maxillary first molar were examined from the slice under the bifurcation level to the apical level using a bright field microscope.

The resorption area was defined as the enclosed area of resorption and the borderline that connected the margins of each discontinuity of root resorption. The percentage of root resorption was calculated as the resorption area per total root area without root canal, as evaluated on images obtained using a Aperio ImageScope (Leica Biosystems Inc., Deer Park, IL, United States (Fig. 4). The percentages of root resorption in six sections were averaged for each sample and compared among the six intervention groups at 21 days.

Statistical analysis

The data were present as mean \pm SD. All samples were remeasured after a 1-month interval to assess intra-observer reliability using intraclass correlation coefficients. All data were analyzed using SPSS version 26 (SPSS, Chicago, IL, USA). The normal distributions of the data were confirmed using the Shapiro-Wilk test. The significance of the differences among the six intervention groups was tested using

one-way analysis of variance (ANOVA) followed by the post hoc Tukey's test; $P < 0.05$ was considered statistically significant.

Results

Weight was measured daily after orthodontic device implantation to assess the health of the rats. The weight of the animals slightly reduced in the first days after implantation, but then continually increased throughout the rest of the observation period. No significant differences in weight were observed between groups. All appliances remained undamaged and delivered exact, consistent forces throughout the study.

The intra-class correlation coefficients ranged between 0.75 and 0.9, thus the measurements were deemed to have an acceptable level of reliability. All data was normally distributed, as confirmed by the Shapiro-Wilk test.

Amount of tooth movement

The average amount of tooth movement by light force were 0.13 ± 0.01 mm, 0.18 ± 0.04 mm, and optimal force were 0.18 ± 0.04 mm, 0.26 ± 0.02 mm at days 14 and 21 respectively ($p = 0.04$). The adjunctive of HFV with the orthodontic force significantly increased the tooth movement using light force by 1.8-fold ($p = 0.01$), 2-fold ($p = 0.01$), using optimal force by 2.1-fold ($p = 0.01$), 2.2-fold ($p = 0.01$) at days 14 and 21, respectively. The combination of light force with HFV led to greater amount of tooth movement (0.22 ± 0.02 mm ($p = 0.7$), 0.35 ± 0.04 mm ($p = 0.01$) compared with optimal force alone at days 14 and 21, respectively (Fig. 5, 6)

Root volume change

The root volume in control (DB: 0.60 ± 0.19 mm³, DPa: 0.60 ± 0.07 mm³) and HFV (DB: 0.60 ± 0.08 mm³, DPa: 0.59 ± 0.11 mm³) were not different from the others experimental group. The light and optimal force showed -0.13% (DB) ($p = 0.93$), -0.06% (DPa) ($p = 0.97$), and -0.27% (DB) ($p = 0.36$), -0.12% (DPa) ($p = 0.35$) change from the control. Adjunction of HFV to light force led to limit root volume: 0.14% ($p = 0.07$) and 0.004% ($p = 0.07$) on DB, and DPa respectively. The distobuccal root in the optimal force group showed the lowest root volume (0.44 ± 0.05 mm³, -0.27%). However, the difference was not statistically significant (Fig. 7, 8a).

Root resorption

Resorption of the DB and DPa roots of the maxillary first molar were evaluated by histomorphometric analysis on day 21. HFV had no effect on root resorption since root resorption lacunae in control (DB: $0.38 \pm 0.01\%$, DPa: $0.29 \pm 0.16\%$) and HFV groups (DB: $0.41 \pm 0.04\%$, DPa: $0.19 \pm 0.02\%$) were both hardly evident. Root resorption was not significantly different in the light force (DB: $0.37 \pm 0.04\%$ $p = 0.46$, DPa: $0.41 \pm 0.03\%$ $p = 0.46$) and light force with HFV (DB: $0.43 \pm 0.01\%$, $p = 0.45$, DPa: $0.26 \pm 0.012\%$, $p = 0.45$) compared to control. However, the increased of root resorption of DB and DPa root were found in optimal force ($1.79 \pm 0.14\%$, 4.9-fold, $p = 0.01$, $1.06 \pm 0.09\%$, 4.1-fold, $p = 0.50$) and optimal force with HFV ($1.82 \pm$

0.26%, 4.84-fold, $p = 0.02$, $1.40 \pm 0.2\%$, 4.85-fold, $p = 0.11$) respectively, with significantly different on DB root.

Discussion

The present study demonstrated that the use of HFV with either light or optimal force increased the tooth movement without causing root resorption in rat model. Furthermore, optimal force with HFV was the mechanical stimuli that lead to maximal tooth movement with minimal root resorption. While light force with HFV resulted in slower movement than optimal force with HFV, light force with HFV may eventually result in a greater amount of tooth movement without root resorption compared to optimal force

Both the rate of tooth movement and risk of complications must be considered in order to achieve effective orthodontic treatment. Root resorption is the most common orthodontic complication and is irreversible. Therefore, investigation of techniques to accelerate tooth movement without root resorption could benefit orthodontic treatment. Vibration appliances can be easily purchased and used at home on a daily basis. However, the effect of vibration on tooth movement has become a source of debate and the optimal mechanical vibration protocol to accelerate tooth movement is still not known. Low-frequency vibration has no impact on tooth movement in animals [11]. In contrast, several animal studies found HFV had a favorable effect on tooth movement, and also stimulated osteoclastogenesis and increased the levels of inflammatory mediators [8–10]. Previous studies in rat model compared the effects of 58–278 Hz on tooth movement, with 120 Hz leading to the greatest amount of tooth movement [9, 10]. In addition, the duration of exposure was not the main factor that increased tooth movement; thus, the present study used the effective shortest exposure time (5 minutes) [9]. The results of this rat model provide conclusive evidence that the adjunction of HFV (125 Hz, 5 min/day) with an orthodontic force accelerates tooth movement without evidence of root resorption. Consistent with previous findings [10, 28], this study indicates that mechanical vibration does not affect the roots. In prior study, utilizing 70 Hz with 15 grams force in a rat model did not result in variations in root resorption area when compared to applying force alone [10]. In another study, application of the low frequency vibration (5, 10, 20 Hz) with 10 g force slightly, but not significantly, decreased root resorption during orthodontic tooth movement [28].

Optimal force is a theoretical ideal for orthodontic treatment that provides the fastest tooth movement [24]. However, the actual optimal force magnitudes for every type of tooth movement are still unknown [18]. Under clinical circumstances, it is almost impossible to control the optimal stress and strain in every area of the periodontal ligament for specific types of tooth movement. Light force may be an excellent choice for safe orthodontic treatment to avoid root resorption caused by excessive stress and strain in the PDL. A combination of HFV with light force could provide several benefits in orthodontic treatment, in terms of increasing the amount of tooth movement and improving safety. Most previous studies focused on using HFV to enhance tooth movement in combination with optimal or greater force magnitudes [9, 10]. Then, this study is the first to reveal that mechanical vibration at 125 Hz combined with light force can enhance tooth movement without causing root resorption.

The histomorphometric analysis provides a high-quality resolution of area of root resorption; however, it may not reveal all areas of resorption around the root, since root resorption can occur in three dimensions due to lateral root resorption and external apical root resorption (EARR). Therefore, this study evaluated root resorption by two approaches: three-dimensional volumetric analysis of the roots by micro-CT and histomorphometric analysis of the root resorption area. Root volume measurements were used to quantify root resorption, by comparing the remaining root volume with the control group. Numerous previous studies used micro-CT to evaluate root resorption [28, 29]. The root volume did not change considerably in response to force exertion or vibration application in this present study. Volumetric analysis of the DB and DPa roots on day 21 revealed no significant differences in root volume between any intervention group and the control group. However, the groups exposed to HFV exhibited a trend toward limit root volume change. Additionally, the DPa roots of the optimal force and optimal force with HFV groups tended to have greatest root volume change. Moreover, the histomorphometric analysis was found the significantly increased of root resorption of DB root in optimal force and optimal force with HFV. Since there might be some over stress and strain in some area of the periodontal ligament of optimal force and optimal force with HFV group. The explanation for the difference in root resorption between DB and DPa is still unknown. However, the previous study of orthodontic tooth movement in a rat model revealed the DB root had the largest and deepest resorption craters [17].

Because the current study was conducted in a rat model, several limitations should be addressed. There are distinct differences between human and rat bone remodeling processes. Humans have a bone remodeling cycle of 1–2 weeks to 2–3 months, whereas rats have a bone remodeling cycle of about 6 days [30, 31].

The finding suggests that employing HFV in conjunction with a light force accelerated tooth movement without indication of root resorption; nevertheless, the underlying biological process require additional exploration. Furthermore, bone remodeling differs between animal and humans. Therefore, further research in the optimal force and mechanical vibration protocol for clinical application is required.

Conclusion

HFV accelerates tooth movement without causing root resorption. The combination of HFV with light force accelerate tooth movement better than the optimal force without causing root resorption in rats, and this protocol might be used as an alternative treatment option to reduce the risk of root resorption.

Declarations

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Conflict of interest

None to declare

Author Contribution

Porntip Tangtanawat: Conceptualization, methodology, investigation, data curation, formal analysis, writing - original draft, visualization

Peungchaleoy Thammanichanon: Methodology, investigation, data curation

Srisurang Sutthapreyasri: Validation, writing – review & editing, supervision, project administration

Chidchanok Leethanakul: Validation, writing – review & editing, supervision, project administration

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Figures

Figure 1

Schematic of study design. Thirty-six Wistar rats (72 sites) were randomly allocated to two observation periods (14, 21 days), then the left and right sides of the maxilla were randomly assigned to the six interventions: control, HFV (125 Hz), light force (5 g), optimal force (10 g), light force with HFV, and optimal force with HFV.

Figure 2

Measurement of tooth movement using micro-CT. The sagittal dimension was used to register the height of contour of the first and second molars to provide a vertical reference plane (yellow line in a). The amount of tooth movement was determined as the distance between the first and second maxillary molars in the transverse dimension at the most distal point of the maxillary first molar (in yellow dashed line in b) at the middle third level, the level at the contour of the crown.

Figure 3

Root volume measurement using micro-CT. Representative image of a day 21 sample scanned and contoured in serial 2-D horizontal sections. The contour components were exported as 3D objects. The 3D volume of the roots was measured with Geomagic® Control X™ software.

Figure 4

Evaluation of root resorption. (a) Histomorphologic analysis of a sample from the light force group at 21 days showing the distopalatal root. The section was stained with hematoxylin and eosin. Scale bar = 200 μM. (b) Measurement of the percentage of root resorption for a sample from the optimal force group on 21 days using Aperio Imagescope software. The yellow circle indicates the area of resorption, the blue circle is the total root area, the green is the circle root canal area. Scale bar = 200 μM.

Figure 5

Micro-CT images of maxillary first molar displacement on days 14 and 21 in the light force, optimal force, light force with HFV, and optimal force with HFV groups. The teeth were scanned in the transverse dimension, which vertically sections the teeth at the height of the contour. Tooth displacement on day 14 a: light force, b: optimal force, c: light force with HFV, d: optimal force with HFV, on day 21 e: light force, f: optimal force, g: light force with HFV, h: optimal force with HFV

Figure 6

Cumulative tooth movement in light force, optimal force, light force with HFV, and optimal force with HFV groups on days 14 and 21. Combining HFV with orthodontic force significantly increased the amount of tooth movement ($p < 0.05$). Optimal force with HFV increased tooth movement by 2.1–2.2-fold compared to optimal force. Light force with HFV increased tooth movement by 1.8–2.0-fold compared to light force.

Figure 7

Representative micro-CT images of the distobuccal (DB) and distopalatal (DPa) roots in each group. The day 21 samples were scanned by micro-CT, contoured as serial 2-D horizontal sections, and the contour components were exported as 3D objects. Root volume was measured with Geomagic® Control X™ software. Representative images of DB roots from the (a) control, (b) HFV, (c) light force, (d) optimal force, (e) light force with HFV, and (f) optimal force with HFV groups, and DPa roots from the (g) control, (h) HFV, (i) light force, (j) optimal force, (k) light force with HFV, and (l) optimal force with HFV groups, showing orthodontic force and vibration did not affect root volume throughout the experiment. Scale bar 250 μm .

Figure 8

*Comparison of the volumes of the distobuccal (DB) and distopalatal (DPa) roots in the control, HFV, light force, optimal force, light force with HFV, and optimal force with HFV groups at 21 days. (a) Three-dimensional root volumetric analysis. (b) Root resorption analysis ($*p < 0.05$).*