

# Effects of mechanical vibration on root resorption in the rat molar induced by a heavy orthodontic force

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*Background:* Orthodontically-induced inflammatory root resorption (OIIRR) is an unwelcome side effect of orthodontic treatment. Mechanical vibration has been suggested as a preventative measure but evidence is limited. The aim of this study was to investigate whether whole body mechanical vibration has a positive influence on OIIRR.

*Material and methods:* Thirty-six 10-week-old Wistar rats were divided into three groups, which received either 30 Hz vibration, 60 Hz vibration or no vibration to serve as a control group. A heavy mesial force of 100 g was applied to the left maxillary first molar using nickel-titanium closed-coil springs. The right maxillary first molar served as an internal control. The vibration groups received 30 Hz or 60 Hz of whole body vibration for 10 minutes per day for 14 days. A volumetric analysis of the extent of root resorption on the mesial-buccal root of the first maxillary molar was examined using micro-computed tomography.

*Results:* When compared with the control group, the animals that received 30 Hz ( $p = 0.21$ ) and 60 Hz ( $p = 0.16$ ) of mechanical vibration did not show a statistically significant reduction in OIIRR. The results did not show a statistically significant difference in the extent of OIIRR between 30 Hz and 60 Hz vibration groups ( $p = 0.78$ ).

*Conclusion:* Mechanical vibration at 30 Hz and 60 Hz, when applied in an experimental model of whole body vibration, showed no significant effect on either physiological root resorption or OIIRR in rat molars loaded by a heavy orthodontic force. (Aust Orthod J 2017; 33: 179-186)

Received for publication: October 2016

Accepted: May 2017

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## Introduction

Orthodontic force applications induce a localised inflammatory response within the periodontal ligament.<sup>1</sup> Not only is this inflammation essential for tooth movement, it also contributes to inflammatory root surface resorption, known as orthodontically-induced inflammatory root resorption (OIIRR).<sup>1</sup>

The incidence of OIIRR has been reported to range from 22–100% of orthodontic patients,<sup>2</sup> with mean losses of root length from 1–2mm.<sup>3</sup> The incidence of OIIRR could be much higher than reported due to its unpredictable nature and difficulty of detection, if small. Fortunately, the level of root shortening in the majority of patients is limited to 2 mm or less.<sup>4</sup> The risk of severe root resorption during orthodontic

treatment (greater than one quarter of the root length, or more than 5 mm) is rare, only affecting 1–3% of patients.<sup>5,6</sup>

Following the application of an orthodontic force, the periodontal ligament on the compression side undergoes sterile necrosis and forms a layer of hyaline tissue.<sup>7-9</sup> Tooth movement occurs after haematopoietic resorptive cells migrate from marrow vasculature in the adjacent bone, and remove the obstructing alveolus and hyalinised zone.<sup>10</sup> Studies in rodents have shown that OIIRR is part of the hyaline zone removal process following orthodontic force application as osteoclasts are activated by signals originating from the sterile necrotic hyaline tissues.<sup>11-16</sup> The role of the activated osteoclasts and their precursors is to remove

the necrotic tissues. During the removal of the hyaline zone, the nearby outer surface of the root can be damaged by osteoclastic resorption.

The aetiology of OIIRR appears to be multifactorial and can be either biologically related or as a result of treatment mechanics. Examples of biological input are genetic factors,<sup>17-20</sup> allergies,<sup>21</sup> medications,<sup>22,23</sup> dental anomalies,<sup>17,24,25</sup> abnormal root morphology,<sup>26</sup> bone density,<sup>27,28</sup> and a previous history of root resorption.<sup>17,29-31</sup> Factors related to treatment mechanics have been suggested to cause increased OIIRR and include force magnitude,<sup>32-34</sup> the duration of treatment,<sup>1,24,25,27,30,35,36</sup> the direction of tooth movement<sup>37-39</sup> and the amount of tooth movement.<sup>40,41</sup>

Several approaches have been proposed to prevent OIIRR and involve a pretreatment assessment of the familial and medical history,<sup>4,18</sup> the application of light forces,<sup>28,32</sup> longer activation intervals,<sup>42,43</sup> a shorter duration of active treatment,<sup>25,44</sup> a radiographic assessment after six months into treatment,<sup>25,45</sup> and a cessation of treatment for two to three months if OIIRR is detected.<sup>45</sup>

Low-level mechanical vibration has been shown to restore bone loss due to prolonged disuse that may be associated with bed rest, space flight or osteoporosis.<sup>46-48</sup> Mechanically stimulated bone may induce osteogenesis instead of adipogenesis,<sup>49,50</sup> resist osteoclastogenesis,<sup>51-53</sup> and finally result in increased bone formation. It has been suggested that the application of mechanical vibration during orthodontic tooth movement can increase the rate of tooth movement.<sup>54,55</sup> Nishimura et al.<sup>54</sup> reported that, when exposed to a resonance vibration frequency of 60 Hz during orthodontic tooth movement, the effect on rat molars was enhanced and there was a trend towards less root resorption in the vibrated molars. However, research on the effects of mechanical vibration on OIIRR is still limited.

The aim of the present study was to investigate the effect of two different whole body mechanical vibration frequencies (30 Hz and 60 Hz) on root resorption expected on rat first molars following the application of heavy orthodontic forces (100 g).

## Materials and methods

### *Animal grouping*

Thirty-six 10-week-old, male Wistar rats were used as the experimental animals. The study was conducted with the approval of the Animal Ethics Committee

of Western Sydney Local Health Network (Protocol No. 5080.06.11). The animals were housed in cages of three and, before commencement, were allowed one week to acclimatise to the new laboratory environment.

Following acclimatisation, the animals were randomly divided into three groups comprising two experimental groups and one control group. The experimental groups were divided according to the vibration frequency (30 Hz or 60 Hz) to be employed during the experimental period. In all groups, the maxillary left first molars were subjected to the orthodontic force and the contralateral first molars served as internal controls.

### *Orthodontic tooth movement*

The animals were anaesthetised with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) for the placement of the orthodontic appliances. A mesially-directed force was directed at the maxillary left first molars of each animal using Sentalloy closed coil springs (GAC, NY, USA), each delivering 100 g of force confirmed by a tension test with a Correx gauge (Dome, CA, USA). The springs were attached to the molars by a ligature wire that looped around the cervical region of the tooth. The springs were extended anteriorly and fixed to the cervical aspect of both maxillary incisors with ligature wire loops. Light-cured hybrid composite resin (Z100, 3M ESPE, MN, USA) secured the tail of ligature wires to prevent wire dislodgement during the experimental period and soft tissue irritation (Figure 1).

### *Mechanical vibration*

The experimental animals were subjected to a mechanical vibration stimulus administered using a Soloflex (Soloflex, Inc., OR, USA) whole body vibration platform (Figure 2). Each cage containing the animals was placed on the vibration platform, and a vibration stimulus of either 30 Hz or 60 Hz was administered for 10 minutes a day for 14 consecutive days.

### *Animal monitoring and care*

Vital signs, such as respiration, body reflexes and eye discharges, of each animal were closely monitored during the intra-operative period. Postoperatively, the animals were placed on a warm pad until they

recovered from the anaesthesia. During the entire experimental period, the behaviour and weight of the animals were monitored and the animals were fed with grounded laboratory chow and water to protect the appliances from damage.

After 14 days of the experiment, the animals were euthanased by carbon dioxide inhalation. The maxillae were dissected and sectioned to include the segment of palate with the right and left first molars. The samples were then stored in 70% ethanol.

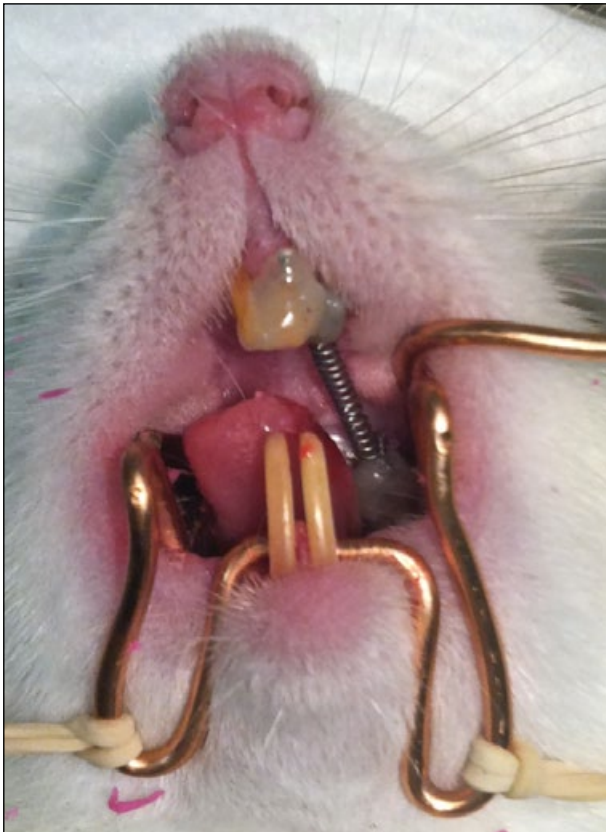


Figure 1. Design of force activation with NiTi coil spring.



Figure 2. Soloflex whole body vibration platform.

### *Micro-CT scanning*

Micro-CT (XRadia MicroXCT-400, CA, USA) was used for specimen scanning. The maxillae were placed in a polymeric container containing 70% ethanol, mounted on a specimen stage, which was set to rotate 180 degrees around a vertical axis. The X-ray tube was set at a voltage of 60 kV and current of 100  $\mu$ A, and each sample was scanned at a resolution of 13.1  $\mu$ m. Each specimen was scanned with 900 radiographic projections followed by acquisition using the in-built software (TXM Reconstructor) to reconstruct all 2D projections to form a Z stack of slices perpendicular to the rotational axis. The reconstructed slices were converted into 16 bit Tagged Image File Format (TIFF) images, which were rendered to form a 3D model using VG StudioMax software (Version 1.2; Volume Graphics GmbH, Heidelberg, Germany) (Figure 3). Significant root resorption was noted on

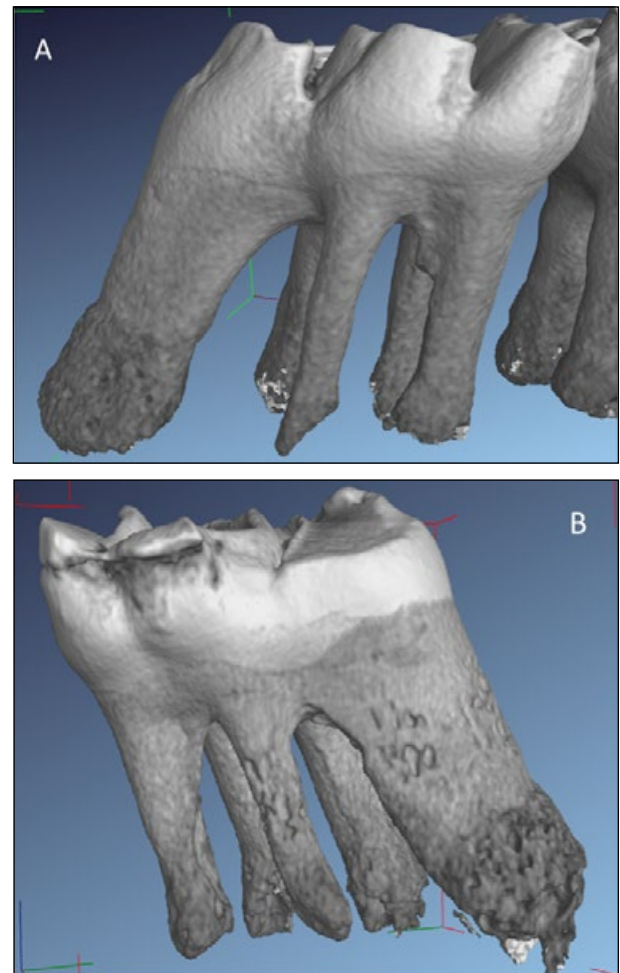


Figure 3. (A) Three-dimensional image of the right first molar. Note minimal root resorption on root surface. (B) Three-dimensional image of the left first molar. Note uneven root surface indicating root resorption.

the mesial buccal root; therefore, a volumetric analysis was carried out. Quantitative volumetric measurement of resorption craters was performed using ImageJ Macro software developed by the Australian Centre for Microscopy and Microanalysis at the University of Sydney. Two-dimensional convex hull algorithms of each 2D slice of the 3D data set were applied to measure the volume of the resorption craters.

### Statistical analysis

All statistical analyses were performed using MATLAB (Version 7.1/R14, The MathWorks Inc., MA, USA). An exploratory Shapiro-Wilks test showed normal distributions of the data in the 30 Hz vibration, 60 Hz vibration and control groups. Student's *t*-tests were applied to compare the volume of root resorption between the following groups:

1. 30 Hz experiment group (orthodontically-loaded molar) and the positive control group (orthodontically-loaded molars);
2. 60 Hz experiment group (orthodontically-loaded molar) and the positive control group (orthodontically-loaded molars);
3. 30 Hz experiment group (orthodontically-loaded molar) and the 60 Hz experiment group (orthodontically-loaded molars);
4. 30 Hz group (non-loaded molar) and the control group (non-loaded molars);
5. 60 Hz group (non-loaded molar) and the control group (non-loaded molars);
6. 30 Hz group (non-loaded molar) and the 60 Hz group (non-loaded molars).

Paired *t*-tests were used to compare the volume of root resorption between orthodontically-loaded and non-loaded molars in each animal for all three groups. All tests were analysed for a statistical significance at a level of  $p < 0.05$ .

The root resorption crater volumes of twelve teeth were remeasured to obtain a standard error of measurement (SEM) and a coefficient of variation (CV%).

## Results

### Animals

The average weight of the animals at the start of the experiment was 363 grams. All animals experienced healthy weight gain during the observation period. One rat from the control group was lost due to anaesthetic misadventure. At the completion of the present study, 24 experimental rats and 11 control animals were available for assessment.

### Accuracy of measurements

Repeated measurements were conducted at different occasions. The standard error of measurement (SEM) was  $1.68 \times 10^{-5} \text{ mm}^3$  and the coefficient of variation (CV%) was 4.60%.

### Volume of root resorption

Root resorption craters were found in both orthodontically-loaded, as well as non-loaded maxillary first molars. In all three groups of animals, a comparison of spring-loaded left maxillary first molars with the contralateral maxillary first molars (internal controls), showed a statistically significant difference in volume of root resorption ( $p < 0.05$ ) (Table I, Figure 4).

A comparison of the spring-loaded maxillary left molars of the 30 Hz vibration group with those of the 60 Hz vibration group showed no significant difference in the volume of root resorption ( $p = 0.78$ ). Similarly, no significant difference in the volume of root resorption was noted when comparing the spring-loaded molars of the 30 Hz vibration group ( $p = 0.21$ ), or the spring-loaded molars of the 60 Hz vibration group ( $p = 0.16$ ), with the positive control group (Table II).

A comparison of the non-loaded right molars of the 30 Hz vibration group with that of the 60 Hz vibration group showed no significant difference in the volume of root resorption ( $p = 0.76$ ). Similarly, there was no significant difference in the volume of root resorption

Table I. Paired *t* test comparing the volume of root resorption crater between non-loaded and unloaded molars in each vibration group.

Comparison between	Mean ( $\times 10^{-5} \text{ mm}^3$ )	Std. error ( $\times 10^{-5} \text{ mm}^3$ )	<i>p</i> value
30 Hz non-loaded & 30 Hz loaded	17.5839	5.3428	0.00719*
60 Hz non-loaded & 60 Hz loaded	16.5240	4.7862	0.0054*
Control non-loaded & positive control	28.4330	6.7867	0.00186*

\* denotes statistically significant difference  $p < 0.05$

when comparing the non-loaded molars of the 30 Hz vibration group ( $p = 1.00$ ), or the non-loaded molars of the 60 Hz vibration group ( $p = 0.78$ ), with the non-loaded molars of the control group (Table III).

## Discussion

Wistar rats were chosen as the model for the present study because they have been used in previous OIIRR studies with a similar tooth movement protocol using coil springs.<sup>56,57</sup> In addition, Wistar rats have also been used in previous studies of the research group to evaluate the effect of vibration on tooth movement.<sup>54,55</sup>

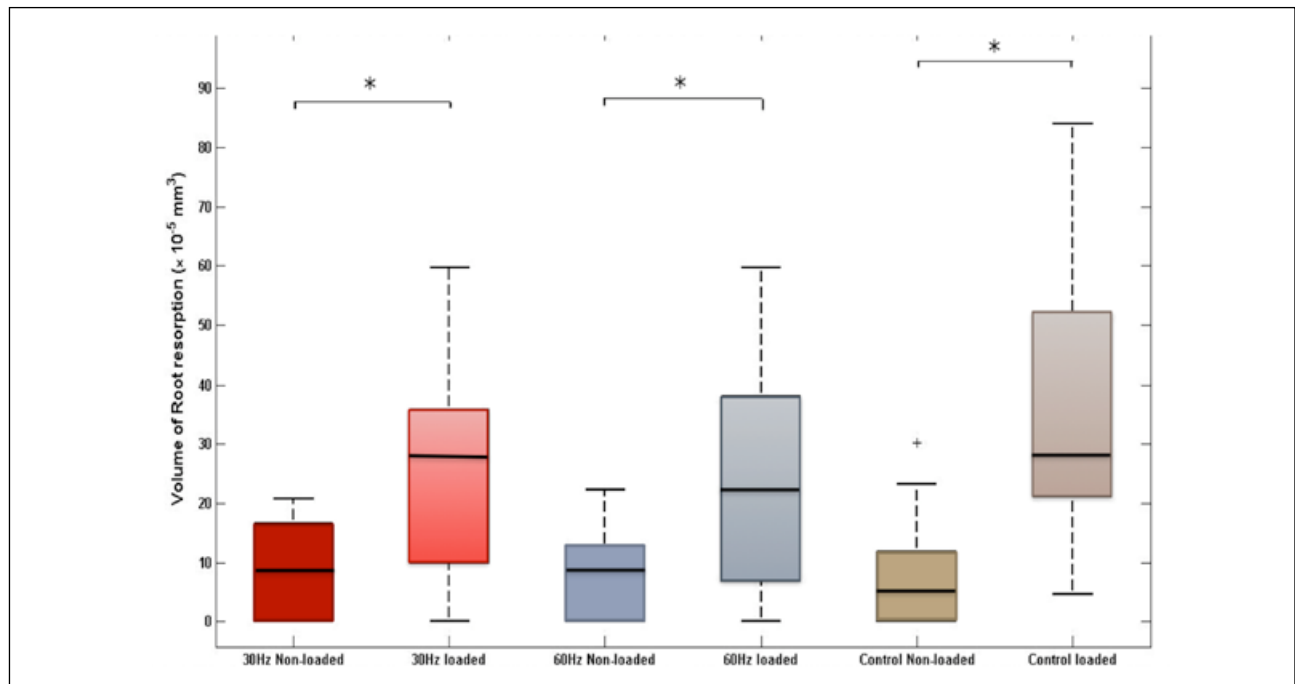
According to Matias et al.,<sup>58</sup> the development of root dentine, cementum, periodontal ligament and alveolar bone in rats is completed by the age of eight weeks. Therefore, mature 10-week-old rats were selected for OIIRR investigation to eliminate any root surface changes resulting from root development. The experimental period of 14 days was applied because, according to Helsing and Hammarström,<sup>59</sup> root resorption craters are detected after seven days of force application. In addition, in previous studies using a similar methodology significant root resorption was evident after 14 days following the application of 100 g orthodontic mesially-directed force.<sup>56,57</sup>

**Table II.** Student *t* test comparing the volume of root resorption crater in loaded molars between different vibration groups.

Comparison between	Mean ( $\times 10^{-5} \text{ mm}^3$ )	Std. error ( $\times 10^{-5} \text{ mm}^3$ )	<i>p</i> value
30 Hz loaded & 60 Hz loaded	2.0631	7.3122	0.78047
30 Hz loaded & positive control	-10.8730	8.4903	0.21428
60 Hz loaded & positive control	-12.9360	8.7814	0.15554

**Table III.** Student *t* test comparing the volume of root resorption crater in unloaded molars between vibration groups.

Comparison between	Mean ( $\times 10^{-5} \text{ mm}^3$ )	Std. error ( $\times 10^{-5} \text{ mm}^3$ )	<i>p</i> value
30 Hz non-loaded & 60 Hz non-loaded	10.0032	3.2471	0.76027
30 Hz non-loaded & non-loaded control	-0.0241	3.8711	0.99509
60 Hz non-loaded & non-loaded control	-1.0273	3.6777	0.78273



**Figure 4.** Box plot shows comparison of the volume of root resorption between groups

The vibration protocol of either 30 Hz or 60 Hz vibration frequencies was conducted for 10 minutes a day according to the recommendation by a whole body vibration platform, Juvent 1000 (American Medical Innovations, LLC, FL, USA), which produces vibration frequencies of 30–45 Hz at a daily usage time of 10 minutes. The second vibration frequency of 60 Hz was chosen based on the average resonance frequency of the rat first molar, which was reported to be  $61.02 \pm 8.83$  Hz.<sup>54</sup> There is currently no consensus in the literature regarding the optimal vibration protocol for tooth movement. Prisby et al.<sup>60</sup> reviewed the effect of whole body vibration in humans and animals and reported that vibration procedures used in earlier studies varied considerably, making it difficult to define the optimal protocol with regards to vibration frequency, duration and magnitude protocol.

The experimental maxillary left first molars were subjected to orthodontic loading with Sentalloy closed coil springs (GAC, NY, USA), each delivering 100 g of force. It was determined from a previous study by Gonzales et al.<sup>56</sup> that root resorption was force dependent and the greatest amount of root resorption was observed in molars subjected to a 100 g force. Heavy force of 100 g was chosen in the present investigation specifically to highlight the possible impact of mechanical vibration in reducing severe OIIRR. The results revealed that there were no statistically significant differences between the vibration groups and a positive control group. This could be explained by the considerable root resorption craters induced by the heavy orthodontic force being beyond the capacity for repair. In addition, whole body mechanical vibration may have no impact on cementogenesis or resistance to osteoclastogenesis at the cellular level, which is dissimilar to bone in which vibration is reported to induce osteogenesis.<sup>49,50</sup>

It is known that rodent molars undergo distal drifting.<sup>61,62</sup> During this continuous and physiological drifting process, the alveolus is subjected to bone resorption on the distal side and apposition on the mesial side, in a process known as remodelling.<sup>63,64</sup> During this physiological drift, root resorption may occur,<sup>65,66</sup> which explained and justified the presence of root resorption observed in the non-loaded molars in the present study. The levels of root resorption in non-loaded molars in all three groups of animals were not significantly different ( $p > 0.05$ , Table III). This finding suggested that whole body mechanical vibration had no effect on the process of physiological root

resorption. Therefore, it follows that the role of whole body mechanical vibration on cementogenesis is questionable.

Several studies that have investigated the effect of intraoral vibration on OIIRR have reported positive results. The concept of having less OIIRR when teeth are subjected to vibration is based on the premise that a vibrational force is cyclical in nature. When an intraoral vibrational device is used in conjunction with orthodontic tooth forces, it is likely that additional intermittent forces are applied to the teeth during orthodontic movement. The application of intermittent forces has been shown to allow cementum healing and prevent root resorption.<sup>67,68</sup> Nishimura et al.<sup>54</sup> studied the rate of tooth movement and root resorption in rat molars subjected to intermittent stimulation by resonance vibration. The findings revealed that the level of root resorption after 21 days was not significantly different between the vibration and non-vibration groups. However, the authors indicated a trend towards less root resorption in the vibration group. It was concluded that resonance vibration might accelerate orthodontic tooth movement without causing collateral damage to the periodontal structures in the form of root resorption. In a cone beam computed tomographic (CBCT) study, Kau<sup>69</sup> investigated the effect of six months of 'AcceleDent' device therapy used in OIIRR. The study reported that root resorption observed was found to be within clinically acceptable limits. However, there was no control group by which to compare the level of OIIRR between vibrated and non-vibrated teeth.

In the present study, the extent of root resorption in orthodontically-loaded molars did not show a statistically significant difference in vibrated or non-vibrated animals ( $p > 0.05$ , Table II). In addition, increasing the frequency of mechanical vibration from 30 Hz to 60 Hz did not show a significant difference in the extent of root resorption in the orthodontically-loaded molars ( $p > 0.05$ , Table II). This finding was contrary to that reported by Nishimura et al.,<sup>54</sup> who suggested that mechanical vibration appeared to intercept the ischaemic response and re-establish the blood supply in the compressed periodontal ligament. The difference could be explained by the young age of the rats (six weeks old) compared with those used in the present study. Immature roots tend to have a stronger repair capacity.<sup>58</sup> In addition, the type of spring used was an expansive spring made of Nickel Titanium wire delivering an average force of 12.8

grams, which was significantly lower than the force that was used in the current investigation. Moreover, the mechanical vibration was induced at the tooth level, in comparison with the whole body vibration applied in the present study.

## Conclusion

Based on the results of the present study, the following conclusions have been made:

1. Whole body mechanical vibration of 30 Hz and 60 Hz has no significant effect on orthodontic-induced root resorption in rat molars loaded with 100 g of orthodontic force.
2. Whole body mechanical vibration of 30 Hz and 60 Hz has no significant effect on physiologic root resorption in rat molars.
3. The effect of whole body mechanical vibration on cementoblast function or cementogenesis and root repair is questionable.
4. Further investigations testing the effect of localised vibration at the tooth level on OIRR are required.

## Acknowledgment

This study was supported by the Australian Society of Orthodontists Foundation for Research and Education.

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