Magnitude Effect of Dynamic Occlusal Guidance Force on Root Resorption in Dog

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Abstract
Its goals are to treat abnormalities of dentition and occlusion that develop during the growth period and to predict and thus prevent abnormalities in the permanent teeth of the adult phase by imparting dynamic occlusal guidance force in an early stage to establish healthy permanent occlusion.

For this study, the authors sought to obtain a detailed picture of tooth movement when a superelastic Nickel-Titanium coil spring was applied. To achieve this, we conducted mechanical tests on Nickel-Titanium coil springs and Cobalt-Chromium coil springs with different coil wire diameters and coil outer diameters. Four kinds of coil spring were attached the space which formed by extraction of maxillary first premolar in dog. Root resorption accompanying molar tooth movement was compared Nickel-Titanium coil and Cobalt-Chromium coil based on micro-CT finding and histopathological findings in beagle dogs.

In Nickel-Titanium spring, no permanent deformation was observed when compression was sufficient to bring the coils into contact. In contrast, with the Cobalt-Chromium spring, permanent deformation was recognized when compression was sufficient to bring the coils into contact.

In the animal experiments at 80-g load, which is considered the optimum orthodontic force, bone resorption was seen on the pressure side and findings of new bone like tissue observed on the tension side. At occlusal guidance force of 240-g load, remarkable bone resorption and root resorption were seen in all of springs. Nickel-Titanium spring exhibited significantly greater root resorption than the Cobalt-Chromium spring.

As these results show, when a Cobalt-Chromium spring is applied to 80-g load, the load decreased as the tooth moves, in the contrast no load decreased in Nickel-Titanium spring. Slight root resorption was seen in the case of 80-g load in Nickel-Titanium spring and Cobalt-Chromium spring.

In clinical practice, a great care is needed in the case of using Nickel-Titanium spring for dynamic occlusal guidance.

Keywords:
dog, root resorption, microCT

Introduction
Occlusal guidance in pediatric dentistry can be either passive or dynamic and the goal of each is to eventually establish sound and healthy dentition and occlusion in the permanent dentition period. Dynamic occlusal guidance aims to treat abnormalities of dentition and occlusion that develop during the growth period and to predict and thus prevent abnormalities in the permanent teeth of the adult phase by imparting dynamic occlusal guidance force at an early stage to establish healthy permanent occlusion. Dynamic occlusal guidance methods may be applied to each patient, one such method being multi-bracket therapy (1).

Research on tooth movement in animals has included histopathological studies and immunohistological studies (2–4). Yang et al. (5) reported a correlation between the...
amount of load and the amount of movement in an orthodontic force experiment on autotransplanted front teeth in dogs. Because tooth movement and root resorption are related (6), there is great interest in how much tooth movement is possible without root resorption. In most orthodontically treated patients, root resorption is small and of no clinical importance (7). However, a recent review indicated that 5% of all orthodontic patients experience more than 5 mm of root shortening (8). Root resorption might be related to force magnitude (9). Harris reported that root resorption probably was induced by periodontal ligament compression and concomitant hyalinization (10). External apical root resorption is an undesirable complication of orthodontic treatment (8).

However, previous research on force in occlusal guidance clearly suggested that conditions and standards in this area have not been adequately established. Few studies have taken the surface area of the periodontal membrane when constant loads were applied to experimental teeth. The process of bone formation during dynamic occlusal guidance or orthodontic treatment is normally evaluated with a combination of histopathological findings and plain X-ray images. However, although plain X-rays allow longitudinal observation, they are only taken from one direction and the X-rays cannot adequately provide detailed three-dimensional visualization of changes in the bone formation. Histopathological images provide detail information of bone formation at different stages, but observation of the same individual over time is impossible because the images were obtained after euthanization. Conventional X-ray micro-computed tomography (CT) has emerged as a non-destructive technique for obtaining three-dimensional images, but is unsuitable for scanning living experimental animals because imaging is time-consuming and requires rotation of the scanned object. Recently, an X-ray CT system optimized for animal experiments was developed (R_mCT® Rigaku; referred to hereafter as R_mCT) (11).

R_mCT is a rotate-format, corn-beam X-ray CT system with an effective focal point size of 7 μm and a photomultiplier fixed as an I-arm that rotates in the vertical plane. This design ensures free space of about 10 cm × 10 cm in the center of the I-arm and allows the experimental animal to be placed on a stable platform and scanned without being rotated. R_mCT is a unique system that is characterized by high resolution, short scanning time, and low exposure, and allows observation of changes in the same living animal over time (12). However, R_mCT was designed for small animals and is unsuitable for larger animals such as dogs. We modified R_mCT so that it could be used with dogs, thus reducing the number of experimental animals used, reducing technical errors, and enabling changes in the same individual to be monitored over time.

There are various methods of tooth movement, but the coil spring is widely used in orthodontic treatment with multi-bracket appliances. It is fitted between two teeth under compression to expand the distance between the two teeth by means of the repulsive force generated by compression. For this study, the authors precisely measured the load on the coil spring in the dog's molar tooth movement, used R_mCT to calculate the root surface area, and then performed the experiment in such a way that the load accurately was kept.

The authors created an experimental model of root resorption under different dynamic occlusal guidance forces and evaluated the root resorption by R_mCT and histopathological findings.

Materials and Methods

Coil spring

Compression tests were done using a universal testing machine with crossheads attached for compression testing. Four coil springs were used in the experiment: Coil spring 1 was Nickel-Titanium (Ni-Ti) 0.254 mm (wire diameter) × 0.762 mm (coil outer diameter), Coil spring 2 was Ni-Ti 0.305 mm × 1.143 mm, Coil spring 3 was Cobalt-Chromium (Co-Cr) 0.254 mm × 0.762 mm, and Coil spring 4 was Co-Cr 0.254 mm × 1.143 mm. All Coil springs were purchased from Rocky Mountain Orthodontics. (Table 1)

Coil spring load and displacement

Coil springs 1, 2, 3, and 4 were used in the experiment. Each coil spring was cut to 20.0 mm for the experiment. The coil springs were fixed to the crosshead of the universal testing machine, and the distance between crosshead and stopper was narrowed using universal testing machine, and the compressive force on each coil spring was measured. Permanent deformation of four coil springs was also examined.

Animal experiment

Twelve 3-year-old beagle dogs (male, approx. 10 kg) that treated scaling of all teeth 2 weeks before were used. After
scaling, the teeth were scanned by R_mCT and the root surface area of the maxillary second premolar to be moved was measured using i-VIEW® on micro-CT. At the same time, the maxillary first premolar was extracted with local anesthesia with 2% xylocaine.

The animals were kept at 24°C, 55% humidity, with a 12-hour light, 12-hour dark lighting cycle, provided with solid feed and sufficient water and health was confirmed based on body weight changes.

The bracket attached to the teeth to fix the coil spring. (Fig.1) Before bracket attachment, the left and right maxillary second and third premolars were etched using K-etchant (Kuraray, Tokyo, Japan) and the brackets were attached using Superbond (Sun Medical Shiga, Japan). The brackets were attached in a fixed position using the molar cusp as a guide. The intraheight between adjacent brackets (InL) was measured on the left and right sides of the maxilla in all 12 animals, and root surface area was measured using micro-CT. The bracket used in this study was Integra bracket (Rocky Mountain Morita, Tokyo, Japan, 0.022 inch slot), the main wire was twist wire (Rocky Mountain Morita, Tokyo, Japan, round-straight 0.546 mm, triflex), and the ligature was ligature wire (Rocky Mountain Morita, Tokyo, Japan, preformed ligature wire, 0.304 mm).

**Table 1**  Coil springs used in the study

<table>
<thead>
<tr>
<th>Wire diameter (mm)</th>
<th>Coil spring 1</th>
<th>Coil spring 2</th>
<th>Coil spring 3</th>
<th>Coil spring 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.254</td>
<td>0.254</td>
<td>0.254</td>
<td>0.254</td>
<td></td>
</tr>
<tr>
<td>Coi outer diameter (mm)</td>
<td>0.762</td>
<td>1.143</td>
<td>0.762</td>
<td>1.143</td>
</tr>
<tr>
<td>Number of coil (20 mm)</td>
<td>26</td>
<td>20</td>
<td>35</td>
<td>41</td>
</tr>
<tr>
<td>Material</td>
<td>Ni-Ti</td>
<td>Ni-Ti</td>
<td>Co-Cr</td>
<td>Co-Cr</td>
</tr>
</tbody>
</table>

This table showed the material, wire diameter, and other specifications of Coil springs 1–4 used in the experiments.

**Coil spring height required for load**

Based on the InL and root surface area, and the compression curves of Coil 1 to Coil 4 in Fig.2 and Fig.3, the required heights of coil spring required for the occlusal guidance force of 80 g and force of 240 g were calculated in each coil spring. Since 240 g was indicated maximum Ni-Ti coil spring load (Fig.2). Coil spring heights obtained from calculation in four coil springs loaded 80 g and 240 g were reconfirmed by universal test machine.

**R_mCT scanning**

We studied changes in dynamic occlusal guidance over time in the same animal using R_mCT (Rigaku Tokyo, Japan). Scanning was done at 90 kV and 88 μA. The animals were prepared for scanning by being intravenously...
anaesthetized with ketamine and xylazine, and restrained on a fixed stand to prevent body movements affecting the scan. We used i-VIEW® as the image reconstruction software (Morita Tokyo, Japan). The main image was scanned at 512 × 512 × 384 pixels and the scan time was 17 seconds. Tomographic images in figures were created from the Y-axis.

Histopathological evaluation
The animals were sacrificed by ketamine overdose 28 days after bracket attachment. After this, perfusion fixation was done using heparin and a 10% phosphate-buffered neutral formalin solution (Wako Pure Chemical Industries Tokyo, Japan). After fixation for 1 week in 10% phosphate-buffered neutral formalin solution (Wako Pure Chemical Industries Tokyo, Japan), decalcification was done at 4°C using K-CX (Falma Tokyo, Japan). Then, paraffin embedding was performed using standard methods and 4μm continuous sections were cut sagittally. Hematoxylin and eosin staining was done and the coil springs were then submitted for histopathological examination and photographed under an optical microscope.

Evaluation of root resorption
Root resorption was evaluated by the pixel number of histopathological images in accordance with the method reported by Asano et al. (13). Images of the prepared sections were loaded into the Image J program. The ratio of the area of the root resorption was presented by the root surface area considered sound dentin and desorbed dentin. The total sound root dentin area measured using Image-Pro Plus 3.0® (Media Cybernetics, Tokyo, Japan) and the area of root resorption by Image J program were measured in square millimeters and those measurements were converted to percentages for statistical analysis.

The animal experiments were conducted in accordance with the Nihon University Rules on Animal Experimentation (Approval Number AP10MD002).

Statistical analysis
Root resorption was evaluated using two-way analysis of variance. Mean and standard deviations were calculated for measurement sites under each condition. Friedman test was used for statistical processing for the load in each coil 1 to 4. For the experiment load, differences between sites were analyzed with one-way analysis of variance and interload multiple comparison test using Tukey’s method were done as the post-hoc test. Statistical analysis was done using the analytical software SPSSII for Windows (SPSS, Tokyo, Japan). The significance level was set at p < 0.05.

Results
Coil spring load and displacement
As shown Fig. 2 and Fig. 3, both Coil spring 1 and Coil spring 2 made of Ni-Ti were compressed with 240-g loads and no permanent deformity, otherwise Coil spring 3 and Coil spring 4 made of Co-Cr were compressed with 240-g load and permanent deformity was seen.

Intraheight between adjacent brackets (InL)
The mean distance and standard deviation of InL (mm) in 3 sites of Coil spring 1 loaded 80 g, Coil spring 1 loaded 240 g, Coil spring 2 loaded 80 g, Coil spring 2 loaded 240 g, Coil spring 3 loaded 80 g, Coil spring 3 loaded 240 g, Coil spring 4 loaded 80 g, Coil spring 4 loaded 240 g were 9.5 ± 0.7, 9.0 ± 0.1, 10.8 ± 0.2, 9.7 ± 0.8, 10.5 ± 0.3, 10.5 ± 0.2, 9.0 ± 0.1 and 8.5 ± 0.3, respectively.

Area of root surface
The mean and standard deviation of the root surface area of maxillary second premolar in Coil spring 1 loaded 80 g, Coil spring 1 loaded 240 g, Coil spring 2 loaded 80 g, Coil spring 2 loaded 240 g, Coil spring 3 loaded 80 g, Coil spring 3 loaded 240 g, Coil spring 4 loaded 80 g, Coil spring 4 loaded 240 g were 3.1 mm ± 0.2, 3.2 ± 0.2, 3.3 ± 0.3, 3.4 ± 0.3, 3.3 ± 0.1, 2.9 ± 0.1, 3.2 ± 0.2 and 3.2 ± 0.1, respectively. (Table 2)
Table 2  Root surface area of Coil spring 1 to Coil spring 4

<table>
<thead>
<tr>
<th></th>
<th>80 g load</th>
<th>240 g load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Coil spring 1</td>
<td>3.08</td>
<td>0.17</td>
</tr>
<tr>
<td>Coil spring 2</td>
<td>3.31</td>
<td>0.26</td>
</tr>
<tr>
<td>Coil spring 3</td>
<td>3.33</td>
<td>0.10</td>
</tr>
<tr>
<td>Coil spring 4</td>
<td>3.21</td>
<td>0.21</td>
</tr>
</tbody>
</table>

The area was measured by i-VIEW®

Coil spring height required for load

For Coil spring 1, mean and standard deviation, of the InL required for 80 g was 11.3 mm (0.65 mm), for Coil 2 was 14.1 mm (0.70 mm), for Coil 3 was 12.7 mm (0.58 mm) and for Coil 4 was 12.5 mm (0.65 mm). For Coil spring 1, mean and standard deviation, of the InL required for 240 g was 14.2 mm (0.68 mm), for Coil 2 was 16.8 mm (0.70 mm), for Coil 3 was 13.0 mm (0.81 mm) and for Coil 4 was 15.5 mm (0.62 mm), which data were obtained from the compression curve in Fig. 2 and Fig. 3.

Animal experiments

R_mCT scanning

Micro-CT images of Coil spring 1 are shown in Fig. 4 (80-g load) and Fig. 5 (240-g load). In the 80-g load, periodontal ligament space expansion, indicated by low density, was confirmed around the root on the pressure side 7 days after tooth movement (arrow). At 14 and 28 days after attachment, bone resorption was confirmed at the furcation accompanying movement of the tooth (arrow). In the 240-g load, resorption at the furcation was confirmed 7 days after attachment (arrow). Fourteen days after tooth movement, remarkable resorption of alveolar bone was confirmed. Twenty-eight days after tooth movement, there was not only marked resorption of alveolar bone but also low-density areas suggestive of root resorption on the pressure and tension sides.

Micro-CT images of Coil spring 3 are shown in Fig. 6 (80-g load) and Fig. 7 (240-g load). In the 80-g load, periodontal ligament space expansion, indicated by low density, was confirmed around the root on the pressure side 7 days after tooth movement (arrow). At 14 and 28 days after tooth movement, bone resorption was confirmed at the furcation accompanying movement of the tooth (arrow). In the 240-g load, bone resorption at the furcation was confirmed 14 days after attachment (arrow). Twenty-eight days after tooth movement, there was not only marked resorption of alveolar bone but also low-density areas suggestive of root resorption on the pressure side (arrows).

Histopathological evaluation

Histopathology images relating to Coil spring 1 are shown in Fig. 4 (80-g load) and Fig. 5 (240-g load). In the 80-g load, bone resorption on the pressure side and new bone like tissue on the tension side were observed. The addition new cementum on the root surface was of observed (arrow), and a slight of root resorption was seen in some case. In the 240-g load, root resorption was evident on the root surface on the
pressureside, which was radiolucent on micro-CT images. Therewas root resorption in the apical region (arrow).

Histopathology images to Coil spring 2 are shown in Fig. 4 (80-g load) and Fig. 5 (240-g load). In the 80-g load, there was bone resorption on the pressure side and new bone like tissue on the tension side. The addition new cementum on the root surface was observed (arrow), and a slight of root resorption was seen in some case. In the Coil spring 2 load in 240g, root resorption was evident on the root surface on the pressure side, which was radiopaque on micro-CT images.

Histopathology images relating to Coil spring 3 are shown in Fig. 6 (80-g load) and Fig. 7 (240-g load). In the 80-g load, there was bone resorption on the pressure side and new bone like tissue on the tension side, a slight of root resorption was seen in some case. The addition new cementum on the root surface was observed (arrow). In the 240-g load, root resorption was evident on the root surface on the pressure side, which was seen as low-density areas on micro-CT images.

Histopathology images of Coil spring 4 are shown in Fig. 6 (80-g load) and Fig. 7 (240-g load). In the 80-g load, there
was bone resorption on the pressure side and new bone like tissue on the tension side, a slight of root resorption was seen in some case. The addition new cementum on the root surface was of observed (arrow). In the 240-g load, root resorption was evident on the root surface on the pressure side, which was seen as low-density areas on micro-CT images.

**Evaluation of root resorption**

Fig. 8 shows the evaluation of root resorption. The root resorption ratios in 80-g load were low and similar in both Ni-Ti and Co-Cr loads, and the mean and standard deviation in root resorption rate in 4 coil springs was 6.2 ± 1.1%. However, the ratio was significantly greater in the 240-g load than the 80-g load for Coil springs 1 to 4. Especially, the ratios of 240 g in Ni-Ti load were significantly higher than that in Co-Cr load.

**Discussion**

The Ni-Ti used in the experiments is a shape-memory alloy with superelasticity and antivibration properties (14). In dentistry in particular, this material has been used as orthodontic wire due to its superelasticity (15). The Ni-Ti coil spring exhibited a constant superelasticity at compressibility of under 300-g load, whereas with the Co-Cr coil spring, displacement and load varied proportionally and there was
permanent deformation when compression was sufficient to bring the coils into contact in this study. Our results demonstrated that the relationship between displacement and load in Ni-Ti coil spring is unlike that of Co-Cr coil spring.

When force is applied to a tooth, the tooth shifts in its alveolar socket, creating pressure in the periodontal tissue in the direction in which the force acts via the periodontal membrane and a state of tension on the opposite side (1, 2, 5, 6). When this state continues for a certain period of time, tissue on both the pressure side and tension side undergoes changes and the tooth moves.

On the pressure side, these changes become degenerative over time and depending on the magnitude of force and application period, these changes evolve from hyaline degeneration to necrosis. The changes are accompanied by resorption and substitutive tissue changes. On the tension side, application of load allowed for the widening of the periodontal ligament space expands and development of the tension force in the periodontal ligament fibers. Continued application of this state results in promotion of ligament fiber regeneration due to segmentation and proliferation of

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**Fig. 6** Micro-CT images and histopathological findings in Coil spring 3 and Coil spring 4 (80-g load)

Coil spring 3: Periodontal ligament space expansion, indicated by low density, was confirmed around the root on the pressure side 7 days after attachment (arrow). At 14 and 28 days after attachment, bone resorption was confirmed at the furcation accompanying movement of the tooth (arrow). There was bone resorption on the pressure side and new bone like tissue on the tension side. The addition new cementum on the root surface was of observed (arrow).

Coil spring 4: Periodontal ligament space expansion, indicated by low density, was confirmed around the root on the pressure side 7 days after attachment (arrow). At 14 and 28 days after attachment, bone resorption was confirmed at the furcation accompanying movement of the tooth (arrow). There was bone resorption on the pressure side and new bone like tissue on the tension side. The addition new cementum on the root surface was of observed (arrow).
fibroblasts. Osteoblasts appear in the alveolar socket and new bone was added to the periodontal ligament fibers under tension. As a result, the tooth moves. Movement thus arises from various cellular activities induced by these mechanical stresses. Mechanical stress-related factors influencing tooth movement include the strength of orthodontic force, direction of action, the age, root morphology, root surface area, and extent of metabolic activity (16).

Root resorption can be detected histologically or radiographically. The detection of severe apical resorption is easily possible using radiographs (17). However, mild apical resorption and resorption at the mesial or distal root surfaces are hardly visible on radiographs and are difficult to detect in the clinical situation. Our results demonstrated that root resorption can be observed more easily with micro-CT than conventional dental X-ray imaging because micro-CT allows three-dimensional observation. Root resorption in cementum is difficult to quantify because the outer border of the cementum is not stable (5-7).

In this study, root resorption during tooth movement was observed at the middle part of the roots and a little in the apical region. This can be explained by the sand-glass shape of the periodontal space, which is thinnest in the middle part of the root (5-7). Therefore, hypoxia and subsequent hyalinization will be most prominent in that area and a close relation between hyalinization and root resorption has been suggested (18). The root resorption at cervical and apical regions was seen during tipping movements (19-21).

At the 80 g, bone resorption was seen on the pressure side and new bone like tissue was seen on the resorption side. At
240 g, marked bone resorption and root resorption were evident in all loads. However, it was thought that bone resorption might be occurred horizontal stress by coil spring and vertical stress by elongation. Significantly greater root resorption with the Ni-Ti wire was seen than with the Co-Cr coil spring.

Coil springs 1 and 2 used in this study both had clearly exhibited consistent superelasticity. This presumably results from long-term continuation of a constant load provided by the coil spring due to its superelasticity: once a certain load is reached, this superelasticity provides a virtually constant load even when displacement increases. In contrast, when a Co-Cr coil spring is applied in clinical practice to provide the appropriate force, this force tends to decrease with increases in the shift of tooth and intraoral adjustment is therefore required to restore the force to its orthodontic level. In contrast, because the Ni-Ti coil spring is able to continuously exert the desired occlusal guidance force, it is an ideal material for effecting physiological tooth movement and is also highly effective in that it offers shorter treatment periods and fewer visits to the dentist. However, care is required when using Ni-Ti coil springs to apply a strong occlusal guidance force, because the same strong force will continue to be exerted, unlike with Co-Cr springs.

To keep root resorption to a minimum when moving a tooth, it is essential that the occlusal guidance force be at or below an appropriate level. In research on tooth movement, no tissue degeneration was seen in experiments using orthodontic force of 45–60 g in dogs (22) and in tooth movement experiments by Nikolai (23) using a canine model, the force applied to the alveolar ridge on the pressure side was large. This ties in with the micro-CT results of our study, which found radiolucent areas suggestive of alveolar bone resorption. Research on a tooth movement in humans has concluded that 2–2.5 g/mm² is the most effective occlusal guidance force (24). Tooth movement without tipping requires a force of 3–4 g/mm², but this increases the rate of biological response in periodontal tissue (24). Schwartz (25) reported that the ideal force for tooth movement was 20 g to 25 g/cm² which force doesn’t go over the capillary blood vessel pressure, and Kondo (26) reported that no change was observed the hemodynamics of periodontal ligament in cat. Besides Owman (27) used the force pressure to dog teeth movement of 80 g as optimal force. Nemoto (28) reported maxillary canine root area was around 300 mg/cm² in human. Based on those reports, we applied the 80-g as optimal load and 240-g load as strong force to dog’s maxillary second premolar, because mean root area of the second premolar teeth were obtained 3.0 mm² in this study. Using the Ni-Ti and the Co-Cr coil springs with a load of 80 g, we found that root resorption was found with 5–6% on dog’s maxillary second premolar, but a load of 240 g, root resorption was 18–20%. Therefore, occlusal guidance force must be used cautiously in clinical practice and particular care must be taken regarding occlusal guidance force when using a Ni-Ti coil spring.

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References