Original Article

Early-stage periodontal ligament compression predicts orthodontically induced root resorption in rats

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ABSTRACT

Objectives: To determine the effect of orthodontic pressure on periodontal ligament (PDL) compression in rats and assess correlation between PDL compression and orthodontically induced root resorption (OIRR).

Materials and Methods: Eight female Wistar rats aged 10 weeks underwent surgery to place 2 mini-screws at the center of the palatal plate. 25 cN coil springs connecting the maxillary first molars and mini-screws were applied bilaterally to generate mesial force. Maxillary first molars were assigned to undergo either bodily or tipping movements. Micro-computed tomography (μCT) scans were taken on days 0, 3, 7, and 14, and histological sections were taken on day 14. OIRR was measured from histological sections, and the corresponding PDL compression ratio was quantified using μCT images.

Results: The PDL was compressed by approximately 76% in tipping movement and 55% in bodily movement after 3 days, and by approximately 47% in bodily and tipping movements after 7 days of orthodontic force application. The extent of OIRR in tipping movement was significantly greater than that in bodily movement. A strong positive correlation between OIRR and PDL compression ratio was observed on day 3; however, no correlation was observed on day 7.

Conclusions: A strong correlation between PDL compression ratio and OIRR was observed at an early stage after the application of orthodontic force regardless of the tooth movement type (bodily or tipping), implying the importance of early stage PDL compression in the induction of OIRR. (*Angle Orthod*. 2024;94:240–246.)

KEY WORDS: Periodontal ligament compression; Orthodontically induced root resorption; Orthodontic tooth movement; Early stage

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Accepted: October 2023. Submitted: April 2023.
Published Online: November 15, 2023

INTRODUCTION

Orthodontic tooth movement (OTM) is caused by the biological response of periodontal tissues to orthodontic forces. Orthodontically induced root resorption (OIRR) is one of the most common and serious adverse effects of OTM. Therefore, it is important to minimize the risk of OIRR during orthodontic treatment. Achieving the maximum rate of tooth movement without damaging the roots and periodontal tissues with optimal orthodontic force is necessary for ideal orthodontic treatment.[1–3]

Since the first report by Sandstedt,[4] various clinical and experimental studies have investigated the process and mechanism of OIRR. Although the biological mechanisms of this process remain unclear, odontoclasts, macrophages, and osteoclasts have been suggested to be involved in OIRR. The site of OIRR corresponds to the area of hyalinization of periodontal ligament (PDL) cells compressed by excessive orthodontic force.[5,6] In addition, tissue changes inducing OIRR are initiated by ischemia-induced apoptosis of PDL cells and subsequent hyalinization caused by the compression of the PDL by orthodontic forces. This is followed by osteoclast and odontoclast formation and change in alveolar bone metabolism, finally leading to OIRR.[7]

Correlation between the magnitude of orthodontic force and OIRR has been reported in human[8] and animal studies.[9] Several studies have reported that the extent of root resorption depends on the applied force system. Schwarz[10] revealed that root resorption can occur if applied orthodontic pressure exceeds the capillary blood pressure. The range of capillary blood pressure is generally 10–40 mm Hg (0.0013–0.0053 MPa).[11] Recently, narrowing of the PDL space by orthodontic force application during tooth movement has been demonstrated via micro-computed tomography (μCT).[12,13] OIRR occurs on the compression side where narrowing of the PDL is observed, suggesting the involvement of PDL space narrowing in OIRR. However, the correlation between the level of PDL compression and OIRR remains unknown. Therefore, the aim of the present study was to assess the serial changes in PDL compression after orthodontic pressure application in rats and determine the correlation between PDL compression and OIRR. In addition, whether the type of tooth movement (bodily or tipping) influenced the extent of PDL compression, OIRR induction, and their correlation, were investigated.

MATERIALS AND METHODS

This study was approved by the Animal Welfare Committee of the Animal Care and Use Committee of Nagasaki University (No. 2010011668-3).

OIRR Rat Model

Eight female Wistar rats aged 10 weeks (weighing 170–180 g) were used in this experiment. Rats were housed in plastic cages at room temperature (22°C–24°C), provided standard pellets and water ad libitum, and acclimatized for a week before the experiment. The experimental timeline is shown in Figure 1A.

Experiments were conducted under general anesthesia. Rats were injected intramuscularly with 0.375 mg/kg medetomidine (Zenoaq, Fukushima, Japan), 2 mg/kg midazolam (Sandoz, Tokyo, Japan), and 2.5 mg/kg butorphanol tartrate (Meiji Seika Pharma Co. Ltd., Tokyo, Japan). The orthodontic procedure used here was a variation of the method described by Nakano et al.[14] Briefly, the orthodontic appliance consisted of: (1) two orthodontic mini-screws (1.3 mm × 5.0 mm; Absoanchor; Anchor Screw; Shohu, Japan) that were placed at the center of the palatal bone near the central incisors, (2) two nickel-titanium coil springs (25 cN; Sentalloy; Tomy, Fukushima, Japan) connecting the maxillary first molars and mini-screws bilaterally, generating mesially directed orthodontic forces, and (3) a 0.014-inch arch-wire (Elgiloy; Rocky Mountain Morita, Tokyo, Japan) that followed the maxillary arch form secured on the mini-screws with composite resin (Figure 1B).

Maxillary molars were allocated to receive either "bodily" or "tipping" movement on each side (Figure 1C). In the bodily movement group, a sliding tube fabricated with self-curing resin was inserted into the Elgiloy arch wire to fix the first molar to the main arch wire using composite resin (Super-Bond; Sun Medical, Shiga, Japan). To eliminate occlusal forces, self-curing resin bite raisers were applied to the maxillary second and third molars.

Measurement of OTM using μCT

μCT (R_mCT, Rigaku, Tokyo, Japan) scans were taken under general anesthesia on days 0, 3, 7, and 14. OTM was measured using the μCT images on day 14. Three measurements were used for the quantification of OTM: the shortest distance between the first and second molar (ShD), distance between contact points (CPD), and maxillary first molar tipping angle (TIA). TIA was measured by the difference between the angles formed by the mesial root axes before and after tooth movement (Figure 1D).

Quantification of OIRR via Histological Analysis

Rats were euthanized via carbon dioxide overdose on day 14. The maxillary bone was removed and fixed in 4% paraformaldehyde, 50 mM sodium solution, and cacodylate buffer (pH 7.4) for 48 hours. Maxillary bone

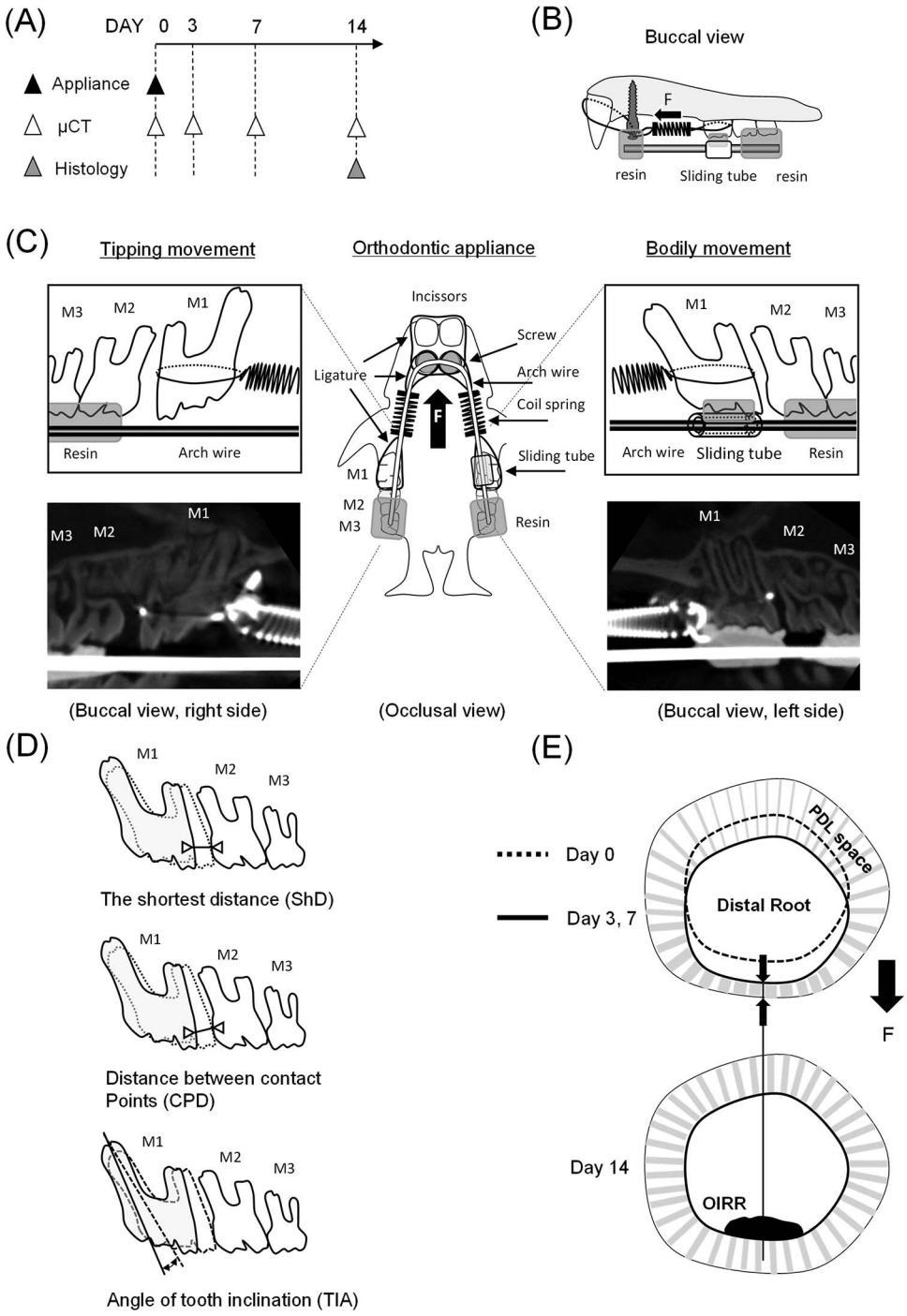

Figure 1. (A) Study Design. Experimental timeline. ▲, day of orthodontic appliance placement; △, day of micro-computed tomography (μCT) imaging; ▲, day of tissue preparation for histology. (B) Schematic diagram of the orthodontic appliance (buccal view). (C) Occlusal and buccal views of the appliance setup. The left first molar was subjected to "bodily movement" via the application of a sliding resin tube. The right first molar underwent tipping movement. μCT images after appliance setup are shown below. (D) Measurements of tooth movement: shortest distance between the first and second molars (ShD), distance between contact points (CPD), and maxillary first molar tipping angle (TIA). (E) Changes in PDL space and the corresponding area of root resorption. Schematic representation of the correlations between the narrowing of the PDL space on days 3 and 7 and OIRR on day 14. Upper: distal root on day 0 (dotted line) and days 3 and 7 (solid line). Black arrows indicate the point coinciding with the center point of the OIRR site on day 14. Lower: OIRR on day 14. F, direction of force application.

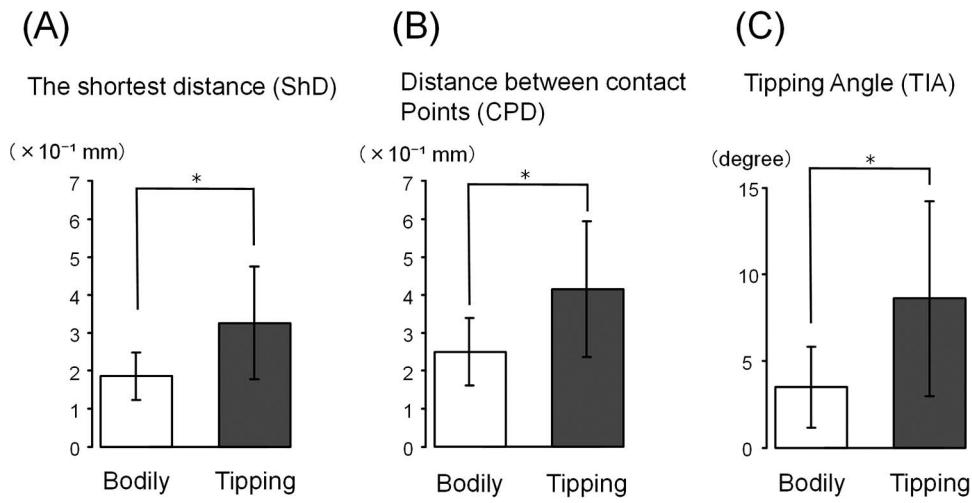

Figure 2. Orthodontic tooth movement (OTM) after orthodontic force application for 14 days. (A) ShD, (B) CPD, (C) TIA. *$P < .05$ compared between bodily and tipping tooth movements.

tissue was then demineralized in ethylenediaminetetraacetic acid solution (Osteosoft, Sigma-Aldrich) for 4 weeks. Then, the tissues were dehydrated and embedded in paraffin. Serial tissue sections were made every 6 μm parallel to the occlusal plane, starting at the cemento-enamel junction (CEJ) to identify the five first molar roots and ending when the central buccal root was no longer visible. Sections were dried, stained with hematoxylin and eosin (HE), and photographed under an optical microscope. The average numbers of HE staining at the OIRR site for each root were 70. OIRR was observed on the proximal surface of the distal root. It was observed in the cervical third of the root, extending from the CEJ to the center of the root. OIRR area and depth were measured in tissue sections using the ImageJ software (National Institutes of Health, Bethesda, MD). The observed groups were examined using histological analysis and μCT to accurately match the OIRR site and PDL space.

Quantification of PDL Compression Using μCT

Thickness of the PDL was measured using μCT images of days 0, 3, 7, and 14. Reference landmarks for the measurements were axial cuts, where the outline of the five roots of the first molar could be observed consistently. After examining all cut levels at the OIRR site in the histological image, the narrowing PDL in the μCT image was measured at the point corresponding to the center of the largest OIRR area observed among those cuts (Figure 1E). The compression ratio was calculated as the ratio of the thickness of the PDL on day 3, 7, or 14 to the thickness on day 0. The following formula was used: compression ratio (%) = [(PDL thickness on day 0 − PDL thickness on day 3, 7, or 14)/PDL thickness on day 0] × 100.

Statistical Analysis

Statistical analysis was conducted using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan). A *t*-test was used to analyze tooth movement. One-way analysis of variance, followed by Tukey's honestly significant difference test, was used to determine the compression ratio.

RESULTS

Measurement of OTM

Average OTM was 186 ± 63 μm for ShD and 249 ± 89 μm for CPD in the bodily movement group. In contrast, ShD and CPD in the tipping movement group were 327 ± 149 and 415 ± 179 μm, respectively. OTM values for ShD and CPD in the tipping movement group were significantly greater than those in the bodily tooth movement group (Figure 2A, B). The tipping movement group showed a significantly larger TIA of the maxillary first molar than the bodily tooth movement group (8.6 ± 5.6° vs 3.5 ± 2.3°, $P < .05$; Figure 2C). These results indicated that, although tooth movement was greater in the tipping movement group than in the bodily movement group, sufficient tooth movement was observed in both groups. However, the pattern of tooth movement differed between the two groups.

Extent of OIRR

Extent of OIRR was determined on day 14 (Figure 3A, B). The OIRR area in the tipping movement group was significantly greater than that in the bodily movement group (6.7 ± 3.3 × 10^{-3} mm^2 vs 3.2 ± 1.6 × 10^{-3} mm^2, $P < .05$; Figure 3C). However, there was

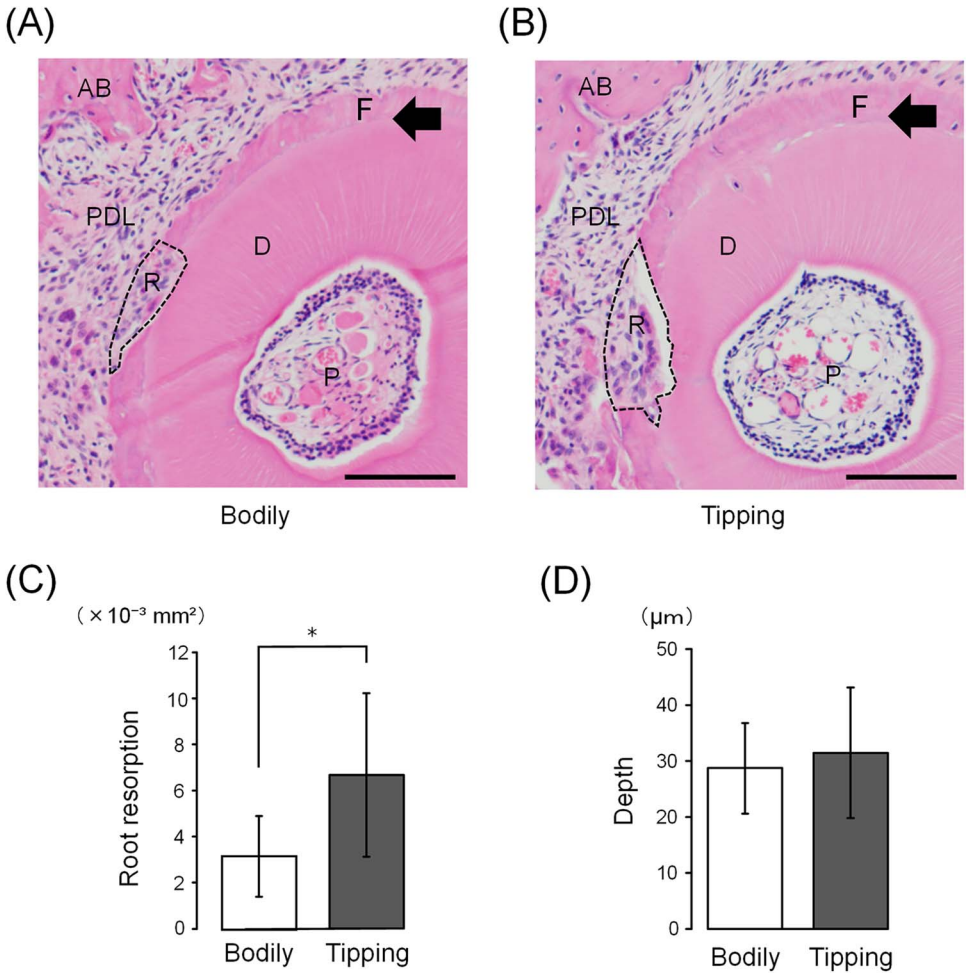

Figure 3. Hematoxylin and eosin (HE) staining. Root resorption in the (A) bodily and (B) tipping movement groups on day 14. (C) Measurement of the area of root resorption. (D) Measurement of the depth of root resorption. Dotted line indicates the root resorption lacuna. *$P < .05$ compared between bodily and tipping tooth movements. Scale bar = 100 μm. AB indicates alveolar bone; D, dentin; F, direction of orthodontic force; P, pulp; R, root resorption lacuna.

no difference in OIRR depth between the tipping and bodily movement groups (29.5 ± 12.1 μm vs 28.7 ± 8.1 μm; Figure 3D).

PDL Compression Ratio

Thickness of the PDL was measured using μCT images of days 0, 3, 7, and 14 at sites where the largest OIRR was observed in histological sections on day 14 (Figure 4A). On day 3, the PDL compression ratio was 55.4% in the bodily tooth movement group and 75.7% in the tipping tooth movement group. On day 7, the PDL compression ratios were 46.4% and 47.3% in the bodily and tipping tooth movement groups, respectively. On day 14, the PDL compression ratios were 0.4% in the bodily tooth movement group and 13.7% in the tipping tooth movement group. There was a statistically significant difference between the two groups on day 3.

Correlation between the Compression Ratio and OIRR

Whether the induction of OIRR was linked to PDL compression ratio was examined. As shown in Figure 4B, using combined data of the tipping and bodily movement groups, a strong positive correlation was observed between the area of root resorption and the PDL compression ratio on day 3 ($r = 0.89$). However, no significant correlation was observed on day 7.

DISCUSSION

When the PDL space is under significant orthodontic force, OIRR is observed on the compression side along with narrowing of the PDL. However, the correlation between the PDL compression ratio and OIRR induction previously had not been investigated.

OIRR is induced when the applied orthodontic force on PDL exceeds the capillary blood pressure of

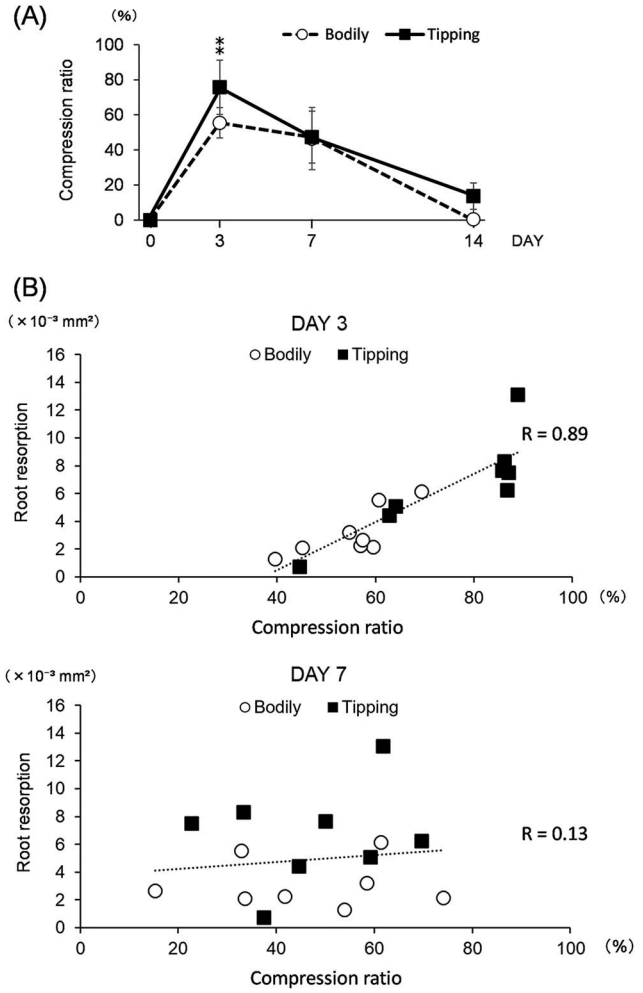

Figure 4. (A) Compression ratio of the periodontal ligament (PDL) on days 3, 7, and 14 compared with that on day 0. Bodily indicates bodily tooth movement; Tipping, tipping tooth movement. Mean values and standard deviations are shown. (B) Correlation between root resorption and the PDL compression ratio on days 3 and 7. $^{**}P < .01$ compared between the different types of tooth movement.

0.0013–0.0053 MPa (0.13–0.53 g/mm^2).[10,11] A heavy orthodontic force narrows the PDL space and collapses the capillaries, thereby inducing tissue necrosis and OIRR. In this study, an orthodontic force of 25 cN was used at a PDL compression pressure of 0.01 MPa (1.0 g/mm^2), which is approximately 2–8 times larger than the capillary blood pressure, and apparent OIRR was observed. However, no studies previously investigated PDL compression prior to the induction of OIRR at the actual OIRR site.

Previous studies reported that OIRR is first observed at 3 days, and distinctly increased 7 days after the application of orthodontic force.[2,15] Therefore, PDL compression was observed on days 3, 7, and 14 after orthodontic force application in this study. The PDL compression ratio was approximately 76% in the tipping movement group and approximately 55% in the bodily movement group on day 3, and approximately 47% in the bodily and the tipping movement groups on day 7. On day 14, thickness of the PDL returned nearly to control (day 0) levels. Also, a strong correlation was observed between the area of root resorption and the PDL compression ratio on day 3. In contrast, no significant correlation was observed on day 7.

Brudvik et al. reported that the PDL was compressed maximally by approximately 70.0% 2–3 days after the application of orthodontic force.[15] Another study showed that the PDL was compressed by 61.7% after 1 day and 43.6% after 7 days of orthodontic force application.[16] These results are consistent with the current findings that the PDL was compressed maximally at an early stage after orthodontic force application and that the thickness of the PDL subsequently returned to control levels. Additionally, they showed that the vascular space within the PDL was compressed up to 77.2% after 1 day. These results suggest that narrowing of the PDL space at an early stage after the application of orthodontic force compresses blood vessels within the PDL and causes ischemia, subsequently inducing hyalinization. Hyalinization occurs 2–3 days after orthodontic force application.[15] Additionally, the OIRR site corresponds to the area of hyalinization in PDL compressed by excessive orthodontic force application.[5,6] However, the correlation between the early-stage PDL compression ratio and OIRR induction previously remained unknown.

This study was the first to demonstrate a strong positive correlation between the PDL compression ratio at an early stage (3 days) after orthodontic force application and the extent of OIRR. In contrast, no correlation between the PDL compression ratio and extent of OIRR was observed 7 days after orthodontic force application. These results indicate that early-stage PDL ischemia due to PDL compression by orthodontic force triggers the induction of OIRR. Therefore, PDL compression during the first 2–3 days after orthodontic force application potentially determines the degree of OIRR induction.

Although tooth movement was observed in both the bodily and tipping tooth movement groups, the bodily tooth movement group showed significantly less tooth movement than the tipping movement group ($P < .05$). In addition, OIRR was also significantly lower in the bodily tooth movement group than in the tipping tooth movement group ($P < .05$). These results are consistent with those of Nakano et al. and Kondo et al.[1,14] Also, this study exhibited that there was no difference in OIRR depth between the bodily movement group and the tipping movement group, indicating that the difference in OIRR area between the bodily and tipping movement groups was not due to OIRR depth. Further studies, including three-dimensional

analyses, are needed to elucidate the differences in OIRR morphology between bodily movement and tipping movement groups.

The PDL compression ratio during bodily tooth movement was lower than that during tipping tooth movement in this study. This may explain the relatively lower OIRR in the bodily tooth movement group than in the tipping tooth movement group. A strong correlation was also demonstrated between the PDL compression ratio and extent of OIRR using combined data from the tipping and bodily movement groups.

Despite their importance, the results cannot be directly applied in clinical settings due to the involvement of other complex factors, such as three-dimensional root morphology, direction of orthodontic force, center of resistance of the tooth/dental arches, presence of cortical bone, age, condition of periodontal tissue, and individual response to treatment, in OIRR. Therefore, further studies are necessary to elucidate the correlation between PDL compression ratio and OIRR and to determine the optimal orthodontic force that will not damage the roots, especially in the early stages of orthodontic force application, in humans.

CONCLUSIONS

- A strong positive correlation was demonstrated between the PDL compression ratio and OIRR at an early stage after orthodontic force application in rats, implying the importance of early-stage PDL compression in the induction of OIRR.
- A lower PDL compression ratio and decreased OIRR were observed in bodily tooth movement than in tipping tooth movement.

ACKNOWLEDGMENTS

This work was supported by grants in aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan to HH (20K10229), AI (21K17187), TK (22K17256).

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