

Root resorption after experimental tooth movement using superelastic forces in the rat

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SUMMARY The purpose of this study was to assess the rate of root resorption in relation to different magnitudes of continuous force during experimental tooth movement using nickel–titanium (NiTi) alloy wire. Four force magnitudes of 0.8, 1.6, 4, and 8 g were applied to the upper first molars of 75 male Wistar rats (300–320 g, 10-week-old) for 1, 7, 14, 21, and 28 days and compared with a control group without an orthodontic appliance. Light microscopic images of the compressed periodontal ligament (PDL) were processed by computer, and the ratio of the root resorption lacuna length to root surface length without the lacuna was analysed and statistically compared using Tukey–Kramer multiple comparison honestly significant difference test.

The experimental groups with 4 and 8 g force showed undermining bone resorption with degenerating tissue and marked root resorption, the 1.6 g group showed only root resorption, while the 0.8 g group was similar to the control. Comparison of the ratios showed that the 0.8 g group was similar to the control with no significant difference. The ratio on day 28 in the 1.6 g group was larger than that in the 0.8 g and control groups, while on days 14, 21, and 28, the ratios in the 4 and 8 g groups were larger than those in the control ($P < 0.01$); these two experimental groups showed the same significant differences.

It is suggested that significant root resorption occurs when the force magnitude exceeds 1.6 g in the rat upper first molar during tipping tooth movement by continuous force, and the amount of root resorption increases with serial force magnitudes from 0.8 to 4 g.

Introduction

Root resorption is one of the adverse effects of orthodontic treatment, which mostly occurs due to application of excessive orthodontic force during treatment. However, orthodontists are unaware of the exact cause of resorption and measures to prevent its occurrence during treatment have yet to be elucidated. Based on orthodontic practice and experimental tooth movement studies using continuous force, it has been reported that root resorption is related to force magnitude (Reitan, 1974; King and Fischlschweiger, 1982; Kirino *et al.*, 1991; Darendeliler *et al.*, 2004; Chan and Darendeliler, 2005; Harris *et al.*, 2006; Gonzales *et al.*, 2008), duration of treatment (Harry and Sims, 1982), direction of tooth movement (Dermaut and De Munck, 1986), and treatment techniques such as Begg (Goldson and Henrikson, 1975; Parker and Harris, 1998; MacNab *et al.*, 2000), while no significant association was found between duration of treatment (Dermaut and De Munck, 1986; Beck and Harris, 1994) and degree or amount of root resorption (Beck and Harris, 1994; Owman-Moll *et al.*, 1996). These findings show that the definitive cause of root resorption has not yet been confirmed.

Regarding the detection of root resorption, Rygh (1977) and Brudvik and Rygh (1994a,b) reported that root resorption occurs beneath the main hyalinized zone in the

compressed periodontal ligament (PDL) on application of continuous force, suggesting an association between the appearances of root resorption and hyalinized tissue. On the other hand, Noda *et al.* (2006, 2007) found no root resorption in the compressed PDL when an interrupted force was applied for controlled tooth movement within the width of the PDL, in which blood vessels and no degenerating tissue were seen at the maximal compressed region. From these results, it can be assumed that root resorption occurs when biological activity is lost in the compressed PDL, where blood circulation is obstructed and tissue degeneration results.

In the present study, continuous forces of four magnitudes (Noda *et al.*, 2000), two light forces, which do not cause tissue degeneration, a heavy force which induces tissue degeneration, and a heavier force applied for a relatively longer period causing tissue degeneration, were used for experimental tooth movement in order to morphologically and statistically assess the association between root resorption and appearance of degenerating tissue.

Materials and methods

The experimental protocol in animals was approved by the Institutional Animal Care and Use Committee of Tsurumi

University and was carried out in accordance with the Guidelines for Animal Experimentation of Tsurumi University.

Seventy-five male Wistar rats (body weight range: 300–320 g, 10 weeks old) were used in this experiment. An orthodontic appliance with two nickel–titanium (NiTi) wire springs (Sentalloy 0.016 inch round wire; Tomy International Co. Ltd., Tokyo, Japan; Figure 1) was used to move the maxillary first molars of the rats under anaesthesia with sodium pentobarbital. Four force magnitudes of 0.8 g (force range: 0.73–0.86 g), 1.6 g (1.54–1.75 g), 4 g (3.73–4.27 g), and 8 g (7.61–8.35 g; Noda *et al.*, 2009) were applied by reducing the NiTi wire with a carborundum point under water cooling. The experimental periods were 1, 7, 14, 21, and 28 days after the start of tooth movement. Simultaneously, control groups without an appliance were included to correspond with these experimental periods. Three rats were allocated to each period in the experimental and control groups.

Histological procedure

After tooth movement, all rats were perfused with 2.5 per cent glutaraldehyde fixative (0.1 M phosphate buffer) for 10 minutes through the ascending aorta under deep anaesthesia. The maxillae were resected and trimmed around the upper first molars. The trimmed tissue blocks were decalcified by immersion in 5 percent ethylenediamine tetraacetic acid (EDTA)-2Na (7 per cent sucrose, pH 7.4, 4°C) for 10 days. After washing overnight with 0.1 M phosphate buffer under cold conditions, 90 µm thick serial sections of the tissue blocks were prepared perpendicular to the root axis using a Vibratome (Series-1000; Technical Products International, Inc., St Louis, Missouri, USA). After post-fixation with 1.0 per cent osmium tetroxide for 1 hour, the sections were dehydrated with a graded series of ethanol and embedded in epoxy resin (Epok 812; Oukun Syoji Co. Ltd., Tokyo, Japan). Serial semi-thin sections (approximately 15 sections), 2.5–3 µm thick, were cut from the resin-embedded thick sections using a diamond knife, and stained with toluidine blue. Finally, a section including the maximally compressed region showing the most suitable condition for image processing was selected from the serial sections.

Image processing and statistical analysis

An area measuring $700 \times 2400 \mu\text{m}^2$, including the maximum compressed PDL on the buccal side, was selected from the semi-thin section for light microscopic examination (Figure 2) and was recorded on positive film (Vano; Olympus Corporation, Tokyo, Japan) for each group ($n = 6$). After scanning (Dimage Scan Dual II; Konica Minolta, Tokyo, Japan), the image data were processed on Powerbook G4 (Apple Japan Inc., Tokyo, Japan), and root surface length including the root resorption lacuna was measured twice using Photoshop CS3 Extended (Adobe Systems

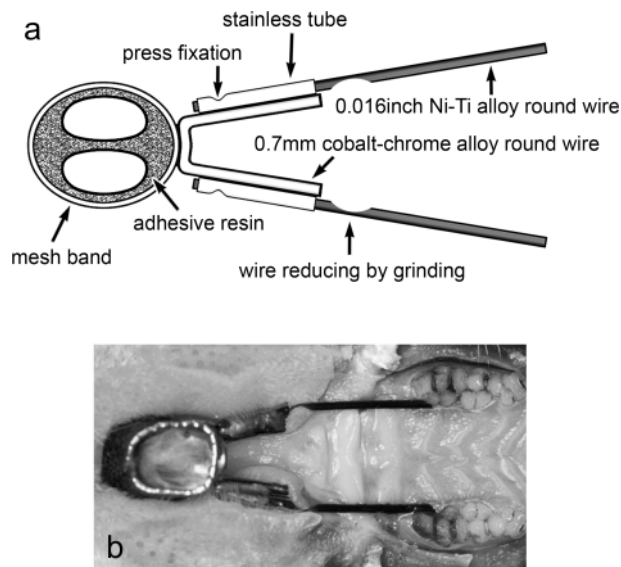


Figure 1 (a) Illustration of an orthodontic appliance with a superelastic force. The appliance consists of a mesh band, 0.7 mm cobalt-chrome round wire, two short stainless tubes, and two 0.016 inch nickel–titanium alloy (NiTi) round wires. The parts without the NiTi wire were assembled with silver solder. The wire was inserted into the tube and was fixed by pressure. The force was adjusted by grinding and reducing the wire under water cooling. (b) An image of the appliance on a rat maxilla. Two wire springs are applied to the upper first molars of both sides, achieving tooth movement in the buccal direction.

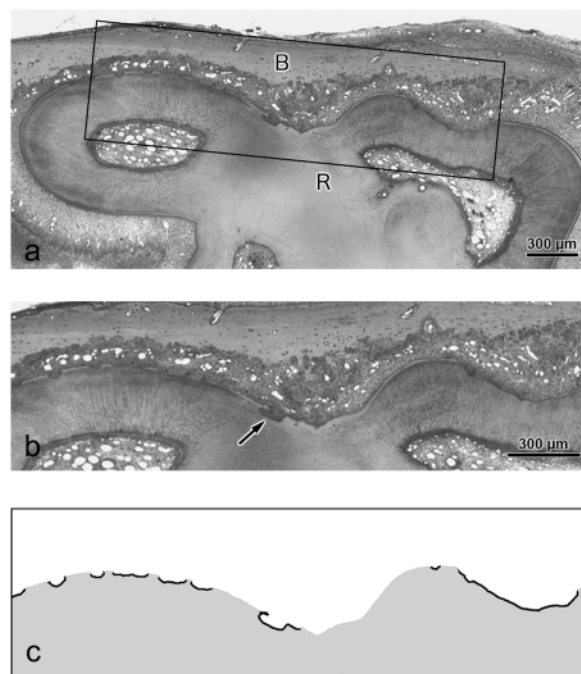


Figure 2 Images of the measurement area stained with toluidine blue and with computer processing. (a) A measurement area of $700 \times 2400 \mu\text{m}^2$, including the maximally compressed region, surrounded by a black line, was selected on the buccal side of the periodontal ligament. R: dental root; B: bone. (b) A higher magnification of the measurement area as shown in Figure 2a. Root resorption with a deep resorbing lacuna (arrow) can be seen on the root surface. (c) Extracted processing image of the cross-section of the root, coloured light grey, as shown in Figure 2b. Black thin lines on the root show the outlines of the resorbing lacunae; scale bar, 300 µm.

Incorporated, San Jose, California, USA). The average of the two values was used in this study. The ratio of the root resorption lacuna length to root surface length without the resorption lacuna (per cent) was calculated in the experimental and control groups in order to assess root resorptive activity and compared using Tukey–Kramer multiple comparison honestly significant difference test (JMP 7; SAS Institute Inc., Cary, North Carolina, USA).

Results

Histological findings

In the 0.8 and 1.6 g force groups, a decrease of PDL width was seen on day 1 after tooth movement (Figure 3). Slight root resorption was seen on day 21 in the former and marked resorption on days 21 and 28 in the latter. No degenerating tissue, staining positive with toluidine blue, was observed at the compressed region in these two experimental groups. On the other hand, degenerating tissue was seen at the compressed region of the PDL from days 1 to 14 in the 4 g group and from days 1 to 21 in the 8 g group (Figure 3). A significant decrease in PDL width was seen on day 1 and undermining bone resorption on day 7 in these groups. No morphological difference was observed in the controls for any experimental periods.

Computer-processed images of root surface with resorbing lacunae in the measured area

Black thin lines indicating the length of resorbing lacuna were seen on days 21 and 28 in the 1.6 g group and on days 14, 21, and 28 in the 4 and 8 g groups (Figure 4). Single, or some scattered, lines were also seen on days 14 and 21 in the 0.8 g group. The total length of the line in the 1.6 g group was visibly shorter than that in 4 and 8 g groups. In the control, no or only some short lines were seen during the experimental period.

Ratio of the root resorption lacuna length to root surface length and statistical analysis

The median values of the ratio in the 0.8 g group showed a flattened linear graph with a slight increase–decrease pattern (Figure 5). There was no statistical difference in any experimental period (Table 1). The ratio in the 1.6 g group increased gradually from days 1 to 14, steeply between days 14 and 21, and tended to plateau between days 21 and 28 (Figure 5). A statistical difference was observed between days 1 and 28 in this group (Table 1). On the other hand, the ratio in the 4 and 8 g groups increased steeply from days 1 to 21. The changes until day 28 showed a decrease in the 4 g group and tended to plateau in the 8 g group (Figure 5). Statistical differences were present among days 21 and 28 and other experimental periods and between days 1 and 14 in these groups (Table 1). In the controls, a slight decrease

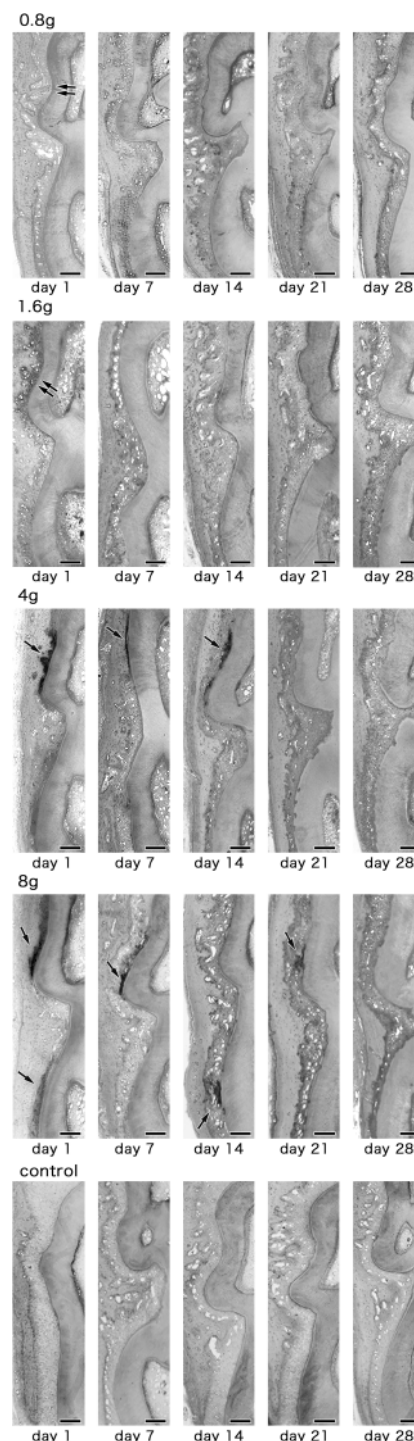


Figure 3 Images of the measurement areas stained with toluidine blue in the 0.8, 1.6, 4, and 8 g experimental groups and in the controls. Decrease of periodontal ligament (PDL) width (double arrows) on day 1 after tooth movement in the 0.8 and 1.6 g groups. Slight root resorption was observed on day 21 in the former and marked resorption on days 21 and 28 in the latter. No degenerating tissue stained with toluidine blue was seen during the experimental period in either group. On the other hand, degenerating tissue was seen at the compressed region of the PDL from days 1 to 14 in the 4 g group and from day 1 to day 21 in the 8 g group. These groups showed a significant decrease in PDL width on day 1 and undermining bone resorption on day 7. In the controls, no morphological difference was seen for any experimental periods. Right side, bone; left side, root; scale bar, 200 μ m.

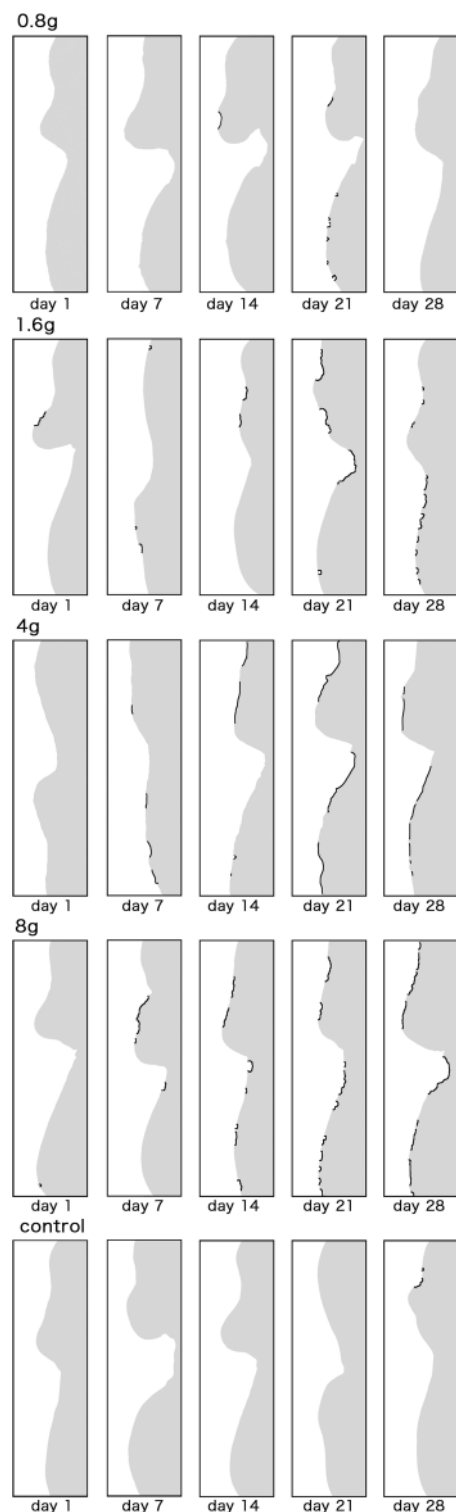


Figure 4 Computer-processing images for obtaining the ratio of the root resorption lacuna length to root surface length without lacuna in the experimental and control groups, expressing root resorption activity. Black thin lines indicate the length of resorbing lacuna, which were seen in large numbers on days 21 and 28 in the 1.6 g group and on days 14, 21, and 28 in the 4 and 8 g groups. Single or some scattered lines were seen on days 14 and 21 in the 0.8 g group, similar to the control that showed no or only some short lines during the experimental period. The total length of the line in the 1.6 g group was visibly shorter than that in the 4 and 8 g groups.

and increase of the ratio was seen during the experimental period, but these were not statistically significant (Figure 5 and Table 1). The highest ratio recorded was 63.2 per cent on day 28 in the 8 g group, indicating severe root resorption, followed by 56.2 per cent on day 21 in the 4 g, 24.9 per cent on day 21 in 1.6 g, 2.8 per cent on day 21 in 0.8 g groups, and 1.4 per cent on day 7 in the controls (Table 2).

Comparing the ratio between the controls and each experimental group (Table 1), there were significant differences among the controls and all experimental groups except the 0.8 g group; the ratio and increase–decrease pattern of this group were similar to the control. The ratio on day 28 in the 1.6 g group was larger than that for all experimental periods in the control ($P < 0.01$ on day 14 and $P < 0.05$ for the periods). On the other hand, the ratios on days 14, 21, and 28 in the 4 and 8 g groups were larger than those at all periods in the control group ($P < 0.01$).

Comparison among the experimental groups (Table 1) showed that the patterns of significant differences between the 0.8 and 4 or 8 g groups were similar to those between the control and the 4 or 8 g groups. Patterns of the differences between the 1.6 and the 4 or 8 g groups were also similar to those between the control and 0.8 g group, where a significant difference was present on day 28 in the former and day 1 in the latter ($P < 0.01$). The ratio on day 1 in the 4 and 8 g groups was less than on days 14, 21, and 28 ($P < 0.01$).

Discussion

Experimental studies (Azuma, 1970; Rygh, 1977; Brudvik and Rygh, 1994a,b; Noda *et al.*, 2000) and clinical reports (Kurol *et al.*, 1996; Kurol and Owman-Moll, 1998) showed that degeneration of PDL tissue preceded root resorption, indicating that the degeneration of the PDL may be one factor affecting root resorption. However, the fundamental factors involved and the trigger for resorption are not clearly known. In the present study, root resorption was assessed on the points of force magnitude and time course using continuous force in close relation to the degeneration of PDL.

Concerning the four force magnitudes used in the present study, the 0.8 and 1.6 g force are regarded as light forces that do not induce degeneration of tissue at the compressed PDL, where blood vessels were seen (Figure 3) indicating preservation of blood circulation (Noda *et al.*, 2000, 2009). On the other hand, forces of 4 and 8 g are regarded as heavy. Heavy forces during tipping result in degeneration of tissue, which stains positive with toluidine blue and is devoid of blood vessels at the maximal compressed region of the alveolar crest (Figure 3; Noda *et al.*, 2000, 2009). It has been reported that root resorption occurs after the appearance of degenerating tissue (Brudvik and Rygh, 1994a; Kurol *et al.*, 1996) and the resorption process occurs after elimination of the tissue (Brudvik and Rygh, 1994b),

Table 1 Results of the Tukey honestly significant difference test of the ratio of the root resorption lacuna length to root surface length without resorption lacuna for all experimental periods in the experimental and control groups 1d, 7d, 14d, 21d, and 28d indicates 1, 7, 14, 21, and 28 days after tooth movement.

| | | 0.8g | | | | | 1.6g | | | | | 4g | | | | | 8g | | | | | cont | | | | |
|------|-----|------|----|-----|-----|-----|------|----|-----|-----|-----|----|----|-----|-----|-----|----|----|-----|-----|-----|------|----|-----|-----|-----|
| | | 1d | 7d | 14d | 21d | 28d | 1d | 7d | 14d | 21d | 28d | 1d | 7d | 14d | 21d | 28d | 1d | 7d | 14d | 21d | 28d | 1d | 7d | 14d | 21d | 28d |
| 0.8g | 1d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 7d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 14d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 21d | | | | | | | | | | | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 28d | | | | | | | | | | | | | * | ** | ** | | | ** | ** | ** | | | | | |
| 1.6g | 1d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 7d | | | | | | | | | | | | | * | ** | ** | | | ** | ** | ** | | | | | |
| | 14d | | | | | | | | | | | | | * | ** | ** | | | ** | ** | ** | | | | | |
| | 21d | | | | | | | | | | | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 28d | * | * | * | | | * | | | | ** | | | ** | ** | ** | ** | | ** | ** | ** | * | * | ** | * | * |
| 4g | 1d | | | | | | | | | | ** | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 7d | | | | | | | | | | | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 14d | ** | ** | ** | | * | ** | * | * | | | ** | | ** | * | * | ** | | ** | ** | ** | ** | ** | ** | ** | ** |
| | 21d | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | * | * | ** | * | ** | * | * | ** | ** | ** | ** | ** |
| | 28d | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | * | * | * | ** | * | ** | * | * | ** | ** | ** | ** | ** |
| 8g | 1d | | | | | | | | | | ** | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 7d | | | | | | | | | | | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 14d | ** | ** | ** | ** | ** | ** | ** | ** | | | ** | ** | | * | * | ** | | ** | ** | ** | ** | ** | ** | ** | ** |
| | 21d | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | | | ** | ** | ** | ** | * | ** | ** | ** | ** | ** |
| | 28d | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | | | ** | ** | ** | * | * | ** | ** | ** | ** | ** |
| cont | 1d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 7d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | | | | | |
| | 14d | | | | | | | | | | ** | | | ** | ** | ** | | | ** | ** | ** | * | * | ** | * | * |
| | 21d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | * | * | ** | * | * |
| | 28d | | | | | | | | | | * | | | ** | ** | ** | | | ** | ** | ** | * | * | ** | * | * |

* $P < 0.05$; ** $P < 0.01$.

suggesting a close relationship between the appearances of root resorption and degenerating tissue. The findings in both the 4 and 8 g groups support these reports. In addition, significant differences were seen on day 28 in the 1.6 g group compared with all experimental periods in the control. This indicates that root resorption occurs at a force magnitude less than that inducing PDL degeneration. A force magnitude of 1.6 g can be converted to approximately 6.42 g/cm² (Noda *et al.*, 2000). Guyton and Hall (2000) stated that capillary blood pressure is a fundamental factor, which affects the vascular active transport system and the pressure measured with the isogravimetric method, considered as functional pressure, is 17 mmHg (23 g/cm²) in humans. Although the capillary blood pressure of the rat PDL has not yet been determined, the interstitial fluid pressure in the PDL is 15.2 mmHg (Kristiansen and Heyeraas, 1989). Nikolai (1975) reported that the force magnitude increases 2.25 times at the alveolar crest when the working point is located at the tooth crown and the type of tooth movement is tipping. Thus, the force magnitude of 6.42 g/cm² in the present study corresponds to approximately 14.5 g/cm², which is close to rat interstitial fluid pressure. Since capillary pressure is generally higher than interstitial fluid pressure (Guyton and Hall, 2000) and plasma migrates from capillary pressure to interstitial fluid pressure by negative force, it could be speculated that root resorption,

i.e. pathological damage, occurs in a compressive condition where the capillary pressure is lower than the interstitial fluid pressure in the PDL. The hypothesis that a force magnitude less than interstitial fluid pressure does not trigger root resorption might be supported by the results in the 0.8 g group (3.21 g/cm²). Slight root resorption was seen but no significant difference was present between this group and the control.

A distinct difference between the 4 and 8 g groups was in the timing of the appearance of degenerating tissue; the former was from days 1 to 14 and the latter from days 1 to 21, indicating that a heavier continuous force delays remodelling of compressed PDL. On the other hand, significant differences in the ratio of root resorption lacuna length to root surface length were seen in the two groups (Table 1), in which the ratios on days 14, 21, and 28 were larger than on day 1, as well as in the control. Additionally, the increase–decrease patterns of the ratios were similar in the two groups. From these results, it is suggested that root resorption increases significantly from 14 days after tooth movement with a continuous force that induces PDL degeneration, regardless of the force magnitude. Therefore, the similar pattern of significant differences seen in both the 4 and 8 g groups might indicate that the process time for root resorption to follow the initial appearance of the degenerating tissue is not influenced by the degree of the heavy continuous force.

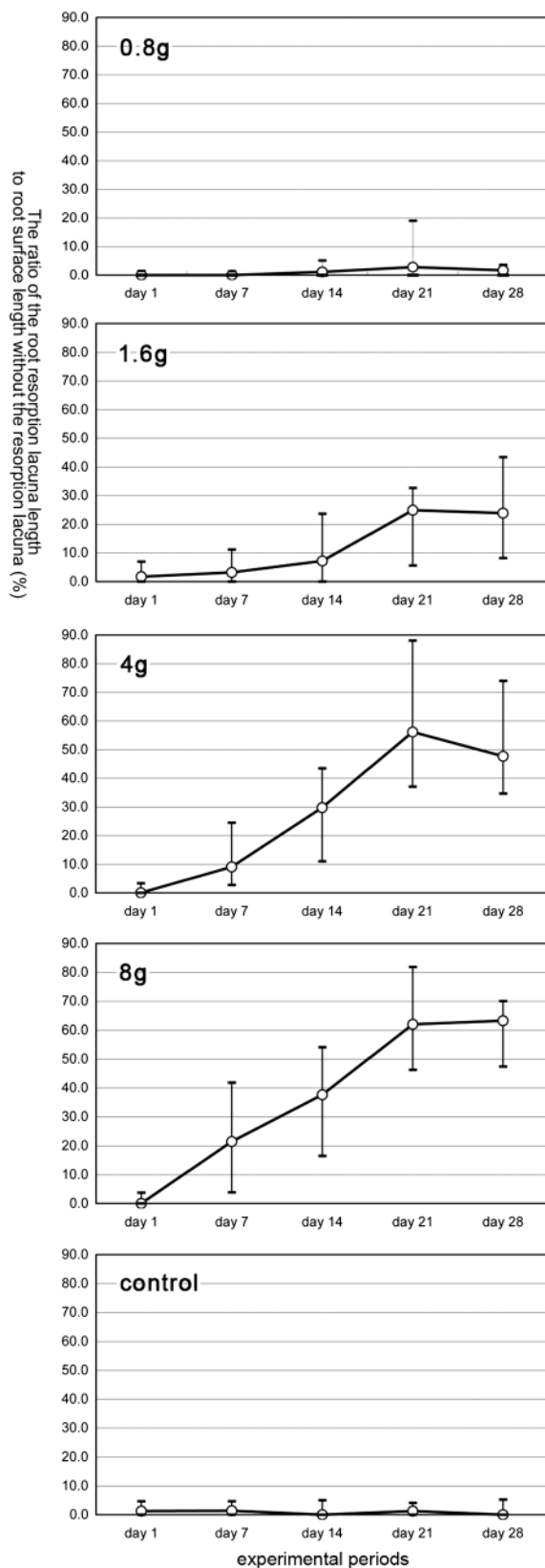


Figure 5 Line graphs expressing the ratio of the root resorption lacuna length to root surface length without the resorption lacuna in the experimental and control groups during the experimental periods.

Table 2 Median and maximum–minimum values of the ratio of the root resorption lacuna length to root surface length without the resorption lacuna during experimental periods in experimental and control groups.

| | Experimental periods | Median (%) | Maximum (%) | Minimum (%) |
|---------|----------------------|------------|-------------|-------------|
| 0.8 g | Day 1 | 0.0 | 1.4 | 0.0 |
| | Day 7 | 0.0 | 1.3 | 0.0 |
| | Day 14 | 1.1 | 5.1 | 0.0 |
| | Day 21 | 2.8 | 19.0 | 0.0 |
| | Day 28 | 1.7 | 3.6 | 0.0 |
| 1.6 g | Day 1 | 1.7 | 7.0 | 0.0 |
| | Day 7 | 3.3 | 11.2 | 0.0 |
| | Day 14 | 7.2 | 23.6 | 0.0 |
| | Day 21 | 24.9 | 32.7 | 5.6 |
| | Day 28 | 23.9 | 43.4 | 8.2 |
| 4 g | Day 1 | 0.0 | 3.3 | 0.0 |
| | Day 7 | 9.0 | 24.5 | 2.7 |
| | Day 14 | 29.7 | 43.4 | 11.0 |
| | Day 21 | 56.2 | 88.0 | 37.1 |
| | Day 28 | 47.7 | 74.0 | 34.6 |
| 8 g | Day 1 | 0.0 | 3.7 | 0.0 |
| | Day 7 | 21.4 | 41.8 | 3.9 |
| | Day 14 | 37.6 | 54.1 | 16.4 |
| | Day 21 | 62.0 | 81.8 | 46.3 |
| | Day 28 | 63.2 | 70.1 | 47.4 |
| Control | Day 1 | 1.2 | 4.6 | 0.0 |
| | Day 7 | 1.4 | 4.6 | 0.0 |
| | Day 14 | 0.0 | 5.0 | 0.0 |
| | Day 21 | 1.2 | 4.1 | 0.0 |
| | Day 28 | 0.0 | 5.2 | 0.0 |

It is known that the degenerating tissue is predominantly resorbed by multinucleated giant cells, regarded as foreign body giant cells (Rygh, 1974; Okumura, 1982; Brudvik and Rygh, 1994a,b; Hellsing and Hammarstrom, 1996; Noda *et al.*, 1997). Rygh (1974) and Brudvik and Rygh (1994b) reported that the multinucleated giant cells closely resemble osteoclasts and odontoclasts (Matsuda, 1992), except for the lack of the ruffled border. On the other hand, Okumura (1982) and Noda *et al.* (1997) reported that multinucleated giant cells have a ruffled border structure enclosing the degenerating tissue. It is well known that calcified tissue is resorbed by the ruffled border structure of multinucleated giant cells and lack of this structure may mean an inability for hard tissue resorption (Chambers *et al.*, 1984; Chambers, 1985; Zaidi *et al.*, 1994; Yoshii *et al.*, 2000). No morphological evidence that giant cells have a relationship with root resorption was found in the present study, but it is speculated that cellular functional diversity may be exercised at the boundary area between the degenerating tissue and root surface.

Conclusions

The following conclusions were derived from the findings of this study.

1. Root resorption occurs when a force magnitude exceeds 1.6 g, assuming it to be more than PDL interstitial fluid

pressure, during tipping tooth movement of the rat upper first molar with a continuous force.

2. There is a force magnitude threshold between 0.8 and 1.6 g, which delineates optimal and pathological tooth movement.
3. The timing of root resorption depends on degenerating tissue appearance; resorption increased significantly 14 days after the start of tooth movement with heavy forces of 4 and 8 g, regardless of the heavy force magnitude.
4. The amount of root resorption is influenced by serial force magnitudes from 0.8 to 4 g but not those exceeding 8 g.

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