Original Article

Prevention of root resorption in hypofunctional teeth by occlusal function recovery

Akiko Hayashi[a]; Hidetaka Hayashi[b]; Toshitsugu Kawata[c]

ABSTRACT

Objective: To clarify whether occlusal hypofunction is one of the key determinants for root resorption during tooth movement and root resorption is prevented by its recovery.

Materials and Methods: The rats were randomly divided into one control and two experimental groups: hypofunctional and recovery groups. In the hypofunctional group, an anterior metal cap and bite plate were attached to the maxillary and mandibular incisors to simulate occlusal hypofunction. In the recovery group, the appliances were removed 7 weeks after their use, and the rats were allowed to bite for 4 weeks after removal. At the age of 16 weeks, the upper first molars were moved and after 0, 7, 14, and 21 days, the maxillae were resected. The resorption area was quantified morphohistologically and tartrate-resistant acid phosphatase (TRAP)-positive cells on the root surface were counted. We also examined the expressions of receptor activator of nuclear factor-κB ligand (RANKL), macrophage-colony stimulating factor (M-CSF), and interleukin (IL)-8 immunohistochemically.

Results: The amount of root resorption and the number of TRAP-positive cells were significantly greater in the hypofunctional group than in the control and recovery groups. Moreover, immunoreactivity for RANKL, M-CSF, and IL-8 was detected in the periodontal ligament and on the root surface in the hypofunctional group.

Conclusion: Occlusal hypofunction is one of the critical factors for root resorption; however, root resorption may be prevented by recovery of occlusal function. (*Angle Orthod.* 2016;86:214–220.)

KEY WORDS: Root resorption; Occlusal hypofunction

INTRODUCTION

Occlusal stimulus is an essential factor for maintenance of the structural integrity of the periodontal ligament (PDL) because the PDL has a functional structure suitable for occlusal pressure.[1] In orthodontic practice, we often encounter malocclusions such as open bite and high canine, or under-occluded teeth. Various atrophic changes have been reported in the PDL of these teeth with insufficient occlusal stimuli known as hypofunctional teeth.[2,3] In addition, the amount of root resorption was shown to be significantly greater in hypofunctional teeth than in control teeth under normal occlusal conditions during experimental tooth movement in rats.[4]

Clinical studies also demonstrated that the severity of root resorption, examined radiographically, was greater in open bite cases than in deep bite cases during orthodontic tooth movement with multibracket appliances.[5,6] Moreover, it is assumed that the root and PDL structures may be less developed because of disuse atrophy resulting from defective occlusal function, and that these structures may recover after occlusal stimuli is regained.[7] However, it still remains unclear whether or not root resorption of hypofunctional teeth is more prominent during experimental tooth movement and can be prevented by the recovery of occlusal stimuli. It is also unclear if root resorption during orthodontic tooth movement is associated with the expressions of receptor activator of nuclear factor-κB ligand (RANKL), macrophage-colony stimulating factor (M-CSF), and interleukin (IL)-8.

The purpose of this study was to clarify if occlusal hypofunction is one of the key determinants for root

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resorption during experimental tooth movement and whether root resorption is prevented by the recovery of occlusal stimuli.

MATERIALS AND METHODS

Animals

Forty-eight 5-week-old Wistar strain male rats (Charles River Labs, Yokohama, Japan) were used in this study. These rats were randomly divided into three groups (n = 16, respectively): two experimental and one control groups. Two experimental groups, hypofunctional and recovery, were established. In the hypofunctional group, an appliance consisting of a metal cap made of band material (3M Unitek Co, Tokyo, Japan) and an anterior bite plate made of new ST lock base (Dentsply-Sankin, Tokyo, Japan) were bonded with composite resin (Clearfil Majesty LV, Kuraray Co Ltd, Kurashiki, Japan) on the maxillary and mandibular incisors, respectively[7,8] (Figure 1A). In the hypofunctional group, the appliances were used for 11 weeks. In the recovery group, the appliances were removed 7 weeks after initiation of the experiment, and the rats were allowed to bite for 4 weeks after the removal. The time schedule of the experiment is summarized in Figure 1B.

Furthermore, at 16 weeks of age, the upper first molars were moved in a palatal direction using an experimental quad helix type appliance (QH) made from a 0.5-mm diameter cobalt-chromium (Co-Cr) alloy wire (Dentsply-Sankin) and 0.4-mm diameter Co-Cr alloy wire (Rocky Mountain Morita, Tokyo, Japan) to produce a 10 g force (Figure 1C). To measure the 10 g force, a tension gauge (Oba keiki, Tokyo, Japan) was used. After 0, 7, 14, and 21 days, each rat was humanely killed (n = 4, respectively).

All animals were fed on powder diet (Rodent Diet CE-2; Japan CLEA Inc, Tokyo, Japan), and water ad libitum under a 12-hour light/dark environment at a constant temperature of 23°C. During the experimental period, the rats were weighed once a week. All experiments were approved by the Animal Experimentation Committee at Hiroshima University, and all procedures were performed under the guidelines for animal research of Hiroshima University.

Tissue Preparation

The animals were deeply anesthetized in diethyl ether, and followed by intraperitoneal injection of chloral hydrate (400 mg/kg). Then, the animals were sacrificed by means of transcardiac perfusion with 4% paraformaldehyde. The maxillae were immediately immersed in the same fixative solution overnight at 4°C. Subsequently, tissue blocks were decalcified in

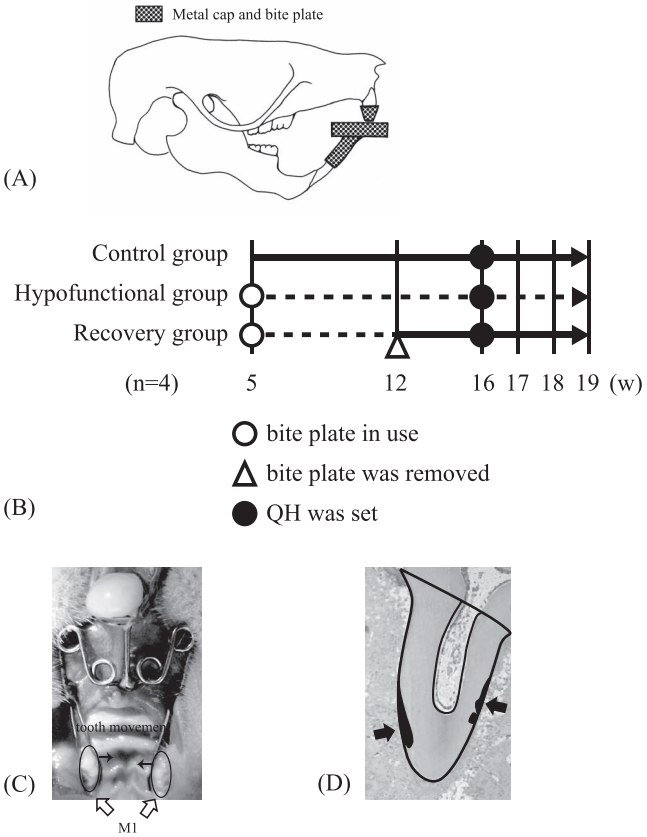

Figure 1. Experimental model. (A) To eliminate occlusal force at the molar region, an anterior bite plate and a metal cap were attached to the mandibular and maxillary incisors, respectively. (B) Time schedule of experiment. Animals were humanely killed 0, 7, 14, and 21 days after the attachment of a quad helix (QH) type appliance. (C) QH type appliance image. The upper first molars were moved in a palatal direction using an experimental QH type appliance made from a 0.5-mm diameter cobalt-chromium (Co-Cr) alloy wire and 0.4-mm diameter Co-Cr alloy wire to produce a 10 g force. (D) HE staining image. In each section, the resorption area and the root area were measured with an image scanner and the ratio calculated as: Percentage of root resorption = the combined area of root resorption lacunae / (the area of whole root – the area of radicular pulp) × 100 (%).

14% ethylene diamine tetra acetic acid (EDTA) at 4°C for 4–6 weeks and embedded in paraffin. Serial sections of 5.0-μm thickness, perpendicular to the long axis of the distobuccal root of the maxillary first molar, were made along the frontal cross-section.

Ten typical sections out of all the serial sections including the longest root canal were stained. The sections were stained with hematoxylin and eosin (HE) and with tartrate-resistant acid phosphatase (TRAP) for immunohistochemical examination of RANKL, M-CSF, and IL-8. The periodontal tissues on the palatal side of the distobuccal root of the upper first molar were observed to investigate the expressions of RANKL, M-CSF, and IL-8 in the root resorption area during experimental tooth movement.

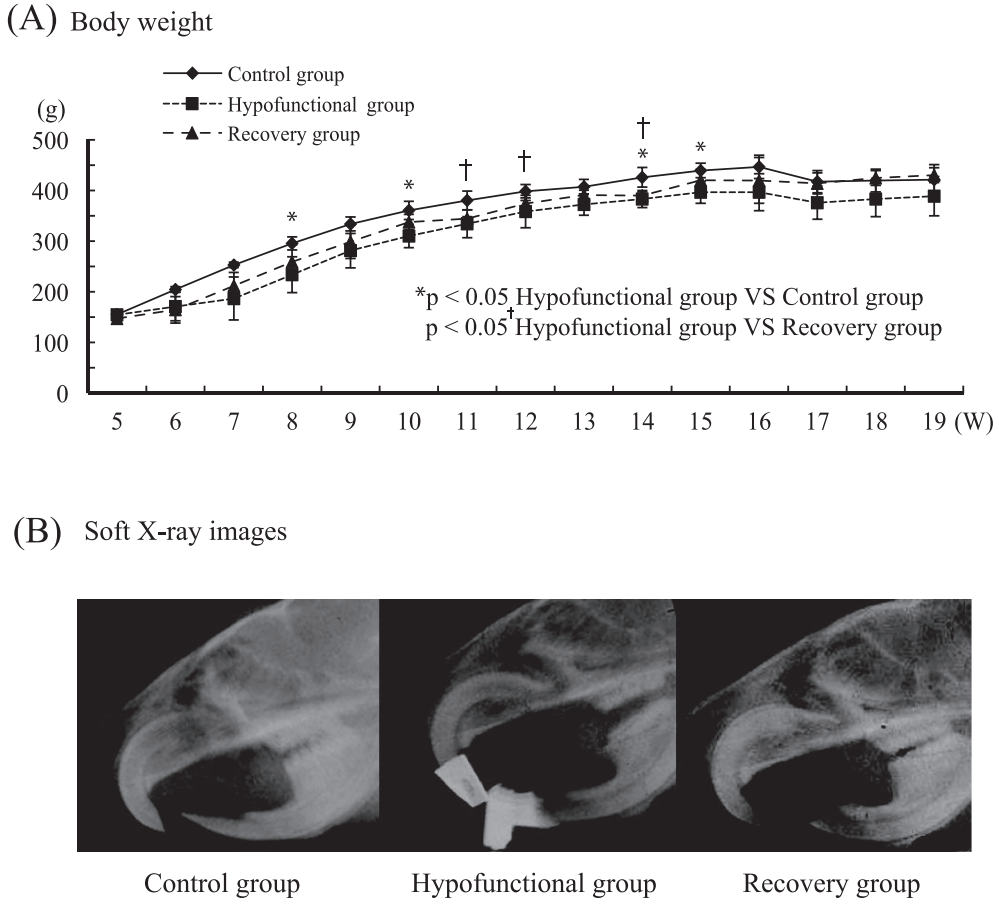

Figure 2. Body weight and soft X-ray images. (A) All of the animals exhibited normal growth without any significant differences among the experimental groups from 16 weeks to 19 weeks. (B) It was confirmed that at the 16 weeks of age.

Immunohistochemical Staining

Tissue sections that included the root canal were prepared from 16- and 19-week-old rats and subjected to immunohistochemical staining for RANKL, M-CSF, and IL-8.

Sections from each group were immunostained with a 1:100 dilution of primary anti-rat RANKL rabbit polyclonal antibodies (Abcam Inc, Tokyo, Japan), followed by anti-rabbit secondary IgG antibody (Hystfine simple stain rat MAX-PO(R); Nichirei, Tokyo, Japan), immunostained with a 1:50 dilution of primary anti-rat M-CSF goat polyclonal antibodies (Santa Cruz Biotechnology Inc, Paso Robles, Calif), followed by anti-goat secondary IgG antibody (Hystfine simple stain rat MAX-PO(G); Nichirei) and immunostained with a 1:5 dilution of primary anti-rat IL-8 rabbit polyclonal antibodies (Abcam Inc), followed by anti-rabbit secondary IgG antibody (Hystfine simple stain rat MAX-PO(R), Nichirei). Immunoreactive sites were finally visualized by 3,3′-diaminobenzidine (DAB) (DAB substrate kit; Nichirei). Counterstaining was performed using hematoxylin. Sections incubated without primary antibody were used as a negative control.

Quantitative Analysis

Ten typical sections out of all of the serial sections, including the longest root canal, were stained by TRAP and HE. The number of TRAP-positive cells on the root surface was also determined.[9]

The resorption area was quantified histomorphometrically. Each of the sections was used to measure the resorption area and the root area with an image scanner connected to a personal computer and an image-analyzing software (BZ analyzer soft BZ-H1A; Keyence, Osaka, Japan). Finally, the root resorption ratio was calculated as shown below[9] (Figure 1 D); the number of TRAP-positive cells and the resorption area were quantified three times for each animal.

Statistical Analysis

The number of odontoclasts and the area of root resorption were measured in each group. These values of the experimental groups were compared with those of

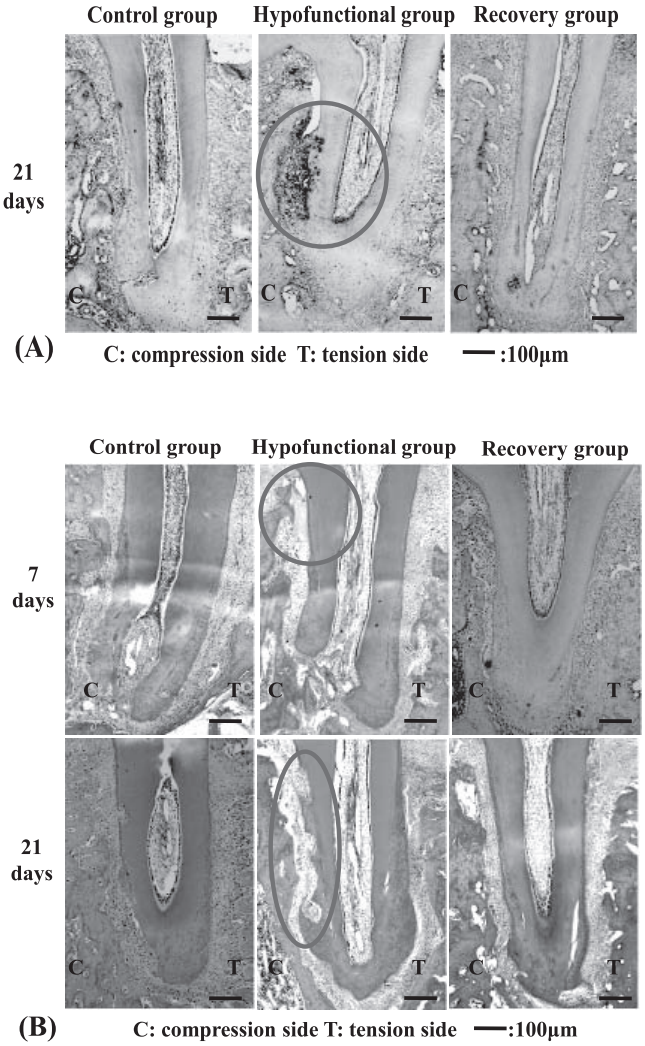

Figure 3. TRAP and HE stainings. (A) Histologic images with TRAP staining at day 21. The larger number of TRAP-positive cells in the hypofunctional group was detected at day 21 after initiation of experimental teeth movement (○). (B) Histologic images with HE staining at day 7 and 21. In the hypofunctional group, hyalinized tissue was observed at day 7 around the compressed area (○). The larger amount of root resorption and hyalinized tissue was observed in the hypofunctional group at day 21 compared with the control and recovery groups (○).

the control group. To determine the statistical significance of mean differences among the three groups of rats, after the normal distribution of data was confirmed by Levene test, we performed repeated one-way analysis of variance (ANOVA) and the Tukey-Kramer test using Statview (Abacus Concepts Inc, Tokyo, Japan) with a confidence level of $P < .05$.

RESULTS

Body Weight

All the animals exhibited normal growth without any significant differences among the experimental groups from 16 weeks to 19 weeks (Figure 2A).

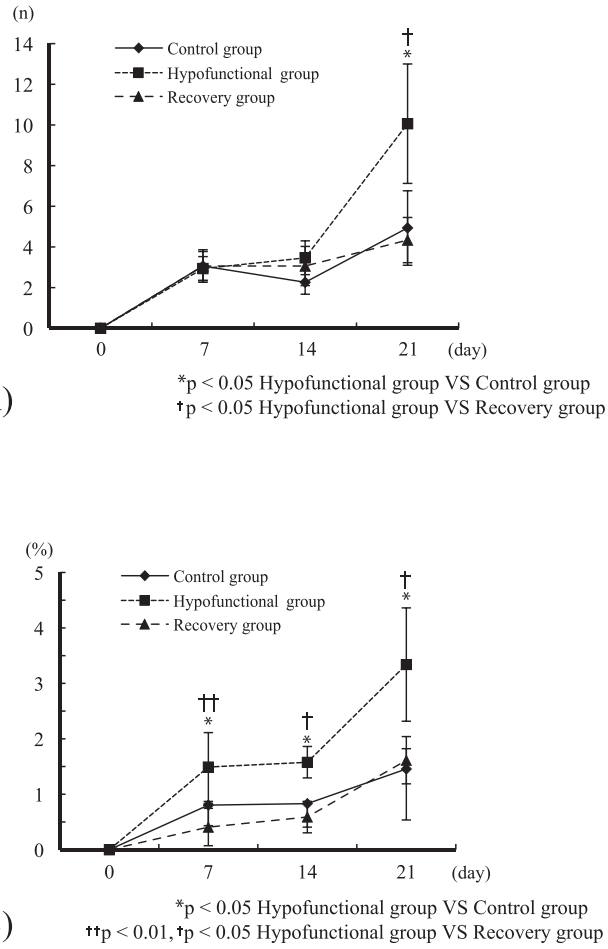

Figure 4. Quantitative analysis. (A) The number of odontoclasts. The number of TRAP-postive odontoclasts in the hypofunctional group significantly increased up to day 21 (about 2.5-fold) in comparison with the control and recovery groups ($P < .05$). (B) The area of root resorption. The root resorption ratio increased significantly from 0 to 21 days in the three groups. The amount of root resorption was significantly greater in the hypofunctional group at 7, 14, and 21 days (about 2.0-fold, respectively) than in the control and recovery groups ($P < .05$).

Occlusal Condition

In order to confirm the condition of the occlusal contact in the molar region, soft X-ray images were taken before sacrifice for each group (Figure 2B). In the hypofunctional group, all of the rats maintained the condition of occlusal hypofunction.

Number of Odontoclasts

In all groups, several resorption lacunae with TRAP-positive multinucleated osteoclasts appeared on the alveolar bone surface of the pressure side on day 7. In the control and recovery groups, bone resorption lacunae with multinucleate TRAP-positive osteoclasts were recognized on days 14 and 21 on the surface of

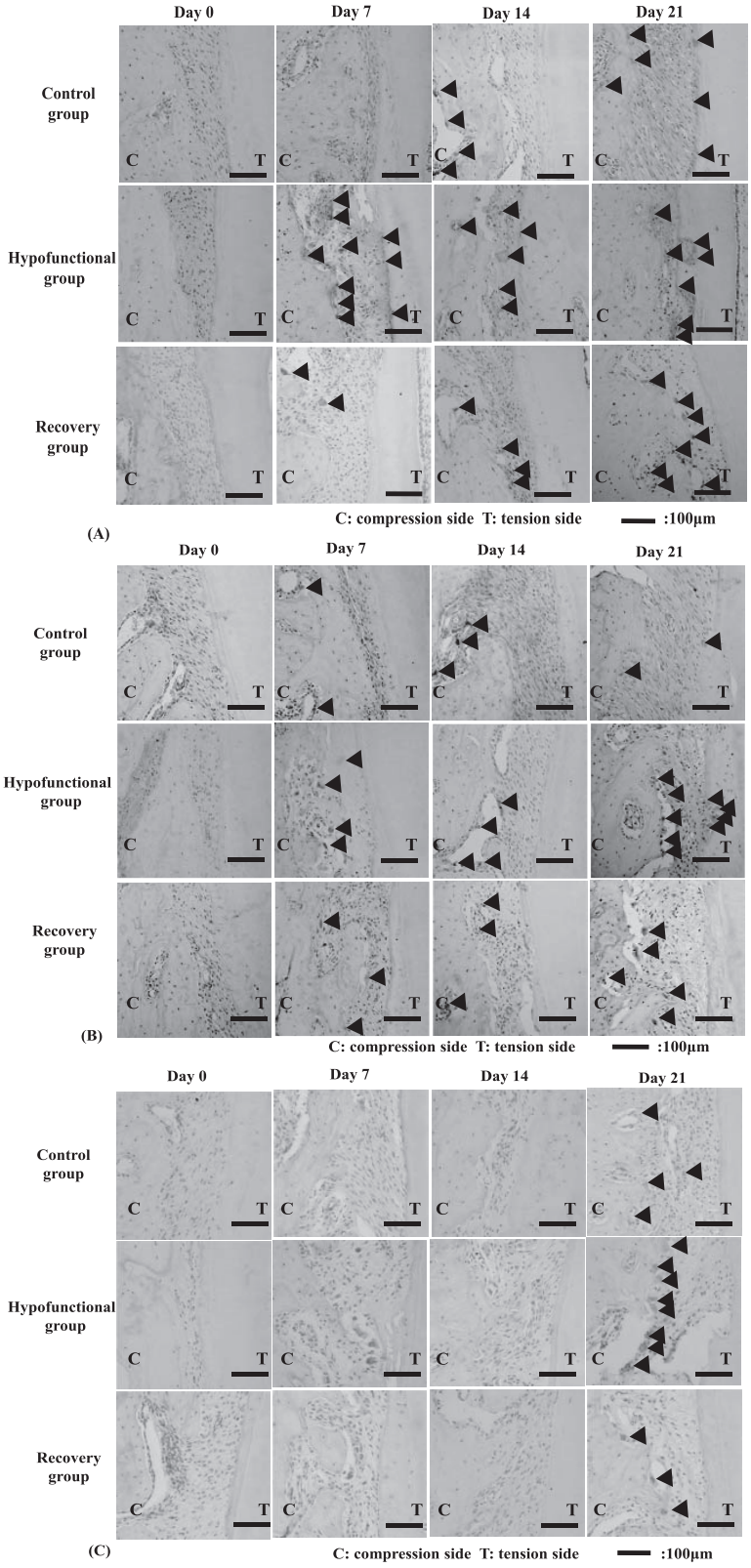

Figure 5. Immunohistochemical staining. (A) RANKL expression. (B) M-CSF expression. (C) IL-8 expression.

the alveolar bone. On the other hand, in the hypofunctional group, many root resorption lacunae with TRAP-positive odontoclasts were observed on days 14 and 21 (Figure 3A).

The number of odontoclasts increased significantly from 0 to 21 days in the three groups. The number of TRAP-positive odontoclasts in the hypofunctional group significantly increased up to day 21 (about 2.5-fold) in comparison with the control and recovery groups ($P < .05$) (Figure 4A), whereas no significant differences in the number of odontoclasts were found between the control and recovery groups.

Area of Root Resorption

As a result of histomorphometric analysis, on day 7, in the control and recovery groups, few resorption lacunae were seen on the surfaces of the alveolar bone and root. On the other hand, in the hypofunctional group, hyalinized tissue was observed. Moreover, the amount of root resorption in the hypofunctional group was larger than in the control and recovery groups (Figure 3B).

The root resorption ratio increased significantly from 0 to 21 days in the three groups. The amount of root resorption was significantly greater in the hypofunctional at 7, 14, and 21 days (about 2.0-fold, respectively) than in the control and recovery groups ($P < .05$) (Figure 4B). On the other hand, there were no significant differences in the root resorption ratio between the control and recovery groups.

Immunohistochemical Finding

The immunoreactivity of RANKL, M-CSF, and IL-8 was evaluated before and after tooth movement. In the control and recovery groups, immunoreactivity of RANKL, M-CSF, and IL-8 was slightly found in the cytoplasm of some fibroblasts and pericytes near the alveolar bone surface. RANKL, M-CSF, and IL-8 positive PDL fibroblasts and osteoblasts on the bone surface were observed on day 21. On the other hand, in the hypofunctional group, many RANKL, M-CSF, and IL-8 positive fibroblasts and odontoclasts were observed in the PDL and on the root surface on day 21, respectively (Figure 5).

DISCUSSION

The present study was designed to investigate the influence of occlusal stimuli on root resorption during experimental tooth movement. We developed an experimental hypofunctional model in the molar region using a bite-raising appliance.[7,8] This method makes it possible to stimulate a hypofunctional condition in the molar region and to return to normal occlusion after removal of the appliance by the result of soft X-ray images at 16 weeks of age. All of the animals also exhibited normal growth without any significant differences among the experimental groups from 16 weeks to 19 weeks, with the exception of the temporary body weight loss by the attachment of the appliance of bite plate and QH, respectively.

In this study, the upper first molar was moved continuously with a 10 g force, which is equivalent to the force used for human tooth movement.[10] It has also been reported that hyalinized cell-free areas were observed in excessively-compressed area with a 50 g force in rat.[11] Hyalinized tissue was observed around the compressed area in the hypofunctional group 7 days after the initiation of tooth movement. It is thus suggested that a 10 g force is regarded as a heavy force for hypofunctional tooth, although the same force exerts appropriate effects on normal tooth. On the other hand, the tooth movement was efficient in the control and recovery groups without hyalinized tissue.

Similarly, more TRAP-positive cells were detected in the hypofunctional group on day 21 than in the control and recovery groups. A significant difference was found in the numbers. The amount of root resorption was significantly greater in the hypofunctional group at 7, 14, and 21 days than in the control and recovery groups.

It is reported that root resorption was induced by multinucleated clast cells related to hyalinized tissues on the compressed side of PDL during tooth movement.[12] Root resorption was also obviously dependent on the force magnitude up to 100 g of super heavy force.[13] It is known that formation of hyalinized tissue is induced more prominently when a heavy force was applied. It is thus suggested that a force of 10 g may be regarded as heavy force for the hypofunctional tooth. This may be a reason why the prevalence of root resorption is higher in hypofunctional group than in the control and recovery groups.

On the other hand, there was no significant difference in the number of odontoclasts and the root resorption ratio between the control and recovery groups. It is suggested that periodontal tissues in the recovery group may easily adapt to orthodontic force on recovery of occlusal function to stimulate the periodontal tissues.

RANKL is known to be produced by stromal cells and osteoblasts,[14] and the differentiation and functions of osteoclasts/odontoclasts are regulated by RANKL/receptor activator of nuclear factor-κB (RANK) system that stimulates osteoclasts/odontoclasts formation.[15] On the other hand, M-CSF is also essential for osteoclastogenesis. A recent immunocytochemical study reported that RANKL/RANK and M-CSF/*c-fms*

were also observed in osteoblasts, osteocytes, fibroblasts, osteoclasts, and odontoclasts during the application of orthodontic forces.[16,17] Furthermore, recent studies have recognized the role of inflammatory cytokines such as IL-8 in transmitting signals for osteoclastogenesis.[18] It is reported that RANKL/RANK, M-CSF/*c-fms*, and IL-8 are expressed in root resorption lacunae around rat molar by heavy orthodontic force.[16,19]

In the present study, in the hypofunctional group, many RANKL, M-CSF, and IL-8 positive fibroblasts and odontoclasts were observed in the PDL and on the root surface at day 21. It is suggested that the expression of RANKL, M-CSF, and IL-8 was induced more prominently when heavy force was applied.[16,19] It is assumed that a force of 10 g is regarded as a heavy force for significantly hypofunctional tooth. This is why the amount of root resorption was larger in the hypofunctional group than in the control and recovery groups.

On the other hand, in the control and recovery groups, the immunoreactivity of RANKL, M-CSF, and IL-8 were localized near the alveolar bone surface at day 21. This result indicated that efficient bone remodeling was performed. It is suggested that the periodontal tissues of the recovery group may possess ability similar to the control group by its recovery of occlusal function which stimulates the periodontal tissues.

In conclusion, it is shown that an optimal force of 10 g exerts an effect similar to that of heavy force to a hypofunctional tooth, which results in extensive hyalinized tissue formation and severe root resorption with RANKL, M-CSF, and IL-8 positive odontoclasts in the PDL and on the root surface. On the other hand, it is also suggested that root resorption may be prevented by recovery of occlusal function which stimulates the periodontal tissues because the periodontal tissues of the recovery group may gain adaptability similar to the control group.

The present study suggested that the magnitude of orthodontic force to a hypofunctional tooth should be smaller than that to a normal tooth. It is also suggested that malocclusions such as open bite and high canine, or under-occluded teeth should be treated earlier in childhood and hypofunctional teeth may need recovery of occlusal function before starting orthodontic treatment.

CONCLUSIONS

- Occlusal hypofunction is one of the critical factors for severe root resorption.
- It is also suggested that root resorption may be prevented by recovery of occlusal function.

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