Original Article

Effects of long-term occlusal hypofunction and its recovery on the morphogenesis of molar roots and the periodontium in rats

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ABSTRACT

Objectives: To investigate the effects of long-term, artificially created, hypofunctional occlusion and its recovery on the morphology of rat molar roots.

Material and Methods: Eighteen 5-week-old Wistar-strain male rats were randomly divided according to their periodontal conditions into normal, hypofunctional, and recovery groups (n = 6 in each). In the experimental hypofunctional and recovery groups, a bite-raising appliance was set to produce hypofunction at the molar region. All groups were analyzed at 16 weeks of age using three-dimensional micro-computed tomography. Root length, width, and area as well as the thickness and the area of the periodontal ligament (PDL) space of the maxillary first molar were calculated.

Results: Roots were longer and narrower in the hypofunctional group than in the control group. The mesial root in particular showed a dramatic change. Root area also decreased significantly in the hypofunctional group compared to the other groups. Moreover, the PDL thickness and area decreased significantly in the hypofunctional group compared to the control group, but increased in the recovery group compared to the hypofunctional group.

Conclusions: These findings suggest that root size and PDL structure may be reduced due to disuse atrophy resulting from a defect in occlusal function, but may be recovered following a gain of occlusal stimuli. (*Angle Orthod.* 2013;83:597–604.)

KEY WORDS: Hypofunctional occlusion; Occlusal recovery condition; Normal occlusion; Root morphology; PDL space

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INTRODUCTION

Occlusal or masticatory mechanical loading is an important regulatory factor for periodontal tissue homeostasis. It has been suggested that the stress of functional occlusion is important for the development and thickening of periodontal collagen fibers.[1] Progressive atrophy of Sharpey's fibers, loss of their regular arrangement, narrowing of periodontal space, and the loss of alveolar bone following the lack of occlusal function has been reported.[2–5]

Histologically, teeth exhibit a hypofunctional periodontium in the absence of occlusal function.[6,7] Periodontal fibers are thinned, reduced in number and density, and become disordered when the function of the teeth is diminished in mice,[3] rats,[8] macaque monkeys,[9,10] and man.[6] Atrophic changes in the periodontal ligament (PDL), such as narrowing of the periodontal space, disorientation of collagen fibers,[11] and vascular constriction have been reported to be associated with loss of occlusal function.[3,4]

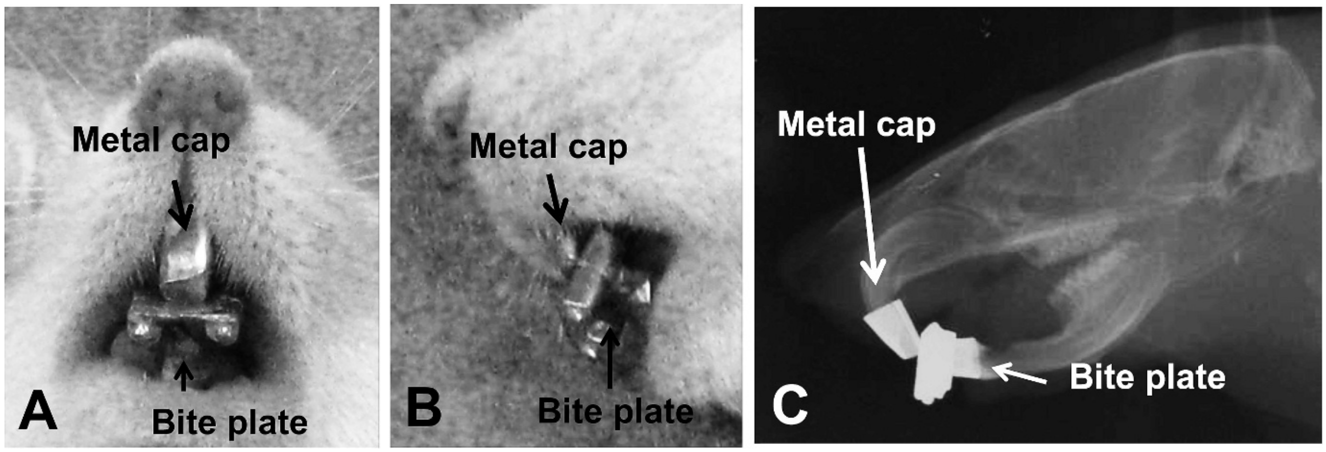

Figure 1. Appliance in situ. Frontal (A) and lateral (B) views. A radiographic image confirmed the occlusal condition (C).

Periodontal hypofunction resulting from the lack of contact with an opposing tooth can be encountered in certain malocclusions such as open bite and ectopic teeth. It has been suggested that a long-term, hypofunctional condition may be one possible etiological factor for root resorption during orthodontic treatment. The amount of root resorption was shown to be significantly greater for hypofunctional teeth than for control teeth under normal occlusal conditions during experimental tooth movement in rats.[12] Clinical studies also showed that the severity of root resorption, examined radiographically, was greater in open bite cases than in deep bite cases before and after orthodontic treatment.[13]

In histological studies of root morphology, the number of histological sections is usually limited to a small number per tissue sample, and it is impossible to obtain histological sections from different aspects using a single tissue sample. Furthermore, the thickness of the PDL can be easily influenced by tissue preparation, such as handling, decalcification, and dehydration of the specimen.[14] To overcome all these limitations and obtain a fully three-dimensional (3D) view of root morphology, a micro-computed tomography (CT) system was developed.

The aim of the present study was to examine the morphology of rat molar root and PDL space three-dimensionally under a long-term hypofunctional nonoccluding condition and to compare these results with those obtained under normal occlusion and occlusal recovery conditions. We also aimed to elucidate the association of occlusal function with periodontal tissue homeostasis.

MATERIALS AND METHODS

Experimental Animals

Eighteen 5-week-old Wistar strain male rats (Charles River Labs, Yokohama, Japan) were included in the experiment. All animals were fed with a powder diet (Rodent Diet CE-2; Japan CLEA Inc, Tokyo, Japan). Water was provided ad libitum and rats were kept in 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 procedures were performed following the ethical regulations defined by the Ethics Committee for animal research of Hiroshima University.

Rats were randomly divided into three groups of six rats each: one untreated control group and two experimental groups. In the experimental hypofunctional and recovery groups, a bite-raising appliance was set to produce hypofunction at the molar region.[15] The appliance consisted 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) bonded with composite resin (Clearfil Majesty LV; Kuraray Co Ltd, Okayama, Japan) on maxillary and mandibular incisors, respectively (Figure 1). In the recovery group, in order to recover occlusal contact, the appliances were removed 7 weeks after initiation of the experiment. All rats were sacrificed at 16 weeks of age. The experimental study design is summarized in Figure 2.

Micro-CT Analysis

After the experimental periods, the rat hemi-maxillae were scanned with a high-resolution x-ray micro-CT system (SkyScan 1172; Bruker, Aartselaar, Belgium) at the Australian Center for Microscopy & Microanalysis. Scanning was performed with a 360° rotation around the vertical axis, a rotation step of 0.3°, and an exposure time of 1.770 seconds. Approximately 1200 radiographs were taken from each sample and were saved as 16-bit tagged image file format files. After image acquisition, the raw data were then processed

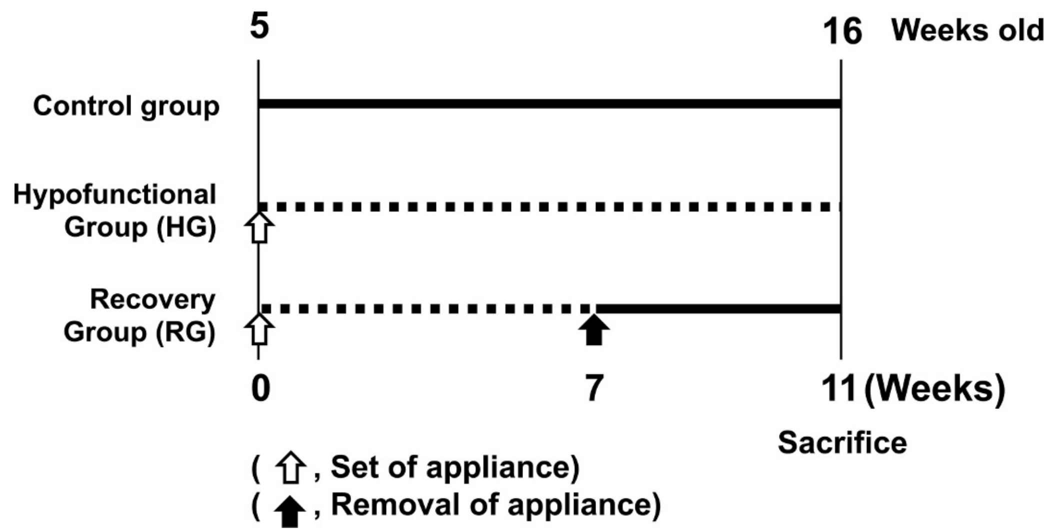

Figure 2. Study design.

using Nrecon (version 1.4.2; Aartsellaar, Belgium). Two-dimensional images were generated as 1024 × 1024-pixel bitmap images having an 8-bit gray scale dynamic range. 3D reconstruction was performed by VG Studio Max software (version 1.2; Volume Graphics GmbH, Heidelberg, Germany).

Morphological Analysis

Root length, width, and area, as well as the thickness and area of the PDL space of the maxillary molar, were calculated on the images using landmarks identified on the roots of the first molar as described below:

Sagittal view.

- *Root length*: measured along the long axis of the root from the cemento-enamel junction (CEJ) and root furcation to the apex (Figure 3A).
- *Root width*: measured perpendicular to the long axis of mesial (M) and disto-buccal (DB) roots at the midpoint of each root (Figure 3A).
- *Root area:* calculated from the CEJ and root furcation to the apex (Figure 3B).
- *PDL thickness:* recorded the distance from the root surface to the alveolar bone of M and DB roots at five points. For this, two perpendicular lines to the long axis of the root divided the root into middle (a, e) and apical (b, d) thirds. Point c was located at the root apex (Figure 3A).
- *PDL area:* The radiolucent area between the root and the alveolar bone was calculated in the sagittal and axial slices (Figure 3B, C, respectively).

Axial view.

- *Root area in the axial aspect:* The root area on a cross-sectional plane passing through the middle

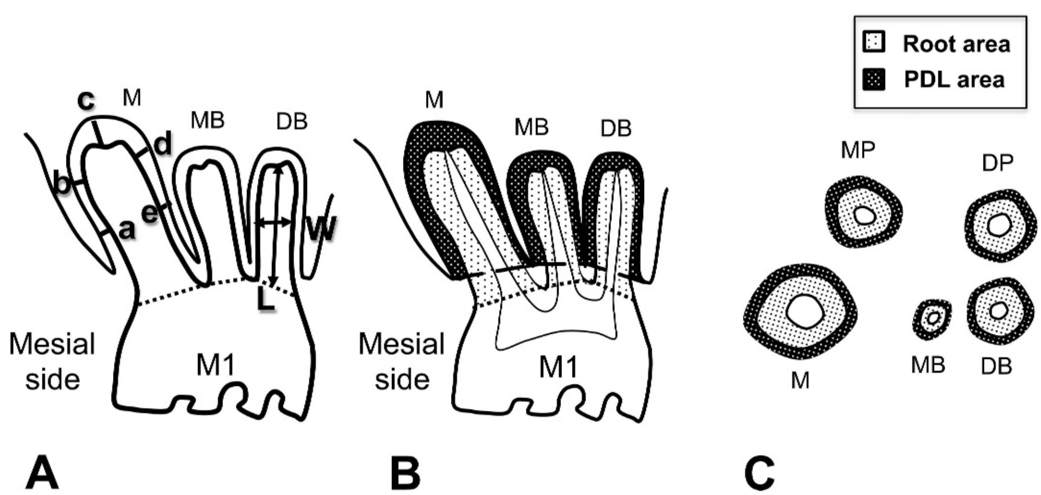

Figure 3. Measurements taken from sagittal (A, B) and axial (C) aspects are indicated. See text for details. M1 indicates maxillary first molar; L, root length; W, root width; M, mesial root; MB, mid-buccal root; DB, disto-buccal root; MP, mid-palatal root; DP, disto-palatal root.

point of each root: M, mid-buccal (MB), mid-palatal (MP), disto-palatal (DP); and, disto-buccal (DB) roots. (Figure 3C).

The same investigator performed all the measurements, and every measurement was repeated three times. The mean value was used as the final measurement. To assess measurement reproducibility, measurements were performed 10 times for one randomly selected root. The standard error was limited to less than 1%.

Statistical Analysis

Data are expressed as the mean ± standard error of the mean. To determine the statistical significance of differences among groups of rats, we performed repeated one-way analysis of variance and the Tukey-Kramer test using Statview (Abacus Concepts Inc, Berkeley, Calif). Confidence level $P < .05$.

RESULTS

The Influence of Occlusal Hypofunction and Its Recovery on Root Length and Width of M and DB Roots in the Sagittal Aspect

The Idem root in the sagittal aspect was significantly longer ($P < .01$) and narrower ($P < .01$) in the hypofunctional group than in the control group (Figure 4A). There were no statistically significant differences in the length and width of the DB root between the two groups, although the DB root tended to be longer in the hypofunctional and recovery groups compared to the control group (Figure 4B).

These results show that roots tend to be longer in hypofunctional and recovery groups, and narrower in the hypofunctional group, than in the other groups.

The Influence of Occlusal Hypofunction and Its Recovery on Root Area in the Sagittal and the Axial Aspect

No significant statistical differences in root area were found between M and DB roots in the sagittal aspect, although the root area of the M root tended to be smaller in the hypofunctional group than in the other groups ($P < .01$; Figure 5A). The area of the M root in the axial aspect was significantly smaller in the hypofunctional group than in the other groups, whereas the areas of MB, DB, DP, and MP roots were almost similar in all groups (Figure 5B). These results show that the M root tends to be significantly smaller in the hypofunctional group compared to other groups.

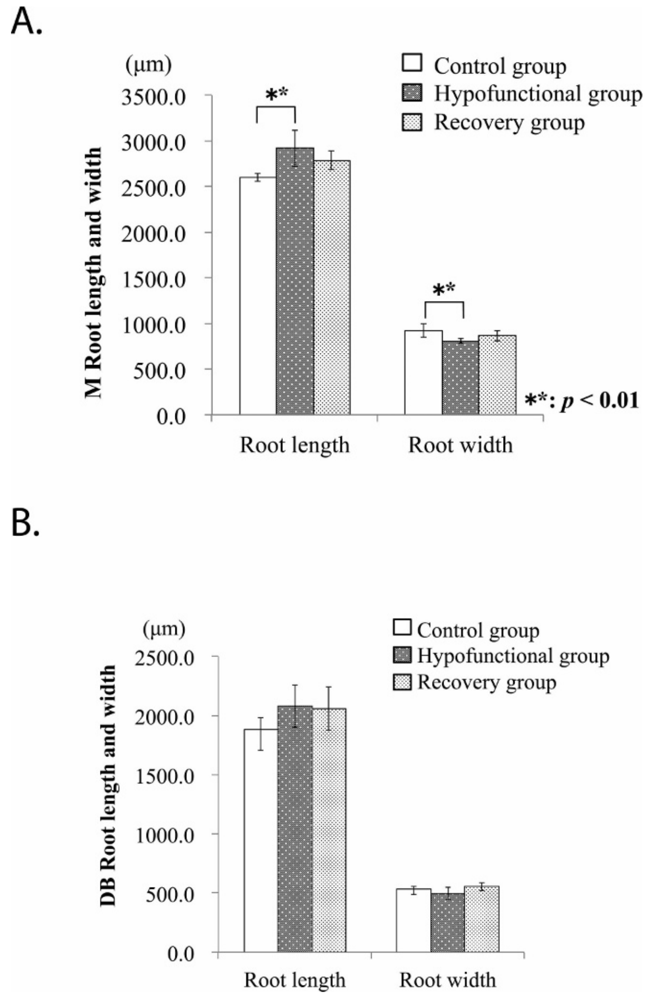

Figure 4. The effect of occlusal hypofunction and its recovery on the length and width of M (A) and DB roots (B) in the sagittal view. M indicates mesial root; DB, disto-buccal root.

The Influence of Occlusal Hypofunction and Its Recovery on the PDL Thickness of M and DB Roots in the Sagittal Aspect

The PDL space of the M root was significantly narrower in the hypofunctional group than in the control group at points a, c, d, and e, and in the recovery group at points b, c, d, and e (Figure 6A). The PDL thickness of the DB root was significantly narrower in the hypofunctional group than in the control group at points a, b, d, and e, and in the recovery group at points d and e. Moreover, the PDL was narrower in the recovery group than in the control group at point a ($P < .01$; Figure 6B).

These results show that the PDL thickness tends to decrease in the hypofunctional group when compared to the control group, but increases in the recovery group compared to the hypofunctional group.

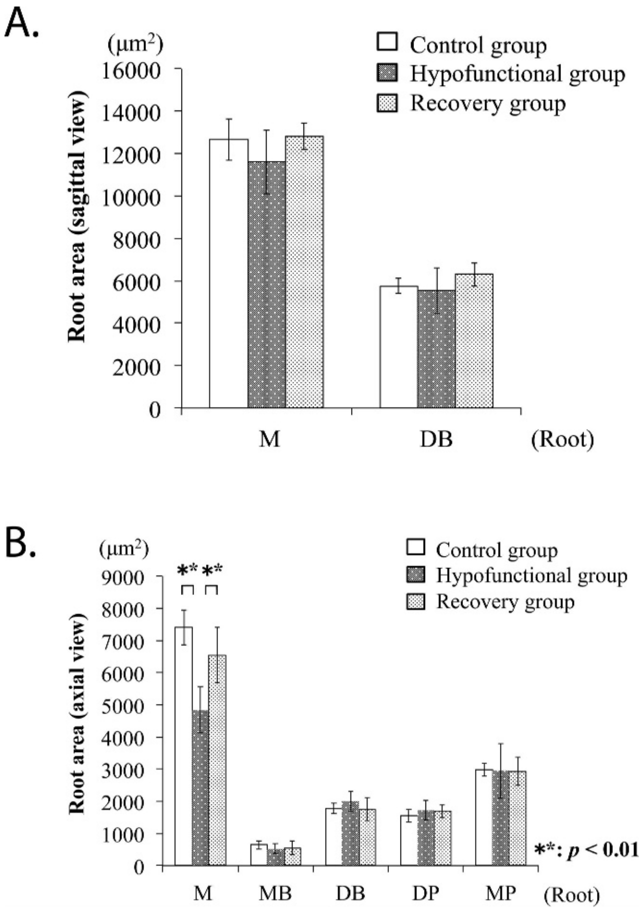

Figure 5. The influence of occlusal hypofunction and its recovery on root area in the sagittal (5A) and axial (5B) views. M, MB, DB, DP and MP indicate mesial, mid-buccal, disto-buccal, disto-palatal and mid-palatal roots, respectively.

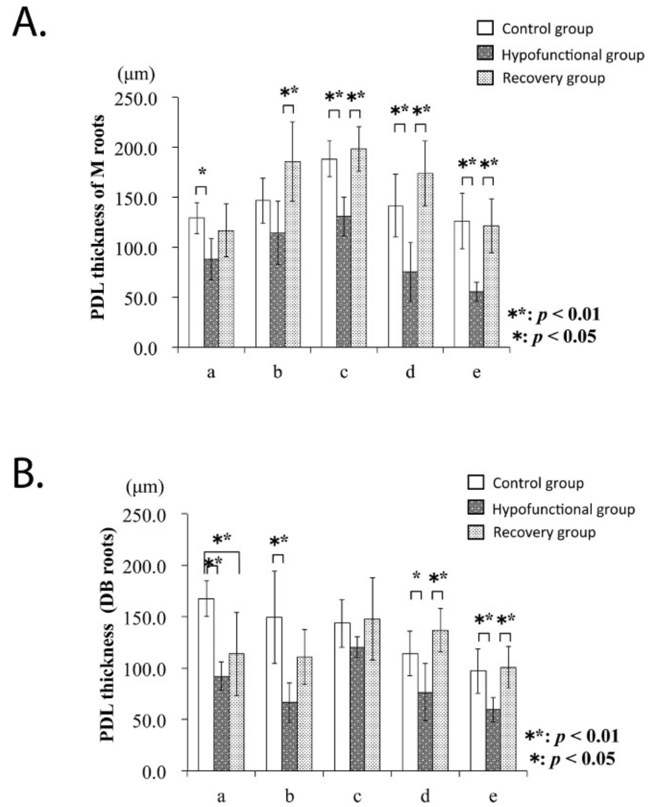

Figure 6. The influence of occlusal hypofunction and its recovery on PDL thickness of M (6A) and DB (6B) roots in the sagittal aspect. The description of points a–e is provided in the *Materials and Methods* section. M indicates mesial root; DB, disto-buccal root.

The Influence of Occlusal Hypofunction and Its Recovery on the PDL Area in Sagittal and Axial Aspects

The PDL areas around the M and DB roots in the sagittal aspect were significantly smaller in the hypofunctional group than in the control group, and the PDL area of the M root was significantly smaller in the hypofunctional group than in the recovery group ($P < .01$; Figure 7A). In the axial view, the area of the PDL around the five roots of the maxillary molar (M, MB, MP, DB, and DP) were significantly smaller in the hypofunctional group than in the control group, and the PDL areas around the M and MP roots were significantly smaller in the hypofunctional group than in the recovery group. Moreover, the PDL areas around the M, DB, and MP roots were significantly smaller in the recovery group than in the control group (Figure 7B).

These results show that the PDL area is smaller in the hypofunctional group than in the control group, but that it tends to increase in the recovery group more than in the hypofunctional group after the reestablishment of occlusal function.

Micro-CT Images of the Upper Molar Teeth in Normal Occlusion, Hypofunctional, and Recovery Groups

The experimental molars in the hypofunctional group showed a greater eruption above the occlusal plane than those in the control and recovery groups. Moreover, 3D reconstructed views showed intact cusps in the crowns of the hypofunctional group. Occlusal hypofunction resulted in a decreased level of alveolar bone compared to the control group, which seemed to return to normal levels in the recovery group (Figure 8).

DISCUSSION

The present study was designed to investigate the influence of occlusal or masticatory mechanical stimuli on the morphology of rat molars and PDL structure. We established an experimental hypofunctional model in the molar region using a bite-raising appliance.[15] This method makes it possible to simulate a hypofunctional condition in the molar region and to reestablish normal

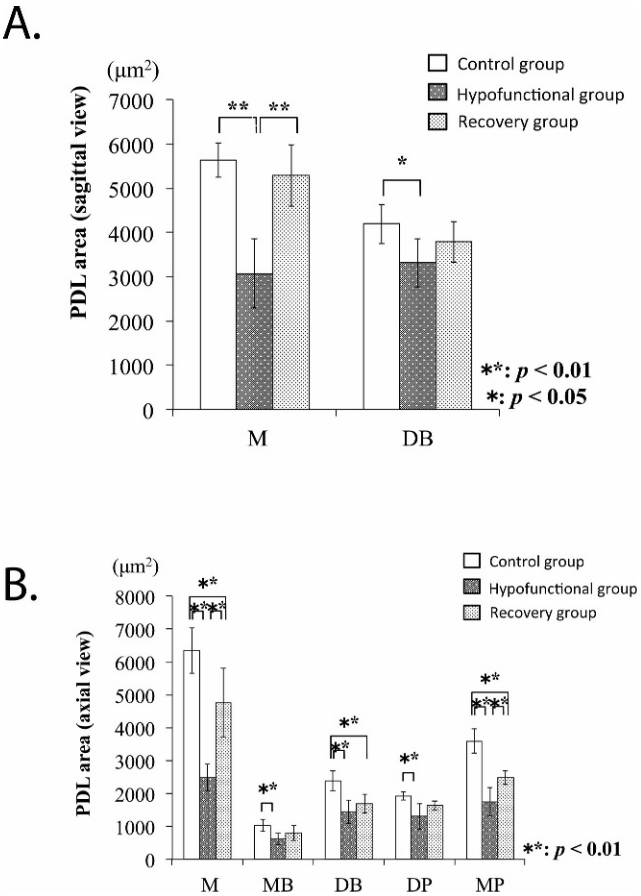

Figure 7. The influence of occlusal hypofunction and its recovery on PDL area in the sagittal (7A) and the axial (7B) aspect. M, MB, DB, DP and MP indicate mesial, mid-buccal, disto-buccal, disto-palatal and mid-palatal roots, respectively.

occlusion after removal of the appliance. Clinically, a tooth without its antagonists will elongate towards the opposing crown; this elongation has also been demonstrated experimentally.[8,16,17] In the present study, micro-CT images of normal and hypofunctional groups and of groups recovering from hypofunctional occlusion suggest that loss of occlusal contact followed by extrusion of the tooth are associated with root elongation of different degrees in the hypofunctional group, but this decreased significantly in the recovery group. From the results of this study it can be postulated that the roots are both shortened by apical root resorption and intruded by functional loads during the recovery period. On the other hand, occlusal hypofunction resulted in a decreased level of alveolar bone compared to the control group, but this level seemed to return to normal in the recovery group. In this regard, occlusal function ensures adequate tooth support. When this homeostasis was disrupted, bone resorption in the alveolar bone crest occurred during the hypofunctional period; however, alveolar bone was restored almost to the level of control groups during recovery period to adapt to the new functional condition. In addition, loss of attrition could only be observed in the hypofunctional group, which suggested that the maxillary molars had no occlusal contact throughout the experimental period.

Atrophic changes in the PDL have been reported to be associated with loss of occlusal function.[11,18] Such changes include narrowing of the periodontal space, disorientation of collagen fibers,[3] vascular constriction, and deformation of the mechanoreceptor structure.[4] Other studies using occlusal recovery models showed widening of the blood vessels in the PDL after the application of occlusal stimuli.[8,19] However, it is difficult to adapt these occlusal recovery models, which were

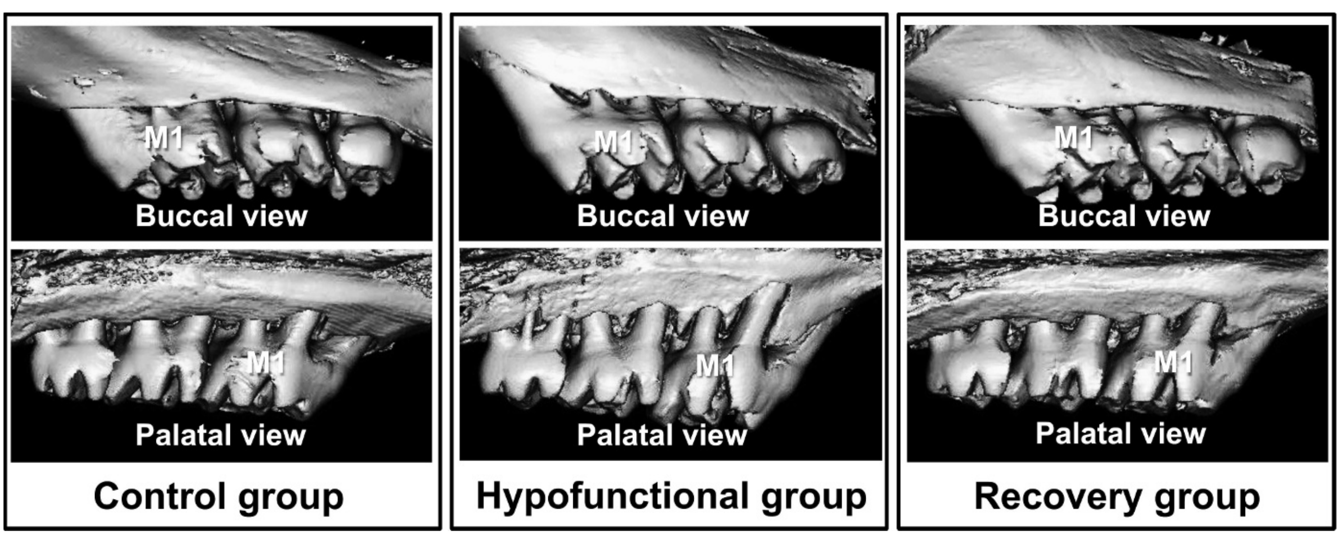

Figure 8. Micro-CT images of the maxillary dentition in normal occlusion (control group), molar hypofunctional, and recovery groups. Upper: buccal views; lower: palatal views. M1 indicates maxillary first molar.

simulated in 7-week-old rats, to a clinical situation, due to the eruption of the first molars in these rat models that was almost finished at 5 weeks old; these rats could naturally bite between 5 and 7 weeks of age before a short-term hypofunctional condition was simulated. Previous studies in male rats showed that alveolar bone turnover and osteoblastic/osteoclastic activity decrease with age.[20–22] In our study, the duration of hypofunctional condition was 11 and 7 weeks for the hypofunctional and recovery groups, respectively. However, since the growth potential of 12-week-old rats is minimal, it was considered that there was no difference between 7-week and 11-week hypofunctional conditions.

With regard to the influence of occlusal hypofunction and its recovery on the length and width of M and DB roots evaluated in the sagittal aspect, we demonstrated that M root was significantly longer and narrower in the hypofunctional group than in the control group, and the DB root exhibited a similar tendency. Moreover, there were no significant differences in root area except for M root, which tended to be smaller in the hypofunctional group than in other groups. In this study it was observed that the mesial root was mainly affected by hypofunction. This could be due to the larger size of the mesial root compared to the rest of the roots. Therefore, the changes on the mesial root were clearly and easily quantified.

Based on these results, it was assumed that a) the roots of rat molars with a loss of occlusal stimuli might elongate toward the opposing crown, and b) since the midpoint of the elongated root in the hypofunctional group shifted towards the apex, the root width and area might decrease.

In the present study it has been shown that most of the PDL measurements suggest a significantly narrower PDL in the hypofunctional group than in the control group and that the PDL was wider in the recovery group than in the hypofunctional group. In addition, the same trend was observed in the PDL area. These findings strongly suggest that atrophic changes in PDL structure might be induced by a loss of occlusal function, but that the PDL mechanoreceptors might be recovered, possibly in association with a widening of blood vessels, in the PDL after the application of occlusal stimuli. Moreover, these data suggest that root elongation present under hypofunctional conditions occurs concomitantly with a decrease in PDL thickness and tooth over eruption.

CONCLUSIONS

- Roots were longer and narrower in the hypofunctional group than in the control group. Root area also decreased significantly in the hypofunctional group compared to the other groups.
- PDL thickness and area decreased significantly in the hypofunctional group compared to the control group but increased in the recovery group compared to the hypofunctional group.
- These findings suggest that root size and PDL structure may be reduced due to disuse atrophy resulting from a defect in occlusal function, but they may be recovered following a gain of occlusal stimuli.

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Erratum

Please note that a title and affiliation for the author have been added to this Letter

Letters From Our Readers

To: Editor, *The Angle Orthodontist*

Re: Effects of long-term occlusal hypofunction and its recovery on the morphogenesis of molar roots and the periodontium in rats. Masahide Motokawa; Akiko Terao; Ersan I. Karadeniz; Masato Kaku; Toshitsugu Kawata; Yayoi Matsuda; Carmen Gonzales; M. Ali Darendeliler; Kazuo Tanne. *The Angle Orthodontist*, 2013;83(4)597–604

I would like to express my congratulations to the authors for publication of this article reporting the effects of long-term hypofunctional occlusion and its recovery on the morphology and PDL structure of rat molar roots.

Various approaches have been used previously to create hypofunctional occlusal conditions in animals: extraction[1] or occlusal reduction[2] of the antagonist teeth, bite opening with composite resin on the occlusal surface of molars,[3] or placing a metal cap on the incisors.[4] The latter was used in this study because it is reversible for occlusal function attenuation and recovery. However, was this method truly effective to create hypofunction of the molars? How far apart vertically was the separation between the molars and did that distance change over the 11 week observation period? Though the molars were separated, the gap may have been smaller than the diameter of the powder diet. As the incisors were covered with the cap, the rats would have no other way but to crush all the hard powders using their molars. Consequently, the first molar might still have been subjected to considerable mechanical stimulation during mastication.

Interestingly, it was found that only the mesial root of M1 was significantly smaller in the hypofunctional group. The authors concluded that the mesial root was mainly affected by hypofunction, which could be due to its larger size. However, it could also perhaps be due to the "wedge-shape" of this hypofunction model. Figure 1(c) shows that opposing molars are more separated mesially. Therefore, there would be less mechanical chewing stimulation mesially. Although the tooth is a rigid object, the stress on the distal root would be larger than that on the mesial root if the force acted principally on the distal occlusal surface of the crown.

The other question I had was this: Was there a change in body weight in the hypofunction condition? Might this affect the normal remodeling of PDL, alveolar bone and even cementum around the molars?

Thank you again for your work in this area and for considering my thoughts on this topic.

Xin Zhao, Postgraduate

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