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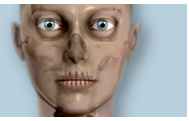

ORIGINAL ARTICLE

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Histomorphometric evaluation of maxillary molar roots and surrounding periodontium following molar intrusion in rats

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Structured Abstract

Objectives – To investigate periodontal tissue changes during and after molar intrusion in rats.

Setting and Sample population – The Department of Orthodontics at Yonsei University. Thirty 12-week-old male rats were assigned to 1 control and 5 experimental groups (n = 5 each).

Materials and Methods – In the experimental groups, two maxillary molars were intruded for 2 weeks; the control group underwent the same procedures without the intrusion force. After 2 weeks of intrusion, rats in one of the experimental groups and in the control group were killed. In the other four experimental groups, the new molar positions were either retained or not retained with an occlusal bite block for 1 or 2 weeks prior to euthanization. Histomorphometric analysis was performed for sulcus depth, osteoclast number per unit alveolar bone length, and root resorption area per unit root surface length.

Results – Sulcus depth increased during intrusion ($P < 0.05$), but decreased after 2 weeks of retention ($P < 0.05$). The number of osteoclasts increased during intrusion ($P < 0.05$), but subsequently decreased regardless of the retention regime ($P < 0.05$). Root resorption area increased after molar intrusion, irrespective of the retention regimen, relative to that of the control group ($P < 0.05$) and was the greatest after 2 weeks of retention.

Conclusion – These results indicated that root resorption occurred during and after molar intrusion and that the surrounding periodontium remodeled accordingly as tooth positions were altered, regardless of retention regime.

Key words: miniscrew; molar intrusion; open bite; retention; root resorption

Introduction

Anterior open bite can be corrected orthodontically without surgical intervention using orthodontic miniscrews. The treatment of this condition primarily depends on molar intrusion, which was hardly feasible before miniscrew application in the orthodontic field.

When molars are intruded to correct an anterior open bite, normal and healthy periodontal tissues generally surround the molars. Therefore, if molar intrusion is carried out in the presence of a healthy periodontium, it can lead to the formation of a pseudo-pocket through gingival coverage of the crown (1). Additionally, several studies have reported apical root resorption after molar intrusion (2–7). The average relapse rate of open bite treatment has been reported to be as high as 10.3–30.3% (8–11), and the intruded molars may largely contribute to the relapse. Based on these studies, some period of retention for the intruded molars is essential to maintain the treatment result. However, because the intrusive movement counteracts the physiologic movement of extrusion, the retention may also cause additional force to the periodontal tissues in retaining the tooth/teeth against the extrusion. Therefore, molar intrusion to correct an anterior open bite may not always be justified, unless the possible adverse effects during and after intrusion are clarified.

A number of studies have investigated changes in periodontal tissues after tooth intrusion. In most studies, not molar intrusion but incisor intrusion was performed, and the response of periodontal tissues following intrusion was controversial. Melsen et al. (12) and Cardaropoli et al. (13) reported that periodontal treatment and orthodontic intrusion of teeth with a reduced periodontium could improve the periodontal condition, while Murakami et al. (14) reported that the gingival sulcus deepened according to the intrusion of the teeth with a normal periodontium. In a study of periodontal changes after molar intrusion, Kanzaki et al. (1) concluded that pressure from the *supra*-alveolar fibers generated by molar intrusion induced alveolar crest resorption and consequently contributed to the maintenance of the gingival sulcus.

However, few studies have investigated the periodontal tissue changes that occurred during molar intrusion as well as during retention following molar intrusion. Because possible adverse effects may worsen during retention, conducting histologic evaluations is essential to gain a better understanding of periodontal tissue changes during and after molar intrusion and to improve the stability of the corrected anterior open bite. Therefore, based on a previous radiographic study (7), we investigated periodontal tissue changes and performed histomorphometric analyses during and after molar intrusion in rats. The null hypothesis tested was that there are no significant differences in the periodontal tissues between intruded molars and untreated molars in rats.

Materials and methods

Materials and experimental procedures

The materials and experimental procedures were reported in detail previously (7). Briefly, thirty 12-week-old rats were randomly assigned to one control group and the following five experimental groups: Intrusion, (+)retention-1 week, (+)retention-2 week, (−)retention-1 week, and (−)retention-2 week (n = 5 each). In all experimental groups, the left maxillary first and second molars (M1 and M2) were intruded with an intrusion spring for 2 weeks, and the left maxillary third molar (M3) was retained as a reference. The control group underwent the same procedures, except no intrusion spring was used. After 2 weeks of intrusion, the animals in the control and intrusion groups were sacrificed, and those in the other four experimental groups were retained with an occlusal bite block [(+)retention] or maintained without an occlusal bite block [(−)retention] for 1 or 2 weeks (Table 1).

A TAD (diameter, 1.2 mm; length, 7.0 mm; BMK Inc., Seoul, Korea) and a 0.016 × 0.022-

Table 1. **Experimental design (7)**

	Age (Weeks)					
Groups	12	13	14	15	16	17
Control (n = 5)	Implantation of TAD	Occlusal bonding material	Occlusal bonding material	Sacrifice	Sacrifice	Sacrifice
Intrusion (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Sacrifice	Sacrifice	Sacrifice
(+)Retention-1 week (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Bite block for retention	Sacrifice	Sacrifice
(+)Retention-2 week (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Bite block for retention	Bite block for retention	Sacrifice
(−)Retention-1 week (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Without retention	Sacrifice	Sacrifice
(−)Retention-2 week (n = 5)	Implantation of TAD	Intrusion spring	Intrusion spring	Without retention	Without retention	Sacrifice

TAD, temporary anchorage device.
In the intrusion group, two maxillary molars were intruded for 2 weeks; a control group underwent the same procedures without the intrusion force. After the 2 weeks of intrusion, in the other four experimental groups, the new molar positions were either retained [(+) Retention] or not retained [(−)Retention] with an occlusal bite block for 1 or 2 weeks.

inch superelastic nickel–titanium alloy wire (L&H Titan; Tomy, Tokyo, Japan) were used to deliver an intrusive force of 0.49 N parallel to the long axis of the molars (Fig. 1) (15). Rats were sacrificed according to the experimental protocol of each group to which they belonged. Immediately after sacrifice, the maxilla, including the teeth, was fixed for 24 h in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), dehydrated in ethyl alcohol, decalcified in EDTA/HCl (Calci-Clear Rapid®; National Diagnostics Inc., Atlanta, GA, USA), and embedded in paraffin. The specimens were cut parallel to the mesiodistal long axis of M1 and M2 at a thickness of 40–50 μm. The sections were prepared and stained with hematoxylin–eosin.

Histomorphometric analysis

Histomorphometric analyses were performed with an image measuring program (Image-Pro PLUS™, ver 3.0; Media Cybernetics, Inc., Bethesda, MD, USA). The mean value of the following measurements from three serial sections was used to reduce the variation in block slicing (Fig. 2).

Sulcus depth

The depth from the free gingival margin to the apical end of the epithelium was termed the sulcus depth, although it sometimes indicated a sulcus or a pocket. Sulcus depth was measured to observe changes in the gingival sulcus and the junctional epithelium during and after molar intrusion. The perpendicular distance from the free gingival margin to the bottom line, which was perpendicular to the root surface at the apical end of epithelium, was measured. The mesiobuccal root of M1 was chosen because it is considered to be the largest root in rats and thereby allows changes to be investigated clearly (16, 17).

Number of osteoclasts on the alveolar bone surface

The length of the alveolar bone adjacent to M1 and M2 was measured by tracing the surface of the alveolar bone; the number of osteoclasts on the traced line was counted. The number of osteoclasts per alveolar bone length was derived by dividing the number of osteoclasts by the alveolar bone length.

Root resorption area

The root length of M1 and M2 and the size of the crater, where the continuity of the cemen-

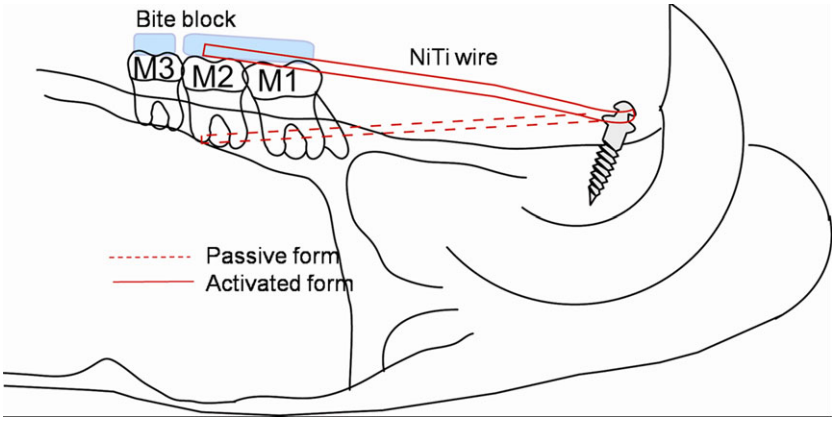

Fig. 1. Intrusion appliance for molar intrusion in the rat jaws (7). M1, M2, and M3 indicate the maxillary 1st, 2nd, and 3rd molars, respectively.

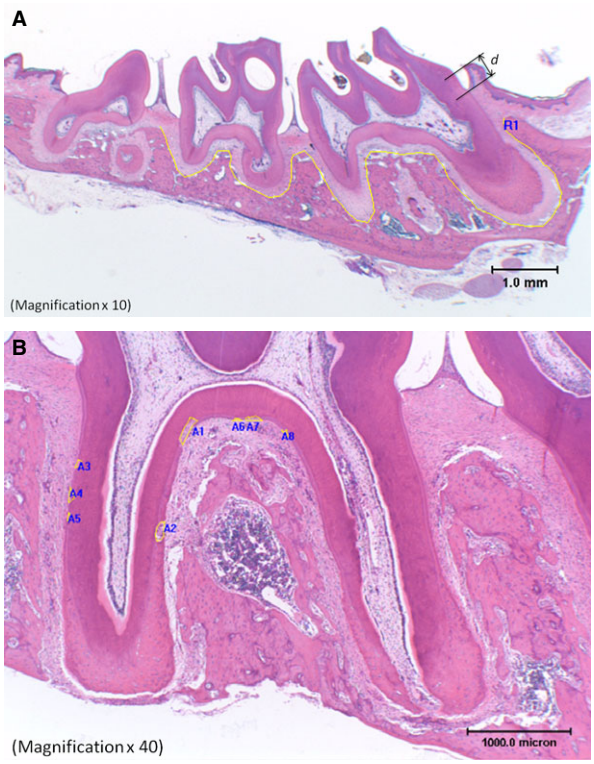

Fig. 2. (A) Histomorphometric analyses of the control and experimental groups. *d*, the perpendicular distance from the free gingival margin to the bottom line, which is perpendicular to the root surface at the apical end of the epithelium on the mesiobuccal root of the 1st molar; R1, the traced line of the alveolar bone surface. The number of osteoclasts located on R1 was counted and divided by the length of R1 to obtain the osteoclast number per unit length. (B) Histomorphometric analyses of the control and experimental groups. A1 to A8 indicate the root resorption cavities. The root length of the maxillary 1st and 2nd molars and the area of A1 to A8 were measured. The root resorption area per unit length was calculated by dividing the root resorption area by the root length.

tum was lost representing root resorption, were measured. The root resorption area per root surface length was calculated by dividing the root resorption area by the root length.

One operator performed all the measurements and conducted the measurements twice with a 2-week interval for 10 randomly selected subjects. Because an intraclass correlation coefficient of 0.97 was obtained (0.95–0.99; $P < 0.001$), the first measurements were used.

Statistical analysis

Statistical analysis was carried out with SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). Data are presented as means ± standard deviations. ANOVA and the post hoc Duncan's multiple range test were performed to compare differences among groups. A P value of <0.05 was considered statistically significant.

Results

Sulcus depth

The sulcus depth of the intrusion group was significantly longer than that of the control group and (+)retention-2 week ($P < 0.05$), but the control group and (+)retention-2 week were not significantly different ($P > 0.05$) (Fig. 3A, Table 2). The (+)retention-1 week group showed significantly longer sulcus depth than the control group ($P < 0.05$). The other two experimental groups showed no significant differences relative to the control and intrusion group ($P > 0.05$).

The epithelial attachment reached the cement–enamel junction in most specimens (Fig. 3B). The junctional epithelium was increased in length in the intrusion group, indi-

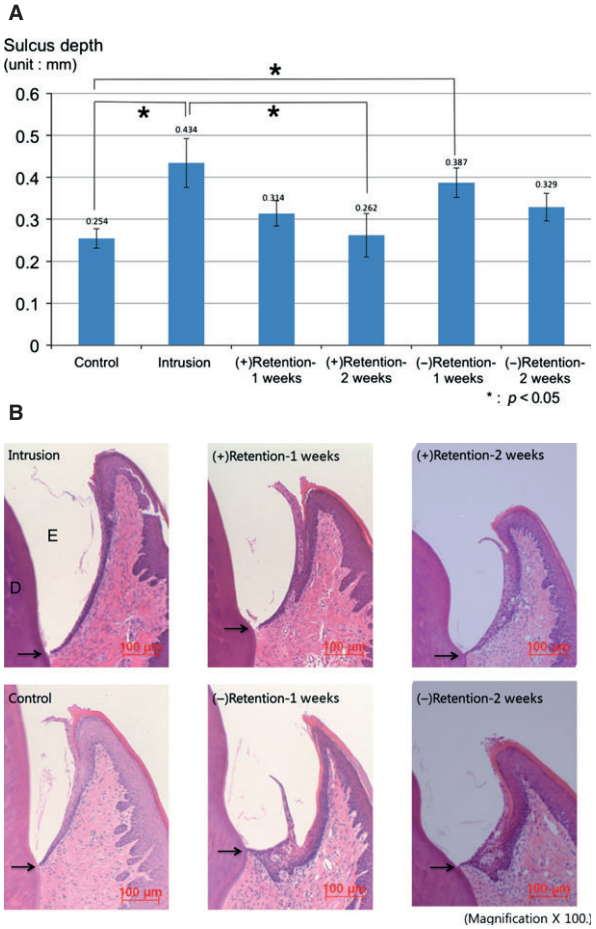

Fig. 3. (A) Changes in the distance from the free gingival margin to the apical end of epithelium (sulcus depth) in the six groups. (B) Hematoxylin- and eosin-stained sections of the sulcus depth in the mesial root of the 1st molar in the six groups. Arrow, cement–enamel junction; D, dentin; E, enamel space.

cating it had been in tight contact with the enamel. The thickening of the junctional epithelium was evident in the (−)retention groups and (+)retention groups and was much greater in the former groups than in the latter groups. Infiltration of inflammatory cells was observed in all experimental groups, especially in high concentrations in and below the junctional epithelium. The sulcular epithelium seemed separated from the enamel, indicating a wide gingival sulcus.

Number of osteoclasts on the alveolar bone surface adjacent to the root

The osteoclast number per length of alveolar bone surface was significantly greater in the intrusion group than in the other groups ($P < 0.01$) (Fig. 4A, Table 2). The number of

Table 2. **Histomorphometric analysis in the control and experimental groups**

	Control	Intrusion	(+)Retention-1 week	(+)Retention-2 week	(−)Retention-1 week	(−)Retention-2 week	Sig.
Sulcus depth (mm)	0.255 ± 0.045^{a}	0.434 ± 0.115^{c}	0.314 ± 0.059$^{a/b/c}$	0.262 ± 0.104$^{a/b}$	0.387 ± 0.070$^{b/c}$	0.329 ± 0.066$^{a/b/c}$	*
Number of osteoclast (number/mm)	0.217 ± 0.017^{a}	1.788 ± 0.597^{c}	1.032 ± 0.506^{b}	0.456 ± 0.216^{a}	0.566 ± 0.348$^{a/b}$	0.253 ± 0.107^{a}	**
Root resorption area (mm^2/mm)	0.000 ± 0.000^{a}	0.005 ± 0.004$^{a/b}$	0.006 ± 0.004^{b}	0.013 ± 0.007^{c}	0.010 ± 0.003$^{b/c}$	0.008 ± 0.000$^{b/c}$	**

In the intrusion group, two maxillary molars were intruded for 2 weeks; a control group underwent the same procedures without the intrusion force. After the 2 weeks of intrusion, in the other four experimental groups, the new molar positions were either retained [(+)Retention] or not retained [(−)Retention] with an occlusal bite block for 1 or 2 weeks.
*$P < 0.05$; **$P < 0.01$; Sig., significance; different letters show post hoc results after ANOVA, indicating significant differences among groups at 0.05 significant level.

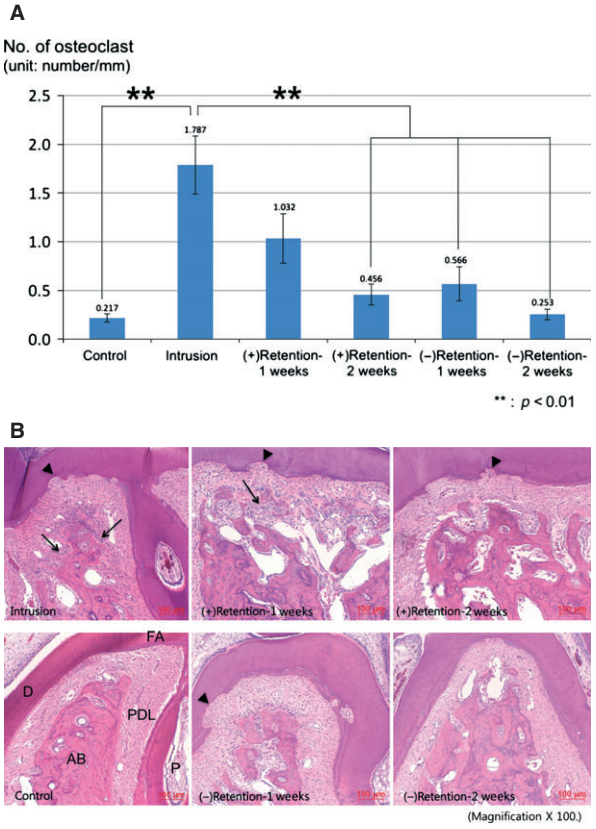

Fig. 4. (A) The number of osteoclasts on the alveolar bone surface in the 6 groups. (B) The number of osteoclasts on the alveolar bone surface (AB) and the furcation area (FA). Arrowheads indicate root resorption cavities; arrow, osteoclast; P, pulp; D, root dentin; PDL, periodontal ligament.

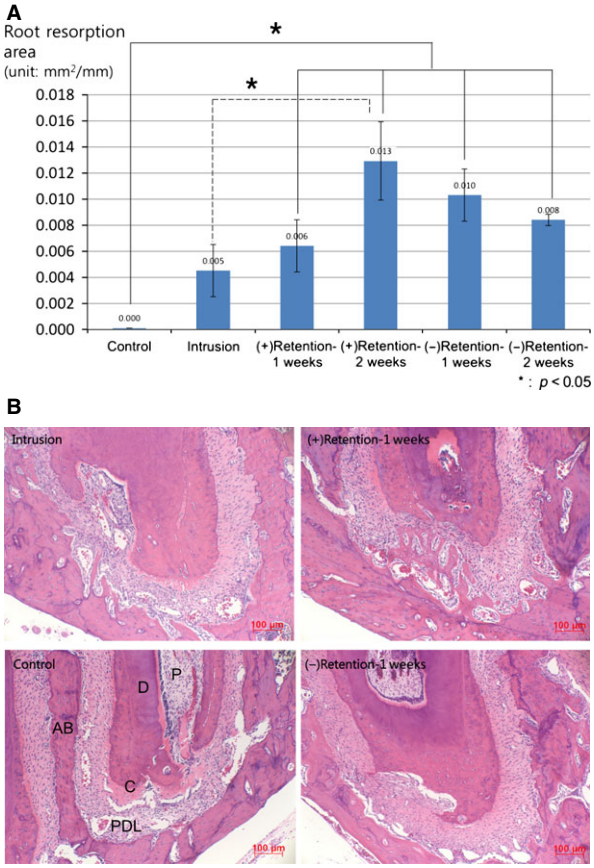

Fig. 5. (A) Root resorption area in the 6 groups. (B) Root resorption area in the apical region of four groups. Note narrowing of periodontal ligament (PDL) space (arrows) in the intrusion and (+)retention-1 week groups, and root dentin exposure due to cementum resorption in the (+)retention-1 week group. AB, alveolar bone; P, pulp; D, root dentin; C, cementum; PDL, periodontal ligament.

osteoclasts in the (+)retention and (−)retention groups was greater than that in the control group; however, the difference was not significantly different.

In the experimental groups, alveolar bone resorption was observed with multiple osteoclasts adjacent to the alveolar bone surface, especially in the intrusion and (+)retention-1 week groups (Fig. 4b). The osteoclasts were mostly located on the crest of the inter-radicular septum and the marginal alveolar bone crest, indicating direct bone resorption. The incidence of blood vessels in the apical and marginal alveolar bones and the inter-radicular septum was high in the (+)retention and (−)retention groups, particularly in the (+)retention-1 week group.

Root resorption area

External root resorption was observed in all experimental groups. The root resorption area of the (+)retention and (−)retention groups was significantly greater than that of the control group ($P < 0.05$) (Fig. 5a, Table 2). The root resorption area of the (+)retention-2 week group was the highest among the groups, showing significant differences from those of the intrusion and control groups ($P < 0.05$). Root resorption was observed more frequently in the furcation area than in the apical area (Fig. 5B). The resorbed area, which reached into dentin, was repaired with cellular cementum, regardless of retention protocol.

Discussion

From radiographic evaluations, we previously demonstrated that apical root resorption

occurred as an immediate response following molar intrusion, while apical movement of the alveolar crest adjacent to the intruded molars was a delayed response compared to root resorption. The present histomorphometric analysis showed that sulcus depth increased during molar intrusion ($P < 0.05$) but decreased and recovered to normal depth after 2 weeks of retention ($P > 0.05$). Osteoclast number showed a similar trend, increasing during molar intrusion ($P < 0.05$) but decreasing after intrusion, regardless of the retention protocol ($P < 0.05$). However, the root resorption area increased during intrusion and showed significant increase in both (+)retention and (−)retention groups compared to the control group ($P < 0.05$). It was the greatest after 2 weeks of retention.

Based on these findings, we can assume that a pseudo-pocket is temporarily formed during molar intrusion, as previously reported (1, 7), and recovers to a normal depth and that the periodontal tissue is remodeled accordingly after molar intrusion, regardless of retention protocol. However, inflammatory signs, such as infiltration of inflammatory cells, thickening of the junctional epithelium, and rete peg formation in the junctional epithelium, were observed even after the sulcus depth decreased. These findings were more evident in the (−)retention groups compared to the (+)retention groups. Additionally, the junctional epithelium in the control, intrusion, and (+)retention groups appeared to have maintained close contact with the enamel surface, although the junction needs to be confirmed with an electron micrograph. Meanwhile, the (−)retention groups showed a wide space between dentin and epithelium, suggesting that extra space existed when the intruded molars returned to the original position after removal of the intrusion appliance.

If the increased junctional epithelium had been tightly attached to the enamel surface by hemidesmosomes during molar intrusion, the sulcus depth would be normal. Otherwise, gingival pockets, which can increase gingival vulnerability, would temporarily exist. Even in the former scenario, however, bacteria present on the tooth surface can enter the mucosal tissue through the hemidesmosomes and produce toxins capable of eliciting inflammation and damage (18). Additionally, the junctional epithelium is a stratified squamous non-keratinized epithelium showing no capacity for differentiation into a keratinized surface epithelium. Therefore, lengthening of the junctional epithelium during molar intrusion may increase the chance of bacterial infiltration and gingiva permeability. Consequently, the gingiva will likely become more vulnerable during molar intrusion, with the vulnerability worsening after removal of the intrusion appliance.

The apical end of the epithelium was maintained at the cement–enamel junction in most specimens, except one (+)retention-2 week sample showing its apical migration. This indicates that in the normal periodontium, the apical end of the epithelium moves with the tooth but that the free gingival margin does not move with the tooth immediately. Murakami et al. (14) reported a similar finding with incisor intrusion in monkeys: The gingiva moved in the same direction teeth were intruded, but only about 60% as far. This may indicate iatrogenic apical migration of the attachment level does not occur during orthodontic tooth movement if carried out in a healthy periodontium. However, when inflammation increases, active proliferation and migration of the junctional epithelium can occur, resulting in a periodontal pocket and apical movement of the attachment level (18). Therefore, meticulous control of oral hygiene should be performed to prevent turning pseudo-pockets into true pockets.

From our previous radiographic evaluation (7), we found that the alveolar crest between two intruded molars moved apically during the retention period, but not during intrusion. The present histomorphometric analysis showed that the osteoclast number was the highest immediately after intrusion and decreased thereafter. This supports our previous radiographic findings that the alveolar crest was remodeled after molar intrusion: Bone remodeling was the most active during molar intrusion, and radiographically apparent alveolar bone resorption occurred during retention. According to Kanzaki et al. (1),

compression from *supra*-alveolar fibers after intrusion leads to alveolar crest resorption and may contribute to decreasing the increased sulcus depth. Because more osteoclasts were observed in the furcation area compared to the marginal region, alveolar bone resorption may occur more extensively in the inter-radicular area.

As reported in several previous studies (2–6), we observed apical root resorption during intrusion in our radiographic evaluation (7) and further root shortening was detected after intrusion, regardless of retention protocol. The current histomorphometric investigation revealed that the root resorption area significantly increased in the (+)retention and (−) retention groups compared to the control group. However, because we measured the crater size, most of the apical root resorption may not have been included. Therefore, this finding likely shows the area of the root resorption cavities rather than apical root resorption.

Like a previous study (15), we found a substantial amount of root resorption in the furcation area rather than around the root apex. This likely resulted because intrusive forces interfere more markedly with blood supply and create more extensive cell-free zones in the inter-radicular region than in the apical and marginal regions (15). Consequently, multiple root resorption cavities and active bone remodeling, including undermining bone resorption, were observed in the furcation area of the intrusion and (+) retention groups. As root resorption seemed to start later particularly in the furcation area (15), the root resorption area would be larger after intrusion than during intrusion. In the (+)retention groups, the extent of root resorption would increase with the length of the retention period (15) because of interference of blood supply and existence of cell-free zones.

Cementum maturity is also of significance for the progression of root resorption (19, 20). While approximately the coronal half of the root has a thin layer of mature cementum, immature cementum covers the apical half of the root and is deposited around the root apex throughout the lifetime in rats (21). Therefore, root resorption cavities may be evident in the furcation area, but hardly distinguishable in the apical area, until the root dentin is exposed, as seen in Fig. 5B. This supports the previous hypothesis that immature cementum around the root apex might be resorbed earlier than apical alveolar bone under pressure from intrusive force, resulting in root shortening (19).

The previous radiographic evaluation and the present histomorphometric analysis show that intrusion and retention of the rat molar are successful and that the surrounding alveolar bone and gingiva are remodeled accordingly. However, we were unable to investigate periodontal ligament changes because of difficulties in the quantitative measurements. Additionally, because this study performed molar intrusion only in a normal periodontium, further investigation of the intrusion of molars with a reduced periodontium is necessary for various clinical situations.

Conclusion

The null hypothesis that there are no significant differences in the periodontal tissues between intruded and untreated molars in rats was rejected. From the previous radiographic evaluation and the present histomorphometric investigation, we concluded that resorption of the tooth root occurred during and after molar intrusion and that the surrounding periodontium remodeled accordingly as tooth positions were altered, regardless of retention protocol. The sulcus depth increased without change to the alveolar crest during intrusion, but decreased with the apical movement of the alveolar crest after intrusion. This indicates that the gingival sulcus deepens during molar intrusion, while the marginal bone remodels thereafter.

Clinical relevance

Molar intrusion using orthodontic miniscrews is widely applied to correct anterior open bite orthodontically. Remodeling of the surrounding

periodontium after molar intrusion is essential to maintain the treatment result as well as the periodontal health. This histologic investigation in rats would provide an insight to gain a better understanding of the periodontal changes that occur during and after molar intrusion.

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