Periodontal tissue changes after retention or relapse following intrusive forces in rat molars

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언제나 제 맘속에 큰 스승님으로 남아계신 유영규 선생님, 손병화 선생님, 만나뵐 때마다 논문에 관심을 보여주시고 용기를 주셨던 백형선 선생님, 유형석 선생님께도 감사의 말씀을 드립니다. 선생님들의 가르치심 덕에 오늘의 제가 있을 수 있었습니다. 논문의 큰 줄기를 잡아주시고 세심하고 예리하게 지적해주시고 도와주신 이기준 선생님, 제가 선생님께 감사드릴 일이 너무나 많습니다. 매 단계마다 귀찮음을 마다않고 친절하고 세심하게 도움을 주셨던 정주령 선생님, 차정열 선생님께도 진심으로 감사드립니다. 조직 사진마다 꼼꼼하게 설명해주시고 저보다 더 큰 열의로 가르쳐주신 문익상 선생님, 여러 번의 귀찮은 질문에 대해 질문보다 항상 더 많이 가르쳐주신 김현실 선생님께도 가슴 깊이 감사드립니다. 힘들고 귀찮은 내색 없이 본인의 일들처럼 열심히 도와주었던 조용민 선생님, 김진욱 선생님, 윤혜림 선생님 덕에 실험이 진행되고 마무리될 수 있었습니다. 매번 표현하지 못했지만, 지면을 빌어서나마 감사의 인사를 전합니다. 어렵고 힘들었던 수련 생활부터 지금까지 늘 힘이 되어주는 동기들–김경석, 김영재, 전재민, 주억–과 실험 내내 든든한 지원군이 되어주었던 의국 후배들–김인실, 이지연, 장정은, 황순신, 김진호–에게도 감사드립니다.

학부 때부터 어려운 결정의 순간마다 중요한 조언을 주신 육종인 선생님, 논문 뿐 아니라 일상의 수많은 고민들에 해결책을 주시는 스승이시면서 동시에 소중한 친구가 되어주시는 김의성 선생님, 늦은 시간까지 연구에 매진하시면서 논문에 매달리는 저에게 뜨거운 격려를 주셨던 허종기 선생님, 박정원 선생님, 김선재 선생님, 신수정 선생님, 교정에 대해서는 물론이거니와 다른 분야에서도 많은 조언을 주시는 이종석 선생님께도 깊이 감사드립니다.

언제나 희로애락을 함께 해주는 소중한 친구들–주연, 혜자, 희진–과, 저에게 가족과도 같은 언니들–난심, 미성, 민경, 은경, 가영, 승은–에게 감사의 마음을 전합니다. 마지막으로 저에게 무한한 사랑을 주시고 든든한 지원군이 되어주시는 사랑하는 가족들께 이 논문을 바칩니다.

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Table of Contents

List of Figures

List of Tables

ABSTRACT

Periodontal tissue changes after retention or relapse following intrusive forces in rat molars

Orthodontic miniscrew implants are commonly used in current practice to intrude molars when correcting anterior openbite. However, there have been few reports on the changes of the intruded teeth and periodontal tissue during retention period following molar intrusion. The aim of this study was to observe periodontal tissue changes after intrusion of posterior teeth in rats using miniscrew implant and its features of retention or relapse.

Orthodontic miniscrew implant was placed behind maxillary left incisor in ten week old rat and 50 gm of intrusion force was applied to the maxillary left 1^{st} and 2^{nd} molars for two weeks with Japanese NiTi wire. Periodontal tissue changes after two weeks of molar intrusion and after a period of one to two weeks of retention or relapse following molar intrusion were observed.

With molar intrusion, mild surface root resorption occurred and it was repaired with cementum after retention or relapse following intrusion. Active bone modeling and remodeling were seen in the alveolar bone adjacent to the intruded teeth, and it was the most evident in the interradicular area. The height of alveolar crest was decreased but there was no statistically significant difference after intrusion with the control group. After retention, however, the alveolar crest between intruded teeth was moved apically ($p < 0.05$). The apical end of epithelium moved with cement-enamel junction causing long junctional epithelium formation after molar intrusion ($p < 0.05$). Periodontal ligament was stretched in the tension side and compressed in the pressure side. The free gingival margin receded and periodontal ligament was remodeled after retention, resulting in normal and healthy periodontium. Most teeth movements of relapse occurred in the early phase, therefore initial retention is important for the stability of treatment.

Key Words : molar intrusion, retention, relapse, anterior openbite, absolute anchorage, orthodontic miniscrew implant

Periodontal tissue changes after retention or relapse following intrusive forces in rat molars

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I. Introduction

Skeletal anchorage simplified orthodontic treatment by counteracting reactive forces and expanded the ranges to be corrected by orthodontic treatment only. Recently, onplant[1], miniplate[2-7] and orthodontic miniscrew implant[8-13] have been used for skeletal anchorage. They are divided according to insertion site, technique and design, but they provide the same function as absolute anchorage for tooth movement. Miniscrew implant has the advantages of immediate loading, various insertion sites, uncomplicated placement and removal procedures, and minimal expense for patients. The success rate of miniscrew implant was reported to be over 90%[14,15], and immediate loading was known not to affect the stability of miniscrew implant[16,17]. Antero−posterior tooth movement is not interfered by miniscrew implant if it is placed with appropriate angulation to bone surface in interproximal area[15].

The correction of anterior openbite with molar intrusion using miniscrew implant[8,10] is an example that the range of orthodontic treatment has been expanded. Miniscrew implant helps to reduce the potential risk of surgery, the troublesome of wearing extraoral appliance and the dependence upon patient compliance during treatment.

In treatment of anterior openbite using miniscrew implant as an absolute anchorage, the occlusal plane was rotated counterclockwise to close anterior openbite as molars were intruded. Since facial soft tissue was changed with molar intrusion, treatment result with molar intrusion is similar to that by orthognathic surgery in respect to facial profile changes. Kuroda et al[2] stated that molar intrusion with skeletal anchorage is simpler and more useful than two−jaw surgery in the treatment of patients with severe anterior openbite. However, since there have been few reports on the changes of the intruded teeth and periodontal tissue during retention period following molar intrusion, relapse rates are controversial and even the effectiveness of anterior openbite correction with molar intrusion has been questioned.

There are some reports of the changes after molar intrusion. It was stated that clinically the changes in alveolar bone height with marginal bone remodeling during molar intrusion were observed but any increase in gingival pocket depth after posterior segmental molar intrusion was not seen, although the temporary formation of a gingival pocket (pseudo−pocket) was observed[4]. The tissues in the marginal alveolar crest showed a resorption and remodeling of alveolar bone and in the interradicular and apical regions had the typical characteristics of pressure zone; periodontal ligament compression and cellfree zone[18].

For the long term stability of treatment it is essential to understand the periodontal tissue changes following molar intrusion and during retention period after molar intrusion, but it has scarcely been reported. Therefore, the aim of this study was to observe periodontal tissue changes after intrusion of posterior teeth in rats using miniscrew implant and its features of retention or relapse.

II. Materials and Methods

1. Materials

29 ten weeks old female Sprague-Dawley rats, averaging 220 - 250 gm in weight, were used. All animals were kept in stainless-steel cages in air-conditioning and subjected to standard 12-hour light / dark cycle. They were fed with a pellet diet (8811M0001, Extrusion, Superfeed Co. Ltd., Gangwon-do, Korea) and tap water ad libitum. They were checked everyday in regard to their health status.

The animals were divided into control and five experimental groups to evaluate periodontal tissue changes following molar intrusion and after retention or relapse following molar intrusion (Table 1). In the 2wk-Intrusion group (n=5), molars were intruded for two weeks. In the 1wk-Retention (n=5) and 2wk-Retention (n=5) groups, intruded molars were maintained for one and two weeks, respectively, after two weeks of molar intrusion. In the 1wk-Relapse (n=5) and 2wk-Relapse (n=5) groups, intruding appliances were disengaged and periodontal tissue changes were observed after one and two weeks, respectively, following two weeks of molar intrusion. In the control group (n=4), the experimental condition was the same as the 2wk-Intrusion group except the intrusion force.

Table 1. Control and experimental groups

	Number of samples	Duration (unit : week)				
		0	1	2	3	4
Control	4	Implantation of miniscrew	Occlusal bonding material →			
2wk-Intrusion	5	Implantation of miniscrew	Intrusion →			
1wk-Retention	5	Implantation of miniscrew	Intrusion →		Retention →	
2wk-Retention	5	Implantation of miniscrew	Intrusion →		Retention →	
1wk-Relapse	5	Implantation of miniscrew	Intrusion →		Relapse →	
2wk-Relapse	5	Implantation of miniscrew	Intrusion →		Relapse →	

2wk-Intrusion, the experimental group in which molars were intruded for two weeks; 1wk-Retention and 2wk-Retention, the experimental groups in which intruded molars were maintained for one and two weeks, respectively, after two weeks of molar intrusion; 1wk-Relapse and 2wk-Relapse, the experimental groups in which intruding appliances were disengaged and periodontal tissue changes were observed after one and two weeks, respectively, following two weeks of molar intrusion.

2. Experimental procedures

The animals were immobilized with ether inhalation and anaesthetized with intraperitoneal injection of Zoletil (Tiletamine 125ml, Zolazepam 125ml; 0.04ml Virbac, 060516 carros, France) and Rompun (Xylazine hydroxychloride 23.32 mg/ml; 0.01 ml Bayer AG, 51368 Leverkusen, German).

Orthodontic miniscrew implant (1.2 mm diameter, 7.0 mm length, BioMaterials Korea Inc., Seoul, Korea) was inserted into the alveolar crest behind maxillary left incisor, impression of upper arch was taken with poly-vinysiloxane (Aquasil Ultra, Dentsply, York, PA, USA) and a dental cast (New Plastone, GC Corp., Tokyo, Japan) was fabricated. With reference to the fabricated cast, the improved superelastic nickel-titanium alloy wire (L&H Titan, Tomy, Tokyo, Japan), 0.016 x 0.022 inches, was bent to transfer an intrusive force of 50 gm[18] parallel to the long axis of tooth by using the direct electric resistance heat treatment method with a heat bender[19] (Fig. 1).

One week after miniscrew implantation, the fabricated wire was attached to miniscrew implant and the occlusal tables of maxillary 1st and 2nd molars on left side. The space between the wire and the neck of miniscrew implant was filled with flowable resin (Esthet-X® Flow, Dentsply, York, PA, USA). GI cement (Ultra Band-Lok™, Reliance Orthod Prod. Itasca, IL, USA) was used to bond wire to molars. Occlusal bonding material, GI cement, was also bonded to maxillary left 3rd molar and three maxillary right molars to prevent unwanted extrusion of teeth(Fig. 1).

After two weeks of molar intrusion, the intruding appliance was disengaged, impression of upper arch was taken and a dental cast was fabricated. In the retention groups, three maxillary molars on each side were splinted by occlusal bonding material again (Fig 2, A). In the relapse groups, all appliances except miniscrew implant were removed (Fig. 2, B).

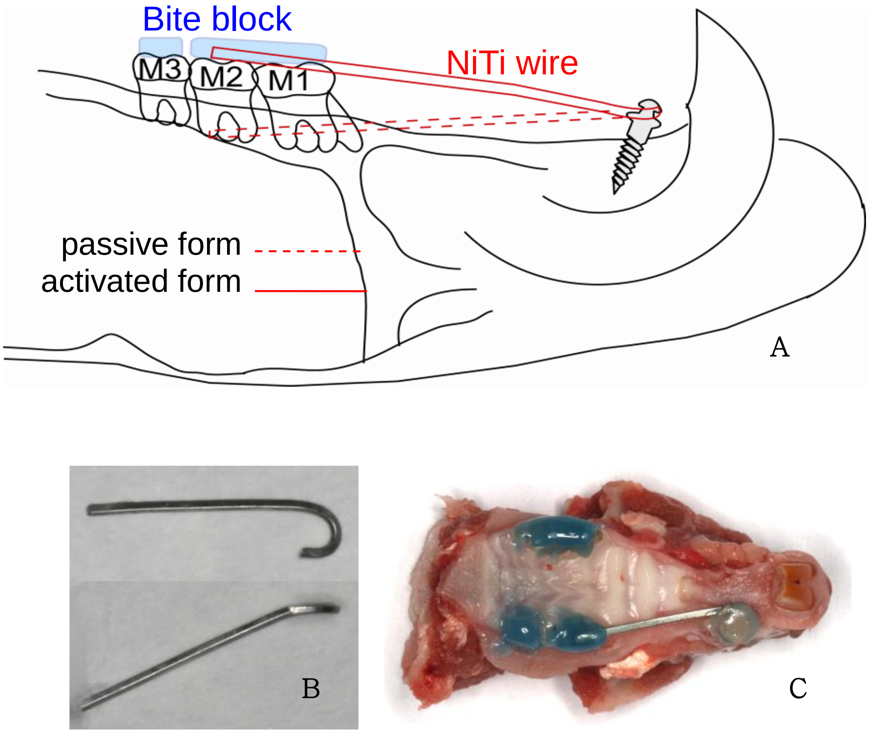


Figure 1. Intruding appliance for molar intrusion in rats. A, Schematic drawing of the appliance; B, Superelastic NiTi wire transformed by heat bender (top, occlusal view; bottom, lateral view); C, Rat which was sacrificed after two weeks of molar intrusion. M1, M2 and M3 – maxillary 1st, 2nd and 3rd molar, respectively.

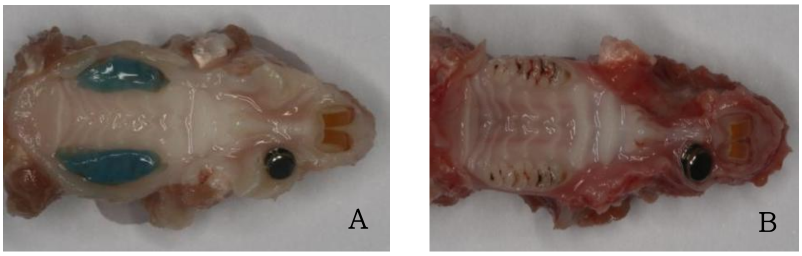


Figure 2. Rats which were sacrificed after retention or relapse following two weeks of molar intrusion. A, Retention group; B, Relapse group.

At the end of each experimental period the animals were sacrificed by cervical dislocation under ether inhalation. Intrusion or retentive appliance was disengaged, impression was taken and a dental cast was fabricated. The appliance was checked everyday under ether inhalation and the body weight of animals was measured every week.

The maxilla was dissected free and divided with a midline, sagittal cut. Each half-cut maxilla was placed on the intraoral periapical film (Insight, Kodak, Rochester, NY, USA). X-ray was exposed at the distance of 30 cm from the film using intraoral radiographic apparatus (AnyRay, E-Woo Technology Co. Ltd., Gyeonggi-do, Korea). The radiographic films were processed in manual method. Micro computer tomography (SkyScan micro CT 1076, Skyscan, Aartselaar, Belgium) was taken on one sample of each five experimental groups (2wk-Intrusion, 1wk-Retention, 2wk-Retention, 1wk-Relapse and 2wk-Relapse groups).

Immediately after sacrifice, the maxilla, including the teeth, was fixed for 24 hours in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), dehydrated in ethyl alcohol, decalcified in EDTA/HCl (Calci-Clear Rapid®, National Diagnosics Inc., Atlanta, GA, USA), embedded in paraffin and cut into 4 ㎛ thick sections in a sagittal direction. The sections were prepared and stained with Hematoxylin-Eosin and Masson trichrome.

3. Measurements

A. Measurements on periapical films

With a digital camera (DFC300FX, Leica Microsystems Ltd., Wetzlar, Germany) connected to a microscope (Leica MZ75, Leica Microsystems Ltd., Wetzlar, Germany), the processed periapical films were converted into digital images which were magnified 6.7 times with measuring tool.

On the digital image a horizontal reference plane was created as a line tangent to cranium below frontal−squamosal intersection at temporal crest[20], and the following categories were measured with image measuring program (Image J, Wayne Rasband, National Institutes of Health, USA).

1) The changes of the vertical position of molar caused by cementum apposition on root apex with aging

In order to evaluate whether cementum apposition, a characteristic in rat molars, would cause the vertical displacement of teeth according to the age of rats, the vertical positions of crown and root were compared. The right maxillary 3rd molar (M3) in the 2wk−Intrusion, 1wk−Retention and 2wk−Retention groups was used for comparison because bonding material had been attached during the whole experimental period but intrusion force had not been applied. The perpendicular distances from the below points to the horizontal reference plane were measured (Fig. 3): (a) frontal−squamosal intersection at the temporal crest, (b) middle cusp tip of M3, (c) distal root apex of M3. To compensate for variance of individual skull size, the ratios of measurements for frontal–squamosal intersection at the temporal crest, which were constant during the experiment, were calculated: b/a and c/a, the relative vertical positions of M3 crown and root, respectively.

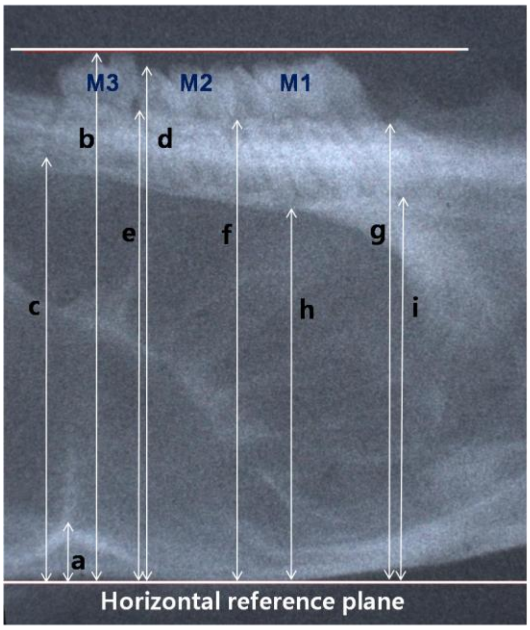


Figure 3. Measurements on periapical film. a, Frontal–squamosal intersection at the temporal crest; b, middle cusp tip of maxillary 3rd molar (M3) – total height; c, M3 distal root apex; d, distal cusp tip of maxillary 2nd molar (M2); e, alveolar crest between M2 and M3; f, alveolar crest between maxillary 1st molar (M1) and M2; g, alveolar crest in M1 mesial side; h, M1 distal root apex; i, M1 mesial root apex.

2) The changes following molar intrusion and after retention or relapse following molar intrusion

To evaluate the amount of molar intrusion and root resorption and the changes of alveolar bone, the perpendicular distance from the below points to the horizontal reference plane were measured (Fig. 3): (b) total height - M3 middle cusp tip, (d) M2 distal cusp tip, (e) alveolar crest between M2 and M3, (f) alveolar crest between M1 and M2, (g) alveolar crest in M1 mesial side, (h) M1 distal root apex, (i) M1 mesial root apex.

Total height (b) was given a value of 1, as a reference, and other measurements were converted accordingly.

B. Histomorphometric analysis

In order to observe periodontal tissue changes immediately after molar intrusion and after retention or relapse, histomorphometric analyses were performed with image measuring program (Image-Pro PLUS™, ver 3.0, Media Cybernetics, Inc., MD, USA) in all the experimental and the control groups. The mean value of measurements from three serial sections was used to decrease the variation of block slicing.

1) Depth from free gingival margin to the apical end of epithelium in the mesial side of maxillary 1st molar

Depth from free gingival margin to the apical end of epithelium in the mesial side of maxillary 1st molar was measured to observe the changes of gingival sulcus and junctional epithelium immediately after intrusion and after retention or relapse period following intrusion. The perpendicular distance from free gingival margin to the bottom line, which was made perpendicular to root surface at the apical end of epithelium, was measured.

2) The number of osteoclasts on alveolar bone surface

The length of the alveolar surface adjacent to root of maxillary 1^{st} and 2^{nd} molars was measured and the number of osteoclasts on the alveolar bone surface was counted. The number of osteoclasts was divided by the alveolar bone length, thus the number of osteoclast per unit length was calculated.

3) Root resorption area

The root length of maxillary 1^{st} and 2^{nd} molars and the size of crater, where the continuity of cementum was lost representing the root length were measured, thus the root resorption area per unit length was calculated.

4. Statistical analysis

The statistical analysis was carried out on SPSS ver 12.0 (SPSS Inc., Chicago, IL, USA). Data were presented as means ± standard deviations. For significance of differences, the data were evaluated by independent *t*-test in comparison between the intrusion (2wk-Intrusion) and control groups, and between the retention and the relapse groups. To compare other variables, one-way analysis of variance (ANOVA) and the post-hoc Duncan's multiple range test were performed. A p value less than .05 was considered statistically significant.

III. Results

1. The changes of body weight

The average of body weights taken every week during the experiment period was calculated. The average body weight decreased 1.56 gm one week after insertion of miniscrew implant (11 weeks old), thereafter it increased gradually. However, the increments of weight for one week were decreased. There was little of weight gain three weeks after the start of the experiment (14 weeks old).

2. Measurements on periapical films

A. The changes of the vertical position of molar caused by cementum apposition on root apex with aging

There was no statistically significant difference ($p > 0.05$) in the relative vertical positions of the middle cusp tip and the distal root apex of maxillary 3^{rd} molars (M3) in 13, 14 and 15 week old rats (Table 2).

B. The changes following molar intrusion and after retention or relapse following molar intrusion

1) The changes following molar intrusion

The cusp tip of maxillary 2^{nd} molar (M2) which had been intruded for two weeks was placed more apically than control teeth, as the root apex of maxillary 1^{st} molar (M1) was.

Table 2. The relative vertical position of molar caused by cementum apposition on root apex in 13, 14 and 15 weeks old rats (p = 0.05)

	13-week (n=5)	14-week (n=5)	15-week (n=5)	Sig.
b/a	8.50 ± 0.31	8.48 ± 0.23	8.46 ± 0.16	NS
c/a	6.76 ± 0.15	6.76 ± 0.96	6.75 ± 0.13	NS

a : the perpendicular distance from the most superior point of frontal-squamosal intersection at the temporal crest to the horizontal reference plane

b : the perpendicular distance from middle cusp tip of maxillary 3rd molar (M3) to the horizontal reference plane

c : the perpendicular distance from M3 distal root apex to the horizontal reference plane

b/a : the relative vertical position of M3 middle cust tip

c/a : the relative vertical position of M3 distal root apex

NS : not significant

However, the differences of the relative height of root apex between the intrusion and the control groups were less than those of cusp tip. The height of the alveolar crest in the intrusion group was lower than the control group, but there was no statistically significant difference in the mesial side of M1 and in the alveolar crest between M2 and M3, and also M1 and M2 (Table 3).

2) The changes after retention following molar intrusion

The relative vertical position of the intruded teeth for two weeks (2wk-Intrusion group) and the maintained teeth after intrusion (1wk-Retention and 2wk-Retention groups) showed no statistically significant difference.

Table 3. The relative vertical position of the intrusion (2wk−Intrusion group) and the control groups

	Intrusion	Control	Sig.
M2 distal cusp	0.964 ± 0.003	0.987 ± 0.002	**
Alveolar crest between M2 and M3	0.892 ± 0.006	0.900 ± 0.003	NS
Alveolar crest between M1 and M2	0.878 ± 0.011	0.882 ± 0.003	NS
Alveolar crest in M1 mesial side	0.868 ± 0.009	0.870 ± 0.007	NS
M1 distal root apex	0.710 ± 0.004	0.725 ± 0.002	**
M1 mesial root apex	0.707 ± 0.012	0.725 ± 0.006	*

M1, M2 and M3; maxillary 1^{st}, 2^{nd} and 3^{rd} molar, respectively.
* : $p < 0.05$
** : $p < 0.01$
NS : not significant

After one or two weeks of retention following intrusion, the relative height of the alveolar crest in the M1 mesial side and between intruded M2 and non−intruded M3 showed no statistically significant difference between after intrusion and after retention. The alveolar crest between intruded M1 and M2 during retention was positioned more apically than immediately after two weeks of molar intrusion.

The relative vertical position of the root apex of M1 was statistically significantly higher after retention following intrusion than immediately after intrusion (Table 4).

Table 4. The relative vertical position in the intrusion and the retention groups

	2wk–Intrusion	1wk–Retention	2wk–Retention	Sig.
M2 distal cusp	0.965 ± 0.003	0.965 ± 0.006	0.968 ± 0.008	NS
Alveolar crest between M2 and M3	0.892 ± 0.006	0.897 ± 0.002	0.896 ± 0.003	NS
Alveolar crest between M1 and M2	0.878 ± 0.011 A	0.865 ± 0.007 B	0.866 ± 0.003 B	*
Alveolar crest in M1 mesial side	0.868 ± 0.009	0.867 ± 0.011	0.863 ± 0.002	NS
M1 distal root apex	0.710 ± 0.004 A	0.727 ± 0.009 B	0.729 ± 0.007 B	**
M1 mesial root apex	0.707 ± 0.012 A	0.721 ± 0.006 B	0.720 ± 0.007 B	**

M1, M2 and M3 – maxillary 1^{st}, 2^{nd} and 3^{rd} molar, respectively; 2wk–Intrusion, two weeks of molar intrusion; 1wk–Retention, one week of retention after two weeks of molar intrusion; 2wk–Retention, two weeks of retention after two weeks of molar intrusion.

* : $p < 0.05$

** : $p < 0.01$

NS : not significant

3) The changes after relapse following molar intrusion

The differences of the relative vertical position of M2 cusp tip between the 2wk–Intrusion and the 2wk–Relapse groups were compared with between the intrusion and the control groups. In the 2wk–Intrusion group, M2 cusp tip was positioned 0.023 apically than the control group, and M2 cusp tip of the 2wk–Relapse group 0.015 occlusally than the 2wk–Intrusion group. Therefore, the relapse rate was calculated 41.67%.

Most of occlusal movements, called as relapse, occurred during the first week of relapse. The alveolar crest between intruded M2 and non−intruded M3 moved occlusally as teeth relapsed, but in the other alveolar crests there was no statistically significant difference with the 2wk−Intrusion group. The root apices of M1 were moved occlusally as the cusp tip of M2 did, but the amount of the root apices movement was bigger than that of the cusp tip (Table 5).

Table 5. The relative vertical position in the intrusion and the relapse groups

	2wk−Intrusion	1wk−Relapse	2wk−Relapse	Sig.
M2 distal cusp	0.964 ± 0.003 A	0.979 ± 0.006 B	0.979 ± 0.002 B	**
Alveolar crest between M2 and M3	0.892 ± 0.006 A	0.899 ± 0.004 B	0.901 ± 0.002 B	*
Alveolar crest between M1 and M2	0.878 ± 0.011	0.878 ± 0.004	0.879 ± 0.008	NS
Alveolar crest in M1 mesial side	0.868 ± 0.009	0.868 ± 0.009	0.866 ± 0.008	NS
M1 distal root apex	0.710 ± 0.004 A	0.730 ± 0.004 B	0.728 ± 0.008 B	**
M1 mesial root apex	0.707 ± 0.012 A	0.726 ± 0.009 B	0.724 ± 0.007 B	*

M1, M2 and M3 − maxillary 1^{st}, 2^{nd} and 3^{rd} molar, respectively; 2wk−Intrusion, two weeks of molar intrusion; 1wk−Relapse, one week of relapse after two weeks of molar intrusion; 2wk−Relapse, two weeks of relapse after two weeks of molar intrusion.

* : $p < 0.05$

** : $p < 0.01$

NS : not significant

4) The differences between the retention and the relapse groups according to duration after molar intrusion

The relative vertical position of M2 cusp tip in the retention and the relapse groups showed a distinct difference. The alveolar crest between intruded M2 and non-intruded M3 moved occlusally in both groups, but there was no statistically significant difference between one week of retention and relapse following intrusion. However, after two weeks of retention or relapse it was placed more apically in the retention groups than the relapse groups. The alveolar crest between M1 and M2 that were intruded for two weeks was positioned more apically in the retention groups than the relapse groups. In the alveolar crest of M1 mesial side, there was no statistically significant difference between with and without retention. It was the same in the root apices of M1 (Table 6).

Table 6. The comparison between the retained and the relapsed teeth according to duration after molar intrusion

	1wk-Retention	1wk-Relapse	Sig.	2wk-Retention	2wk-Relapse	Sig.
M2 distal cusp	0.965 ± 0.006	0.979 ± 0.006	*	0.968 ± 0.008	0.979 ± 0.002	*
Alveolar crest between M2 and M3	0.897 ± 0.002	0.899 ± 0.004	NS	0.896 ± 0.003	0.901 ± 0.002	*
Alveolar crest between M1 and M2	0.865 ± 0.007	0.878 ± 0.004	*	0.866 ± 0.003	0.879 ± 0.008	*
Alveolar crest in M1 mesial side	0.867 ± 0.011	.0868 ± 0.009	NS	0.863 ± 0.002	0.866 ± 0.008	NS
M1 distal root apex	0.727 ± 0.009	0.730 ± 0.004	NS	0.729 ± 0.007	0.728 ± 0.008	NS
M1 mesial root apex	0.721 ± 0.006	0.726 ± 0.009	NS	0.720 ± 0.007	0.724 ± 0.007	NS

M1, M2 and M3 - maxillary 1st, 2nd and 3rd molar, respectively; 1wk-Retention, one week of retention after two weeks of molar intrusion; 1wk-Relapse, one week of relapse after two weeks of molar intrusion; 2wk-Retention, two weeks of retention after two weeks of molar intrusion; 2wk-Relapse, two weeks of relapse after two weeks of molar intrusion.

* : $p < 0.05$

NS : not significant

3. Micro computer tomographic findings

Intruded M1 and M2 were placed more apically than control teeth and they were maintained well during retention period. In the relapse groups where the intruding appliance was disengaged without retainer, intruded M1 and M2 showed a similar vertical position to M3 that was not intruded. In the relapse groups, the difference of the vertical position of teeth according to the duration of relapse after intrusion was not evident (Fig. 4).

Rough root surface with multiple small craters was observed in the 2wk-Intrusion group, but it disappeared after retention or relapse. In M1 and M2 of the 2wk-Intrusion group, the mesial surface of roots, from which the intrusion force was originated, showed root resorptions in apical 1/3 area (Fig. 4, G). In the retention and the relapse groups root resorptions were also observed, especially in short and small roots. There was no distinct difference of roots in furcation area between the experimental and the control groups.

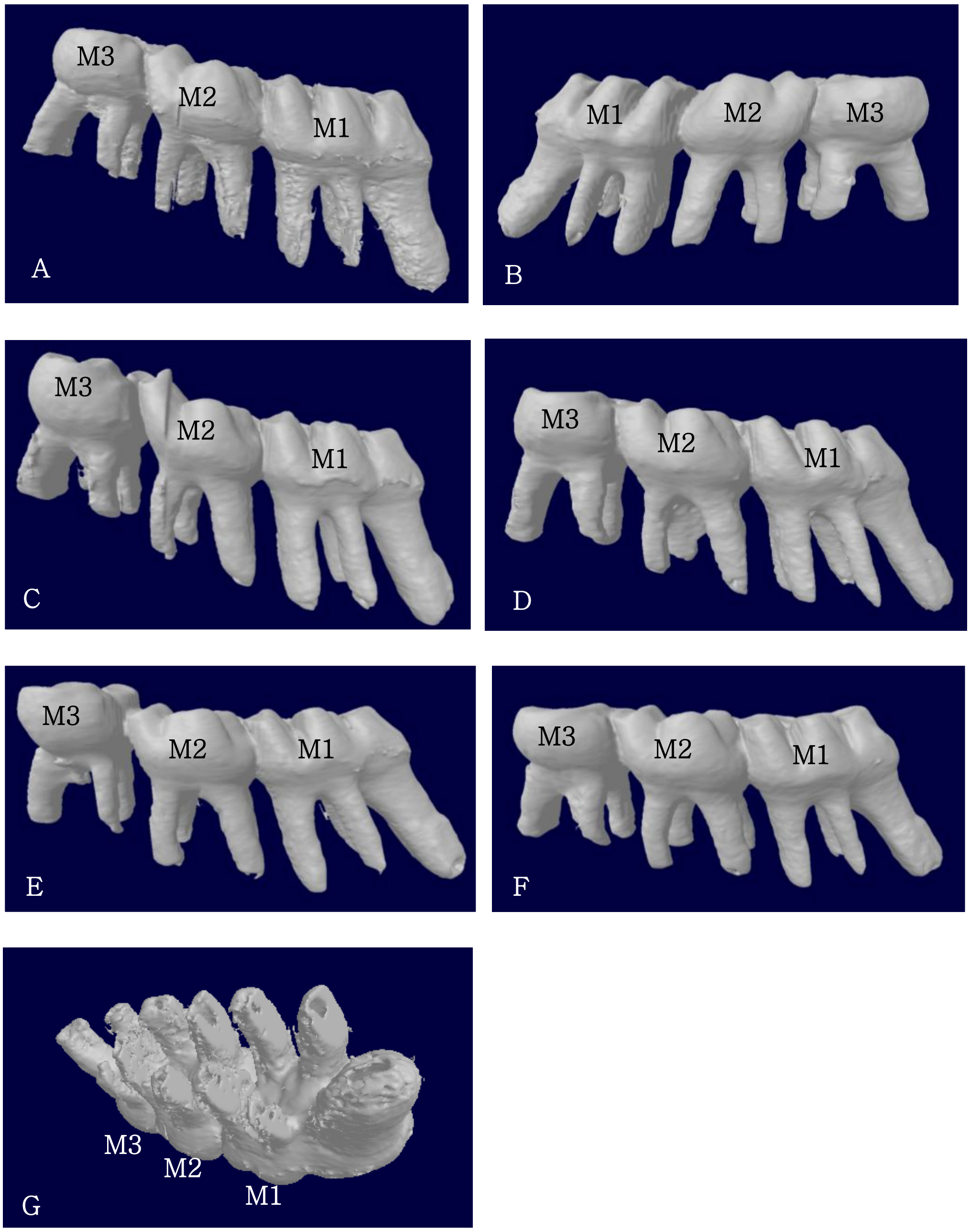


Figure 4. Micro-CT 3D images of teeth (Buccal view). A, 2wk-Intrusion group; B, Opposite side of 2wk-Intrusion group (control side); C, 1wk-Retention group; D, 1wk-Relapse group; E, 2wk-Retention group; F, 2wk-Relapse group; G, Bottom view of 2wk-Intrusion group. M1, M2 and M3 - maxillary 1^{st}, 2^{nd} and 3^{rd} molar, respectively.

In the alveolar socket adjacent to furcation area of the 2wk-Intrusion and the retention groups, the interradicular alveolar bone showed similar configurations to the control group. Otherwise, in the relapse groups the smooth texture of the interradicular alveolar bone was not observed and the surface was rougher than in other groups. But the roughness was decreased according to the duration of relapse (Fig. 5).

It was shown that the root apices of the intruded molars penetrated into the nasal cavity in the 2wk-Intrusion group. Even though the perforated hole of the apical alveolar bones in the control group was apparent, its number and size were much smaller in the control group than the 2wk-Intrusion group. The perforated wholes were also seen in the retention groups, but the size was decreased in the 2wk-Retention group compared to the 1wk-Retention group. In the relapse groups, the perforated area was notably diminished, therefore the differences between the 2wk-Relapse and the control groups were not evident (Fig. 5).

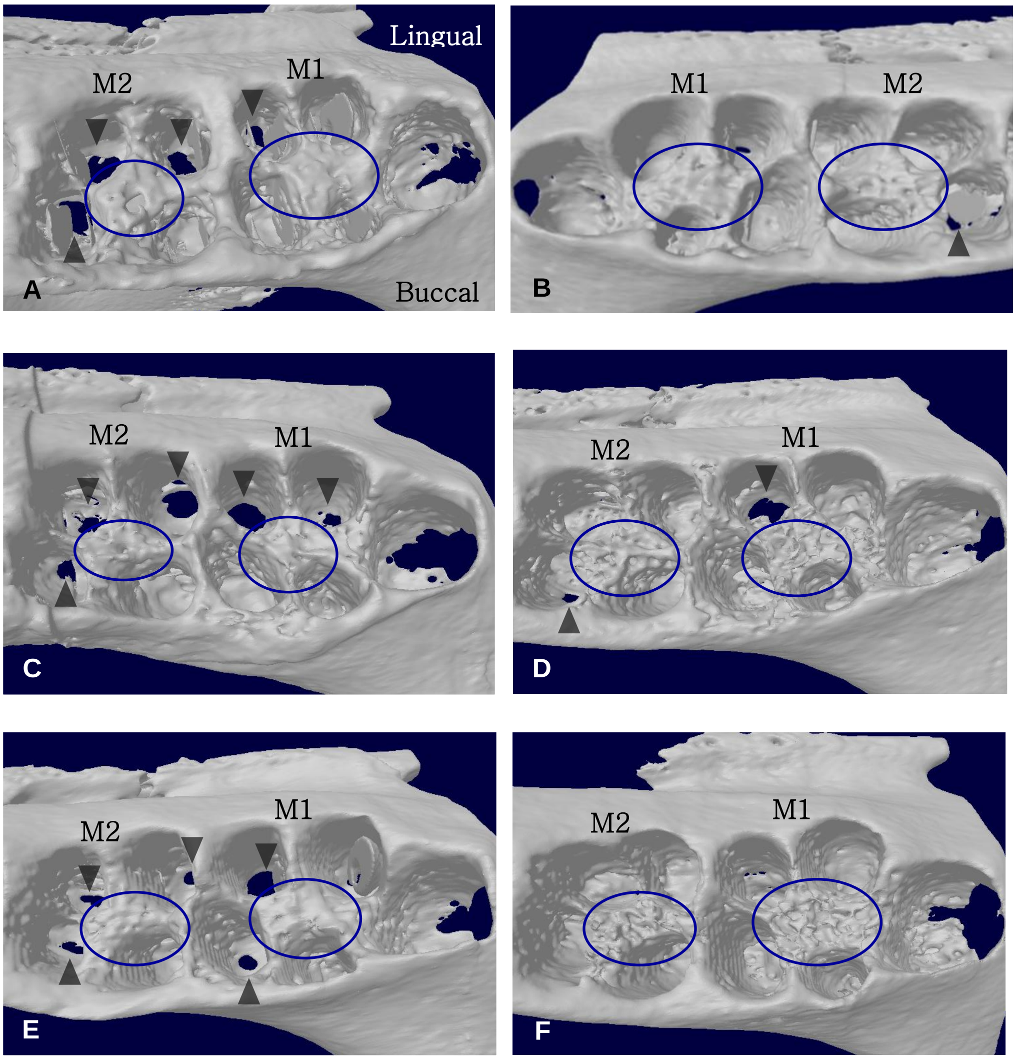


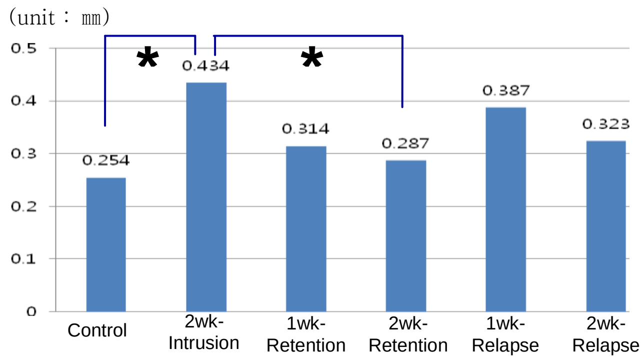
Figure 5. Micro−CT 3D images of alveolar socket (Occlusal view). A, 2wk−Intrusion group; B, Opposite side of 2wk−Intrusion group (control side); C, 1wk−Retention group; D, 1wk−Relapse group; E, 2wk−Retention group; F, 2wk−Relapse group. M1 and M2 − maxillary 1st and 2nd molar, respectively. (Top, lingual; Bottom, buccal side). Arrowheads, the perforated hole of the apical alveolar bone (the holes in M1 mesial apex were excluded because they were seen in all groups); circles, interradicular alveolar bone adjacent to furcation area.

4. Histologic findings

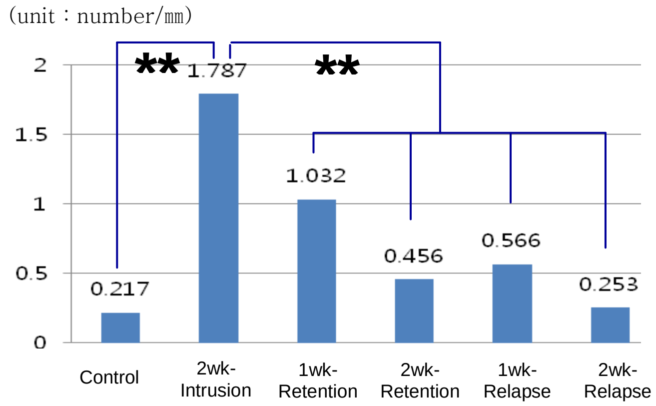
A. Depth from free gingival margin to the apical end of epithelium in the mesial side of maxillary 1^{st} molar

Depth from free gingival margin to the apical end of epithelium after two weeks of molar intrusion was increased statistically significantly compared to the control group and decreased again after two weeks of retention following intrusion. Even though it had been decreased after retention or relapse, there was no statistically significant difference between the 2wk-Intrusion group and other experimental groups except the 2wk-Retention group. In the retention and the relapse groups it was measured more than the control group, but there was no statistically significant difference (Fig. 6, A).

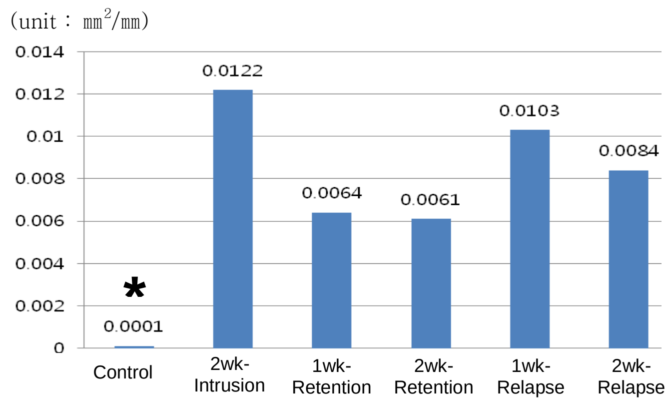
The epithelial attachment reached the cement-enamel junction in both the control and the experimental specimens. The junctional epithelium was lengthened with molar intrusion, consequently the depth from free gingival margin to the apical end of epithelium was increased. However, there was no sign of edema or swelling of the gingiva. The thickness of junctional and sulcular epitheliums was increased in the retention and the relapse groups and it was more evident in the relapse groups. (Fig. 7).



A. Depth from free gingival margin to the apical end of epithelium



B. Number of osteoclasts



C. Root resorption area

Figure 6. Histomorphometric analyses of the control and the experimental groups. A, Depth from free gingival margin to the apical end of epithelium; B, Number of osteoclasts; C, Root resorption area.

* : $p < 0.05$

** : $p < 0.01$

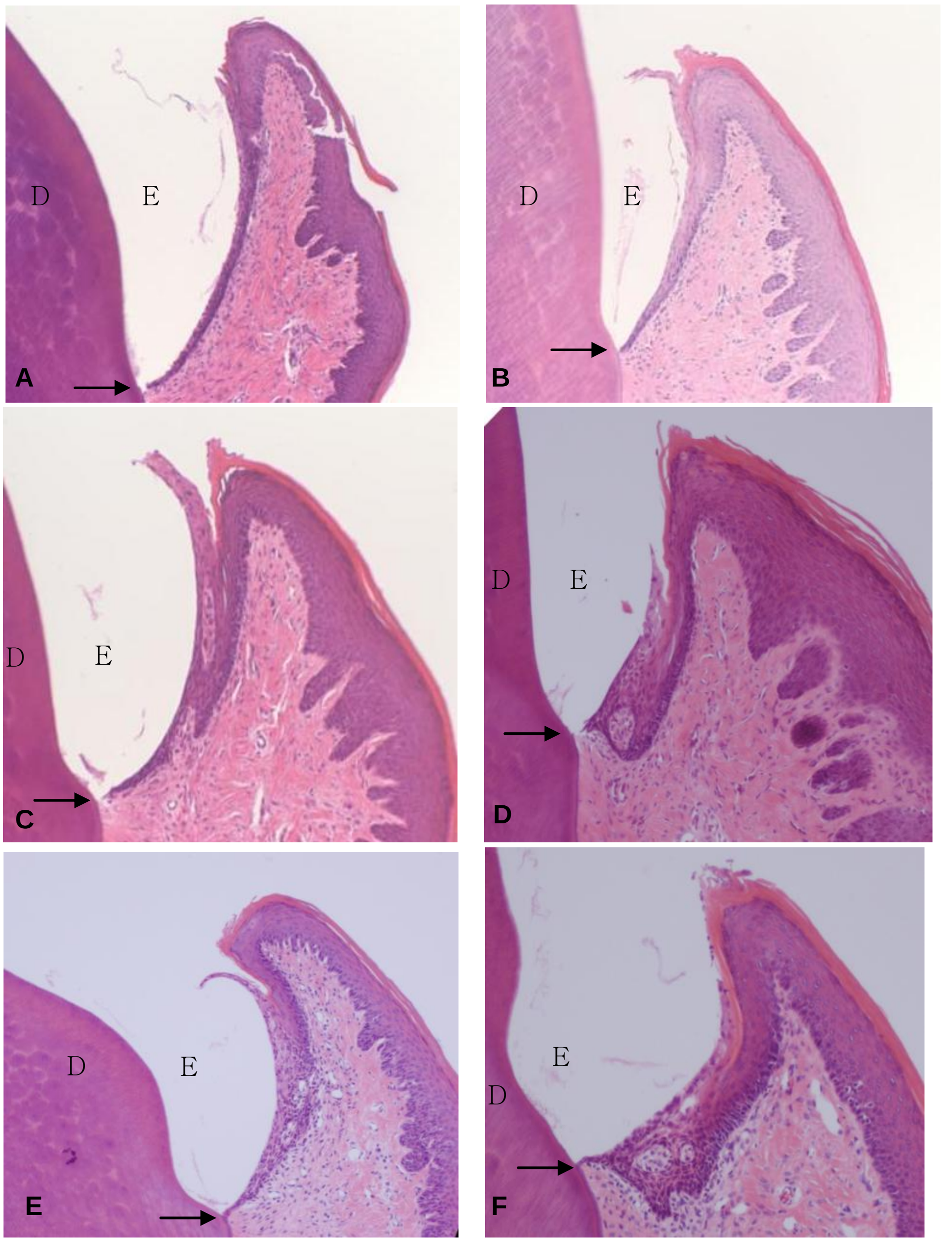


Figure 7. Changes of gingival sulcus and junctional epithelium in the mesial side of maxillary 1st molar. A, 2wk−Intrusion group; B, Control group; C, 1wk−Retention group; D, 1wk−Relapse group; E, 2wk−Retention group; F, 2wk−Relapse group. Arrow indicates CEJ (cement−enamel junction); D, dentin; E, enamel cavity. (Magnification X 100)

B. Osteoclasts on the alveolar bone surface adjacent to root

The number of osteoclasts per unit length of alveolar bone surface after two weeks of molar intrusion was increased statistically significantly compared to the control group and it was decreased again after retention or relapse following molar intrusion (Fig. 6, B).

1) Interradicular area

In the experimental groups, active modeling and remodeling of alveolar bone was seen compared to the control group. The incidence of blood vessels in the apical and marginal alveolar bones as well as the interradicular alveolar bone was high in the retention and the relapse groups, especially the 1wk−Retention group (Fig. 8).

After two weeks of molar intrusion many osteoclasts were seen, but the number of osteoclasts was decreased after retention or relapse following molar intrusion. Osteoclasts on the alveolar bone surface were observed more in the retention groups than the relapse groups (Fig. 9).

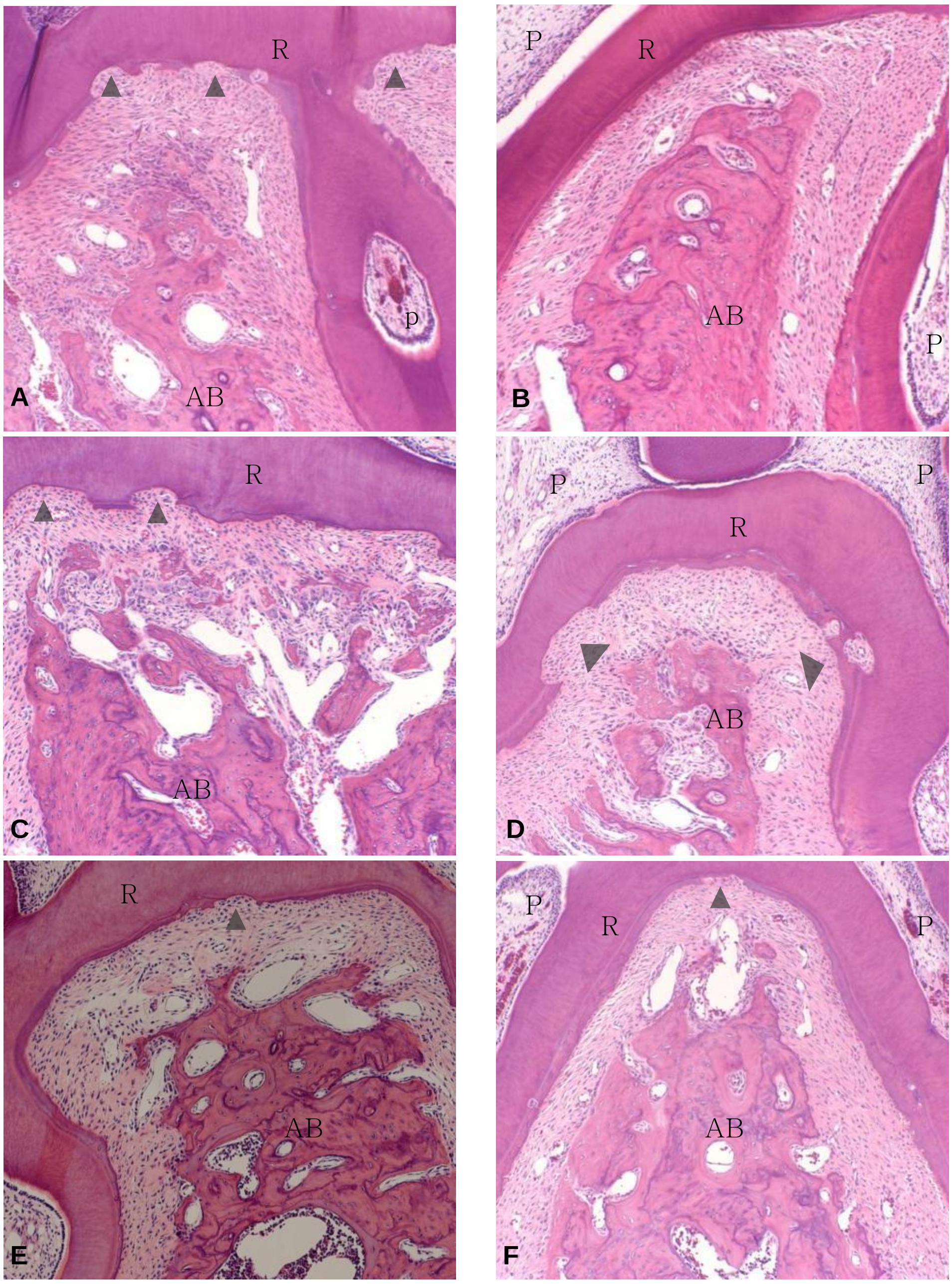


Figure 8. Interradicular alveolar bone and furcation area of the intruded teeth. A, 2wk−Intrusion group; B, Control group; C, 1wk−Retention group; D, 1wk−Relapse group; E, 2wk−Retention group; F, 2wk−Relapse group. Arrowheads indicate root resorption area; AB, alveolar bone; P, pulp; R, root dentin. (Magnification X 100)

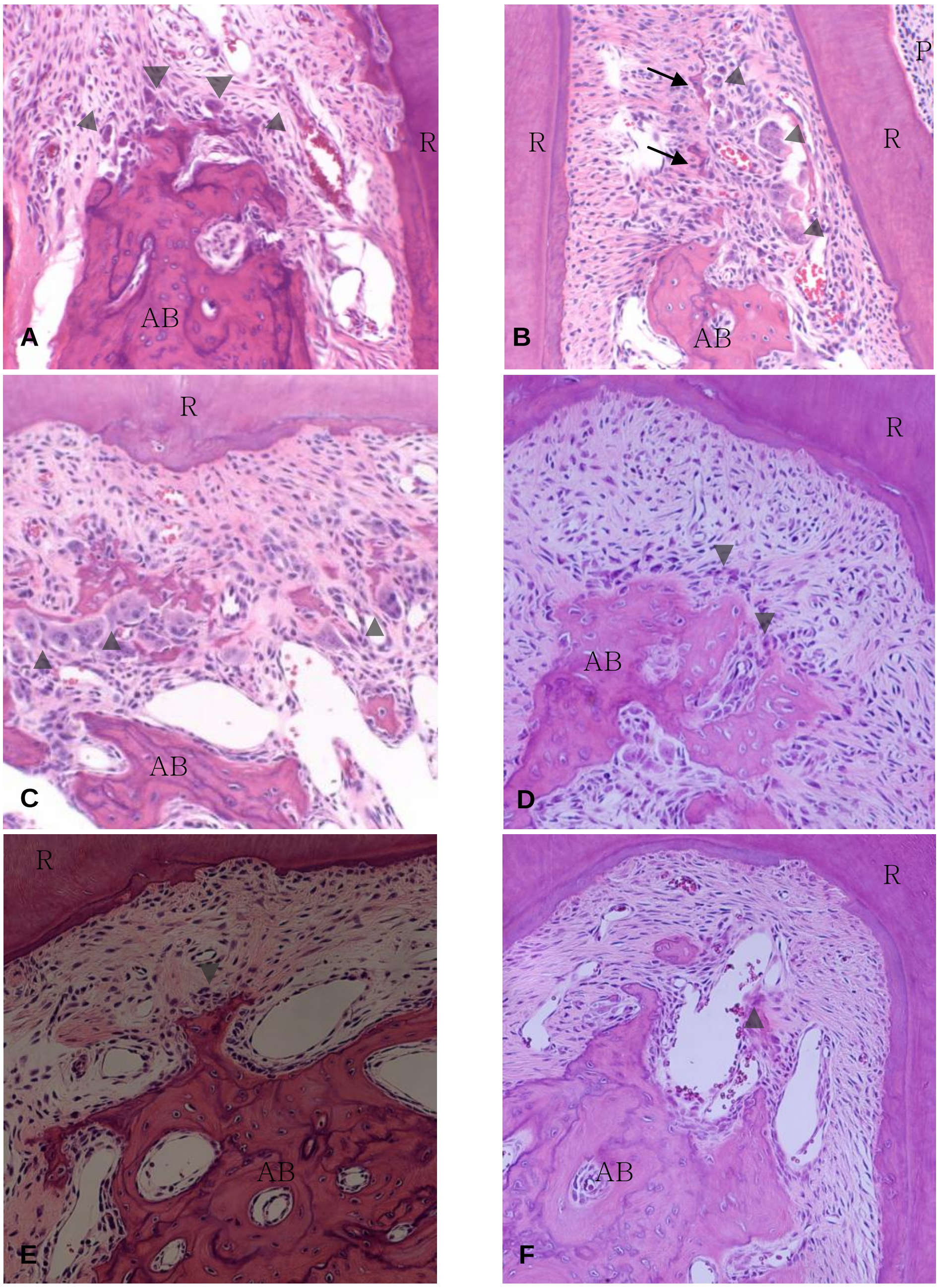


Figure 9. Osteoclasts in the interradicular area. A and B, 2wk−Intrusion groups; C, 1wk−Retention group; D, 1wk−Relapse group; E, 2wk−Retention group; F, 2wk−Relapse group. Arrowheads, osteoclasts; arrows, alveolar bone remnants; AB, alveolar bone; P, pulp; R, root dentin. (Magnification X 200)

2) Interdental area between maxillary 2^{nd} and 3^{rd} molar

The apical end of epithelium of maxillary 2^{nd} and 3^{rd} molars was located at CEJ in both the experimental and the control groups. The transseptal fibers ran straight across the interdental septum in the control group, while they were stretched toward intruded tooth after two weeks of intrusion. The orientation was changed after retention or relapse. The transseptal fiber bundles were imbedded approximately perpendicular to the cementum and ran parallel to the imaginary line between CEJs of two adjacent teeth.

Direct and undermining bone resorptions on the marginal alveolar bone adjacent to intruded teeth were observed in the 2wk−intrusion group. In other experimental and the control groups, however, osteoclasts adjacent to alveolar bone surface were hardly observed and osteoid tissue bordered by osteoblasts was seen on the alveolar crest and into the resorptive lacunas (Fig. 10).

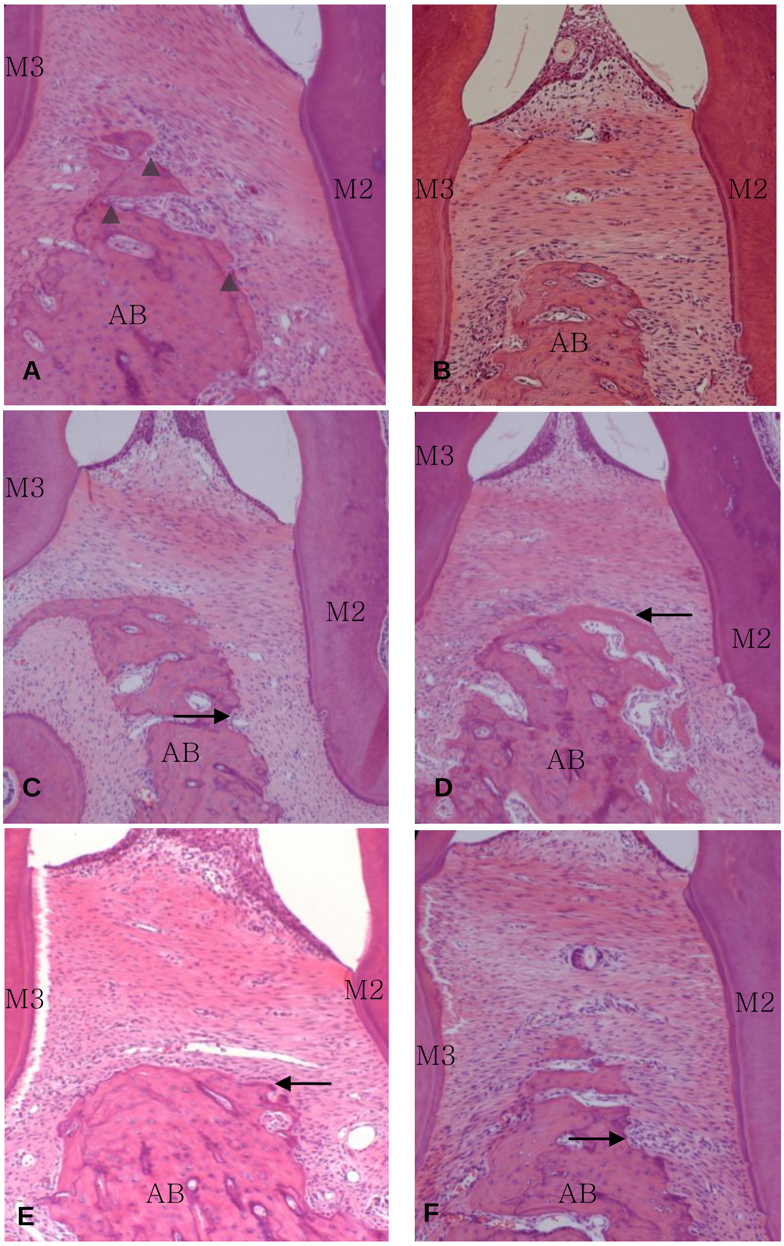


Figure 10. Interdental area between intruded (M2) and non-intruded (M3) teeth. A, 2wk-Intrusion group; B, Control group; C, 1wk-Retention group; D, 1wk-Relapse group; E, 2wk-Retention group; F, 2wk-Relapse group. Arrowheads, osteoclasts; arrows, osteoid tissue bordered by osteoblasts; M2, maxillary 2nd molar; M3, maxillary 3rd molar; AB, alveolar bone. (Magnification X 100)

C. Root resorption area

Surface root resorptions were observed in all experimental groups. The area of root resorption in all experimental groups was statistically significantly larger than the control group (Fig. 6, C). Root resorption area after retention or relapse following molar intrusion was decreased compared to the 2wk-Intrusion group, but there was no statistically significant difference between the 2wk-Intrusion and other experimental groups. Root resorption partly reached into dentin was repaired with cementum after retention or relapse (Fig. 8).

D. Periodontal ligament

The oblique periodontal ligament (PDL) fibers that were intruded for two weeks were stretched toward the direction of intrusion. The apical and interradicular PDL fibers were compressed and the density of cells was increased after two weeks of intrusion, while the apical PDL of control group showed a radial shape. The PDL stretching toward apex decreased gradually according to the duration of retention. A radial shape of the apical fibers similar to the control group was observed in the 2wk-Retention group. In the relapse groups, the stretched oblique fibers were not seen and the density of cells in the apical PDL was decreased compared to the 2wk-Intrusion group, in which the apical fibers were compressed by intrusion. PDL in the 2wk-Relapse group looked more similar to the normal PDL than in the 1wk-Relapse group (Fig. 11).

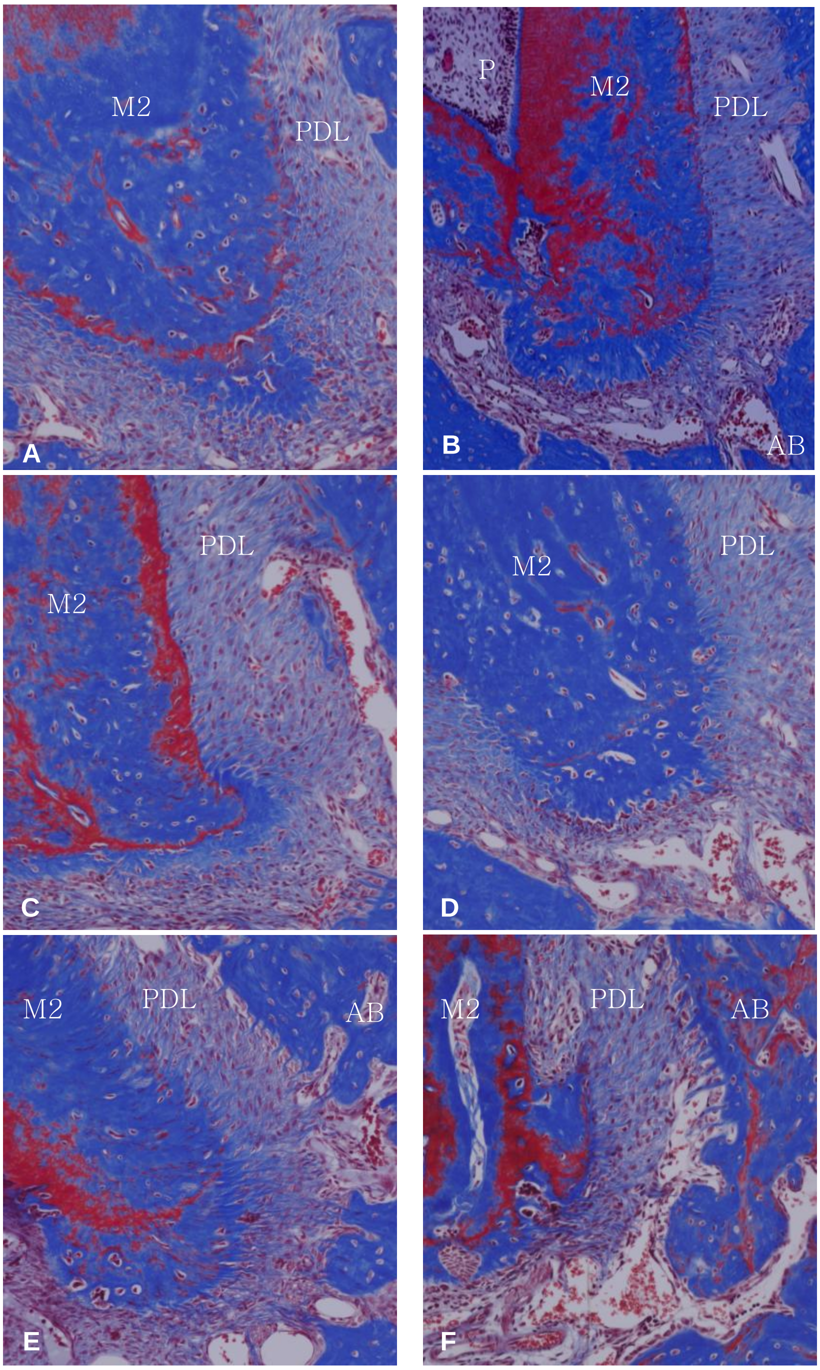


Figure 11. Masson trichrome stained sections of root apex of maxillary 2nd molar. A, 2wk−Intrusion group; B, Control group; C, 1wk−Retention group; D, 1wk−Relapse group; E, 2wk−Retention group; F, 2wk−Relapse group. M2, maxillary 2nd molar; AB, alveolar bone; P, pulp; PDL, periodontal ligament. (Magnification X 200)

IV. Discussion

Molar intrusion was nearly impossible before orthodontic miniscrew implant provided absolute anchorage for tooth movement. Consequently, there were few reports of molar intrusion unlike incisor intrusion and periodontal tissue changes after retention or relapse following molar intrusion. Molar intrusion is thought to be different from incisor intrusion in that molar is a multiradicular tooth which has a furcation area, while incisor has a single root, and persistent vertical force is applied to the occlusal table. Studies about molar intrusion have been performed in beagle dogs using miniplate[3,4,11,12]. When using beagle dogs there were limitations that it was difficult to generalize the results because of relatively small sample sizes and the response of molar to intrusive force in beagle dog could be different from human molar because it had no occlusal tables. In this study, the subjects were rats, which had occlusal tables like human molars, even though they didn't work for occluding, and were useful to generalize the results by increasing the sample size.

Rats used in this study were ten weeks old female. Rats become sexually mature at age of six weeks[21], there is no more increase of bone maturity score since nine weeks after birth[22]. In rats females are more advanced in skeletal maturity than males from birth to adulthood and the cephalocaudal maturity gradient is seen in the development process[22]. Shimomoto et al[23] reported that bone formation at the periosteal surface of the alveolar and jaw bones was very active in five week old rats but declined gradually with age and bone formation rate in rat alveolus significantly decreased since nine weeks after birth.

Since intrusion force in rats was not possible to be delivered directly from miniscrew implant placed in the interdental alveolar bone as human jaws, the alveolar crest behind maxillary incisor, where there was enough cortical bone to stabilize the miniscrew implant with no important anatomic structures, was chosen for miniscrew implantation. A miniscrew implant, 1.2 mm in diameter and 7.0 mm in length, was placed and intrusion force of 50 gm was delivered to molars indirectly.

Japanese nickel titanium (NiTi) alloy wire possesses characteristics of superelasticity and low hysteresis[19]. With superelasiticity the stress value remained fairly constant, therefore the additional activation of wire after initial application was not required. Low hysteresis, which means there is small difference between loading and unloading of wire, delivered constant intrusive force to teeth during two weeks of intrusion. It is possible to bend wire into other configurations without losing the superelastic quality by direct electric resistance heat treatment method. Thus Japanese NiTi wire which was supported by miniscrew implant in the anterior part of maxilla could deliver the intrusive force to molars indirectly.

Bondevik[18] reported that when rat molar were intruded, the reaction to forces of different magnitude, varying between 0.29 and 0.98 N, was essentially the same but the incidence of cellfree zones and root resorption lacunae seemed to increase as the force increased. Based on this finding, the intrusion force was decided to be 50 gm, which was thought to be the lowest force level for the heat−bended NiTi wire to deliver. The intrusion appliance was confirmed not to inhibit normal growth of rats by checking changes of body weight.

Cementum apposition and occlusal bonding material could affect the vertical position of molars. Cementum apposition on root apex with aging did not make a statistically significant difference in the vertical position of molars during experimental periods. In order to evaluate whether the bonding materials which attached wire to occlusal tables of molars would cause the vertical displacemenet of teeth, pre−experimental study was performed. It was found that the occlusal bonding material did not affect the relative vertical positions of teeth for two weeks ($p > 0.05$). Bresin[24] reported that lower molars to which bite block had been bonded during four weeks were intruded in four week old rats. However, it is evident that the alveolar bone formation rate is much higher in four week old rats in their most active period of skeletal growth than over ten week old rats when skeletal growth is almost finished[22,23].

Periodontal tissue changes after the application of intrusive force were discussed in following orders; junctional and sulcular epitheliums, alveolar bone, root resorption and periodontal ligament.

After two weeks of molar intrusion, the depth from free gingival margin to the apical end of epithelium was increased and the marginal, interradicular and apical alveolar bones were resorbed. The number of osteoclasts per unit alveolar bone surface immediately after molar intrusion was statistically significantly larger than other experimental and the control groups. However, the alveolar crest height measured on periapical films immediately after molar intrusion did not show statistically significant difference from the control group. The findings that crown moved more apically than root apex after molar intrusion indicated a surface root resorption on root apex resulting in root shortening. Root resorptions partly reached into dentin were observed on overall root surfaces, and it was confirmed from numerous root craters seen in the 3D reconstructed CT images. Periodontal ligament was stretched toward the direction of intrusion.

In the retention groups, intruded teeth were maintained well. The changes due to relapse were mostly occurred during the first week of relapse. Periodontal tissues were remodeled correspondingly to the altered teeth position after retention or relapse. The increased depth from free gingival margin to the apical end of epithelium was decreased again and so did the number of osteoclasts. Surface root resorptions were repaired with cementum and the configuration of stretched oblique and compressed apical PDL fibers was changed similar to normal PDL.

The depth from free gingival margin to the apical end of epithelium was increased and junctional epithelium was lengthened as teeth were intruded.

In every experimental specimen, the epithelium ended at the CEJ just as it did in the control teeth, and there was no apical migration whatever. It indicated that in normal periodontal tissue the apical end of epithelium moved in the apical or occlusal direction with tooth as much as tooth movement. Murakami et al[25] reported the same finding from incisor intrusion in monkeys. It was also stated that the gingiva moved in the same direction that the teeth were intruded, but only about 60% as far. After one or two weeks of retention, the depth from free gingival margin to the apical end of epithelium was decreased. It could be inferred that the further recession of free gingival margin was occurred during retention, because the apical end of epithelium was not changed. However, the change in the relapse groups may not be the same as that in the retention groups, because the position of the apical end of epithelium was changed with tooth movement.

Alveolar bone resorptions in the marginal, interradicular and apical area were induced by intrusion, and they were remodeled actively during the initial phase of retention and looked similar to normal alveolar bone in the later phase.

Not only alveolar bone resorptions but root resorptions were more evident in the interradicular region as compared to the marginal and apical regions. Several possible explanations were stated. Intrusive forces would interfere more markedly with the blood supply and create more extensive cell free zones in the interradicular region than in the apical and marginal regions[18]. It has been also suggested that bone morphology is a factor affecting the occurrence of cellfree zones and the nature of bone resorption[26] and cementum maturity is of significance for the progression of root resorption[27].

In the two weeks of molar intrusion group, the density of osteoclasts was higher in the interradicular alveolar crest than in other regions and a couple of alveolar bone remnants were observed above the interradicular alveolar crest. In the one week of retention following molar intrusion (1wk-Retention) group, many osteoclasts were observed in the interradicular alveolar crest as compared to the control and other retention (2wk-Retention) and relapse (1wk-Relapse and 2wk-Relapse) groups, even though there was no statistically significant difference.

The height of alveolar crest moved apically with molar intrusion, but there was no statistically significant difference between immediately after molar intrusion and the control groups. After one or two weeks of retention, the alveolar crest between two intruded teeth was positioned apically ($p < 0.05$). On the other hand, the alveolar crest between intruded maxillary 2^{nd} molar and non-intruded maxillary 3^{rd} molar was positioned occlusally with the relapse of tooth after one or two weeks of relapse ($p < 0.05$).

Numerous reports indicate that alveolar bone resorption is the consequence of increased pressure in the periodontal ligament, while deposition of osteoid tissue is elicited by a stretching of the fibers. Bone resorption on the alveolar crest cannot be explained in the same way. It may be a consequence of pressure exerted against the crest by free gingival fibers as tooth is depressed. This was confirmed by the experiment of supracrestal fiberotomy[18]. Kanzaki et al[4] also reported the same findings that the amount of alveolar bone resorption was smaller in fiberotomy group compared with nonfiberotomy group on the alveolar crest.

In the studies from molar intrusion of beagle dogs, monkeys and human, mild root resorptions in the root apices and the furcation areas were observed[5,6,11-13]. The findings from premolar[27-31] and incisor intrusions[32,33] of humans indicated similar results and it was also observed in rat molars. Most root resorptions were occurred in the apical third of root, and it resulted in root shortening. During the first week of retention following two weeks of molar intrusion, root resorption was aggravated a little. During the second week of retention, additional root shortening was not observed. The retention groups showed more apical root resorptions than the relapse groups. Cementum immaturity may be related to apical root resorptions. Immature cementum might be resorbed earlier than apical alveolar bone under increased pressure around apical PDL following intrusive force. Root resorption partly reached into dentin was repaired with cementum. Stenvik and Mjor[27] had stated that the defects created would be repaired by tissue resembling bone and cementum if the teeth were left in situ after the force was removed.

The oblique fibers of periodontal ligament were stretched and the apical and interradicular fibers were compressed after intrusion. PDL was also remodeled according to the new position of teeth and PDL after two weeks of retention looked more similar to normal PDL than after two weeks of relapse.

Intrusion is not favorable for retention because tooth is moved opposite to the physiologic movement of extrusion. Furthermore, the interradicular and the apical periodontal tissues compressed during intrusion are reorganized more slowly than in other sites, because repair of resorbed roots as well as adjacent alveolar bone resorption are required simultaneously. Additionally, intrusion is less stable than rotation and mediodistal movement because periodontal fibers, which are generally thought to resist occlusal forces, can also strongly resist intrusive force and an effective method for retention has not been established for intruded molars[7]. In previous study, it was demonstrated that the application of bisphosphonates in animals was an effective pharmacologic method of retention that inhibited alveolar bone from remodeling around moved teeth[34]. It can be applied to retention of intruded molar in animals and even humans after further experiments.

In this study, it was confirmed that rat molars were intruded using orthodontic miniscrew implant and they were maintained to the altered position. Periodontal tissues were remodeled to healthy periodontium after the retention period. The changes of vertical position in the relapse groups mostly occurred in the first week of relapse.

V. Conclusion

Orthodontic miniscrew implants are commonly used in current practice to intrude molars when correcting anterior openbite. However, since there have been few reports on the changes of the intruded teeth and periodontal tissue during retention period following molar intrusion, relapse rates are controversial and even the effectiveness of anterior openbite correction with molar intrusion has been questioned. The aim of this study was to observe periodontal tissue changes after intrusion of posterior teeth in rats using miniscrew implant and its features of retention or relapse.

Orthodontic miniscrew implant was placed behind maxillary left incisor in a ten week old rat and 50 gm of intrusion force was applied to the maxillary left 1^{st} and 2^{nd} molars for two weeks with Japanese NiTi wire. Periodontal tissue changes after two weeks of molar intrusion and after a period of one to two weeks of retention or relapse were observed. The results were as followings;

1. When molar was intruded, mild root resorption was occurred and the depth from free gingival margin to the apical end of epithelium was increased due to formation of long junctional epithelium. Periodontal ligament was stretched in the oblique portion toward the direction of intrusion and compressed in the interradicular and the apical regions. Alveolar bone in the marginal, interradicular and apical areas was resorbed with tooth intrusion.

2. After retention, craters created by surface root resorption were repaired with cementum, consequently the size of the craters was decreased, and shortening of the root was observed. The free gingival margin was recessed and the periodontal ligament was remodeled correspondingly to the altered tooth position and resulted in a close resemblance to healthy periodontium. Alveolar crest between intruded teeth was positioned more apically than immediately after molar intrusion. The periodontal tissues showed more active remodeling after one week of retention compared to two weeks of retention following molar intrusion.

3. After relapse, root resorption craters were decreased compared to immediately after intrusion and repaired with cementum. With the relapse of teeth, the depth from free gingival margin to the apical end of epithelium was decreased and the stretched periodontal ligament moved occlusally. Alveolar crest between intruded teeth showed the same vertical position as immediately after two weeks of molar intrusion, even though the intruded teeth moved occlusally.

It was confirmed from this study that after retention, root resorption which occurred by molar intrusion was repaired with cementum and periodontal tissues were remodeled correspondingly to the altered tooth position, resulting in a final normal and healthy periodontium. Most teeth movements of relapse occurred in the early phase, therefore initial retention is important for the stability of treatment.

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국문요약

백서 구치의 압하 후 보정 및 재발 양상

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최 윤 정

교정용 미니스크류 임플란트를 이용하여 여러 구치부 치아들을 압하시킴으로써 전치부 개방교합을 치료하는 것이 보편적인 치료 방법이 되어가고 있다. 그러나 압하된 치아 및 그 주위 조직이 보정 기간 중 일어나는 과정에 대해서는 연구된 바가 거의 없다. 본 연구는 백서 구치를 압하시킨 후 보정 혹은 재발시켰을 때 조직 반응을 연구하고 기간에 따른 변화 양상을 관찰하는 것을 그 목적으로 하였다.

교정용 미니스크류 임플란트를 10주된 백서의 상악 왼쪽 전치 후방 치조정에 식립하고, 압하력을 전달하도록 Japanese NiTi wire를 미니스크류 임플란트에 연결하여 상악 왼쪽 제 1, 2 대구치에 2주간 50 gm의 압하력을 가하였다. 구치를 압하시킨 직후의 조직 변화 및 구치 압하 후 1, 2주 동안 그 위치를 유지시키거나 재발시켰을 때의 조직 변화를 관찰하였다.

구치 압하로 경미한 치근 흡수가 나타났으며, 압하된 치아를 보정 혹은 재발시키는 동안 치근 흡수 부위는 백악질에 의해 재생되었다. 압하된 치아 주위의 치조골에서 활발한 골 재형성 및 개조 현상이 관찰되었으며, 특히 치근 사이의 치조골에

서 가장 활발하게 나타났다. 구치 압하로 치조정 높이는 감소하였으나 통계적 유의차를 보이지는 않았다. 그러나 유지 기간 후 압하된 두 치아 사이의 치조정은 치근첨 방향으로 이동되었다 (p < 0.05). 상피의 최하방점은 법랑-백악 경계를 따라서 이동하였으며 그 결과 구치 압하로 인한 긴 접합상피가 형성되었다 (p < 0.05). 치주인대는 인장부는 신장되고, 압박부는 눌린 형태가 관찰되었다. 그러나 보정 기간을 거치면서 치은연이 퇴축되고 치주인대는 재형성되어 건강하고 정상적인 치주조직을 나타내었다. 재발에 의한 대부분의 치아 이동은 초기에 발생하므로 초기 보정 기간이 치료의 안정성을 위해 중요할 것이다.

핵심되는 말 : 구치부 압하, 보정, 재발, 전치부 개방교합, 절대적 고정원, 교정용 미니스크류 임플란트