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The Influences of Molar Intrusion on the Inferior Alveolar Neurovascular Bundle and Root Using the Skeletal Anchorage System in Dogs

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ABSTRACT

The skeletal anchorage system (SAS) was developed as intraoral rigid anchors for open-bite correction by intrusion of molars. Since the application of SAS is a new modality in orthodontic treatment, the influences of radical molar intrusion on the root and the inferior alveolar neurovascular bundle were unknown. The purpose of this research is to verify the effect of molar intrusion on the neurovascular bundle, the level of osseointegration of bone screws, and root resorption. The results of this study showed mandibular molars were intruded 3.4 mm on the average over 7 months in dogs. The miniplates were well stabilized with osseointegrated bone screws and the peri-implant soft tissues showed slight inflammatory changes. Neither nerves nor blood vessels were damaged. Root resorption was observed but was repaired with new cementum. We concluded that the SAS utilizing transmucosal titanium miniplates as an immovable orthodontic anchorage could provide a new modality for molar intrusions without serious iatrogenic problems.

KEY WORDS: Skeletal anchorage system, Molar intrusion, Inferior alveolar neurovascular bundle, Root resorption, Titanium miniplate.

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The skeletal anchorage system (SAS) was developed as an intraoral anchor for orthodontic tooth movement.¹⁻³ In SAS, osseointegrated titanium miniplates with monocortical screws provide rigid temporary anchorage for molar intrusion, which is nearly impossible using traditional orthodontic mechanotherapy. Since titanium miniplates transmucosally fixed on the buccal cortical bone of the mandible and/or maxilla with monocortical screws provide immovable anchorage, it has become possible to treat some anterior open-bite cases without any extraoral anchorages or orthognathic surgery. In our previous report, we demonstrated that mandibular molars were successfully intruded


Since the application of SAS for molar intrusion is a fairly new modality in orthodontic treatment, the effects of molar intrusion on root resorption and position of the inferior alveolar neurovascular bundle were completely unknown. However, we did observe that, in some anterior open-bite cases, the intruded mandibular molars moved into the mandibular body and reached the inferior alveolar neurovascular bundles. When viewing the radiographic films, the mandibular canal seemed to remodel and drift to avoid being compressed in those patients. The close relationship between the root and the mandibular canal was reported to cause temporary paresthesia of the lower lip during orthodontic treatment,^{4,5} while in our clinical evaluations, no neurosensory dysfunction occurred following extensive intrusion of the mandibular molars.

Stability and osseointegration of titanium implants have been extensively studied in the field of dental implants and have shown good results.^{3,6} However, the information regarding titanium miniplates, temporarily transmucosally fixed on the buccal cortical bone for orthodontic anchorage, remained unknown.


The purpose of this research is to clarify the effect of mandibular molar intrusion on the position of the inferior alveolar neurovascular bundles to verify the prospect of osseointegration by temporarily implanted titanium miniplates and to study root resorption accompanied with intrusion. We employed an animal model system using dogs to histologically elucidate the effects of molar intrusion. We hypothesized that molar intrusion induced repositioning of the inferior alveolar neurovascular bundles to prevent nerve and blood vessel damage and that the transmucosal titanium miniplates with monocortical screws integrated well into the host's bone to provide sufficient anchorage against orthodontic force loading.


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Experimental animals and titanium miniplates

Six adult female 12-month-old beagles were used in the animal model system of this investigation. All the permanent teeth were already fully erupted and mandibular growth was complete in the experimental animals. The titanium miniplates used for SAS tooth movement were specifically designed as shown in [Figure 1](#) . First, the bone-contact area of the miniplates were sandblasted to increase surface roughness to enhance osseointegration. Second, the transmucosal portion was finished as a smooth and polished surface to reduce alveolar mucosal stimulation. Finally, 3 continuous hooks were designed for easier application of orthodontic force at the intraorally exposed portion of the titanium miniplates. All of the miniplates and bone screws were made of pure titanium. Sankin Kogyo Ltd (Tokyo, Japan) developed the titanium miniplates used in this study and the titanium bone screws (2 mm in diameter, 5 or 7 mm long) were produced by Leibinger Co (Freiburg, Germany).

Surgical procedure and application of intrusive force on teeth


For surgical application of titanium miniplates, experimental animals were premedicated with ketamine HCl at a dose of 10 mg/kg of body weight and were anesthetized with sodium pentobarbital (Wako Chemicals, Tokyo, Japan) at a dose of 25 mg/kg of body weight. Local anesthesia (2% lidocaine, Fujisawa Yakuhin Co, Tokyo, Japan) was applied at the implantation sites of the miniplates. A 2-cm mucosal incision was made over the buccal vestibule in the fourth premolar region. The mucoperiosteal flap was reflected inferiorly to the lower border of the mandible. The miniplates were fixed on the buccal side at the lower border of the mandible with 3 titanium monocortical bone screws. Another monocortical bone screw was fixed on the lingual alveolar bone between the fourth premolar and first molar ([Figure 1](#) ). Additionally, 2 titanium bone markers were inserted into the buccal cortical bone parallel to the margin of the alveolar bone. The bone markers were used as reference points to measure tooth movement by superimposing the tracings of radiographs. The mucoperiosteal flap was repositioned and the surgical wound was sutured with the long arm of the miniplate intraorally exposed. The left sides of the experimental mandibles were used for tooth movement and the other side as a nonloaded control.

In order to apply intrusive force on the molar, a cast metal crown was attached on the molar crown and a super elastic closing coil spring (Tomy International Co, Tokyo, Japan) was tied to both the head of the lingual bone screw and the hook of the buccal titanium miniplates. After a 3-month implantation-healing period, an intrusive force was applied on each mandibular molar that varied between 100 and 150 g over a period of 4 months or 7 months. The coil springs were adjusted or changed every 3 weeks. Five fourth premolars were intruded for 4 months or 7 months and 2 premolars and 3 third premolars remained unintruded as controls ([Table 1](#) ). The experimental periods were determined according to the actual clinical application period of intrusive force on open-bite cases for 7 months and to verify the time course for changes of tooth movements. During the experimental period, the tooth and the transmucosal portions of titanium miniplates were cleaned by brushing and oral rinsing. The protocols described above were reviewed and approved by the Animal Ethics Committee, Tohoku University School of Dentistry, and animal care took place in accordance with their guidelines.

Radiographic evaluation of tooth movement and root resorption

To evaluate the amount of molar intrusion, standardized dental radiographs were taken using a film holder attached to the titanium miniplates and third premolar. The standardized radiographs, with a 200-mm focus-object distance and 2-mm object-film distance, were taken every 4 weeks. The radiographs were magnified 5 times with a mounted slide projector. The titanium miniplate and screws, teeth,

lower border of the mandible, inferior alveolar neurovascular bundle, and bone markers were traced.

The amount of tooth movement at the 5 fourth premolars, defined as the distance between the 2 lines connecting the mesial and distal root apices, was measured by superimposing those tracings on the titanium bone markers, as shown in [Figure 2](#) . Actual tooth movement was calculated by dividing the measurements by 5. To assess the amount of root resorption, the distance from the bifurcation to the root apexes was measured on the tracings using calipers and the difference was divided by 5 to evaluate the actual amount of root shortening.

Statistical analysis

A Student *t*-test was employed for statistical analysis of tooth movement and a paired *t*-test was used for root shortening. The statistical differences were evaluated between 4 months and 7 months after tooth movement and between the root length before and after tooth movement in the 4-month and 7-month groups. A *P* value less than .05 was considered statistically significant.

Vital staining to analyze bone remodeling during molar intrusion

Two experimental animals underwent a vital staining procedure⁷⁻¹¹; these 2 dogs were treated with 5% oxytetracycline (Sigma, St Louis, Mo, USA), 0.16% Alizarin red (Wako Chemicals), and 1% calcein (Dojin Kagaku, Tokyo, Japan). Each vital staining reagent was diluted in saline, fructolact solution (Otsuka Seiyaku, Tokyo, Japan), and saline. Each dog was subcutaneously injected at doses of 20 mg/kg, 75 mg/kg, and 8 mg/kg of body weight, respectively. For the experimental animals with the molar intruded for 7 months, oxytetracycline was injected every week during the first 7 weeks, Alizarin red was injected the next 4 weeks after a 1-week interval, and calcein was injected during the final 8 weeks after a 1-week interval. For the other experimental animal with the molar intruded for 4 months, tetracycline was injected during the first 6 weeks every week after a 1-week interval from the beginning of tooth movement, and then calcein was injected every week for 6 weeks.

Tissue preparation and histological evaluation of tooth movement and remodeling of mandibular canals

Each experimental animal was anesthetized with pentobarbital and then sacrificed with 4% paraformaldehyde in pH 7.4 phosphate-buffered saline (PBS) by perfusion from the carotid artery. After the middle part of the mandibular bodies including the third and fourth premolars were dissected, specimens were further fixed in the same fixative for another 2 days at 4°C. The specimens were divided into 2 groups that were subject to a decalcified histological evaluation, undecalcified evaluation for vital staining, and contact microradiography. Additionally, the alveolar mucosa around the titanium miniplates and intact mucosa, which was the control epithelium, was dissected out and further fixed in the same fixatives.

For decalcified samples, the specimens were thoroughly rinsed in PBS, decalcified in Prunk and Ryuklo solution or formalin-formic acid solution for 4 weeks or for 4 months, respectively, neutralized in 5% sodium sulfate, and rinsed thoroughly in distilled water. The specimens were then dehydrated in a graded series of ethanol and were embedded in paraffin. Six- μ m-thick sections were cut and stained with hematoxylin and eosin. The epithelia were also embedded using the same procedure without decalcification and 5- μ m-thick sections were cut and stained with hematoxylin and eosin.



Undecalcified specimens were thoroughly rinsed in distilled water, dehydrated in a graded series of ethanol and embedded in Rigolac (Wako Chemicals). Undecalcified sections, 100 μ m thick, were cut using a saw microtome (SP 1600, Leica, Bensheim, Germany), polished to 60 μ m in thickness, and observed under fluorescent microscopy.

Contact microradiograph analysis

Undecalcified sections prepared as described above then underwent contact microradiography to analyze osseointegration of titanium bone screws and root resorption. The sections including a titanium miniplate and bone screw were microphotographed at 60 kVp and 25 mA for 10 minutes using a soft X-ray system (Softex, CMR type, Tokyo, Japan). Contact microradiograph films were developed in D19 (Eastman Kodak, Rochester N.Y., USA) for 5 minutes, stopped for 1 minute, and fixed in Fujifix (Fuji Film Co Ltd, Tokyo, Japan) for 5 minutes.

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Tooth movement

Mandibular molars were intruded into the mandibular body in the 7-month experimental group ([Figure 3](#) ). During the first 1 month of intrusive force application, tooth movement was minimal, according to the radiographic films. However, the intrusion began to be apparent in the second month. The average amount of intrusion gradually and linearly increased and reached 2.1 mm after 4 months and 3.4 mm after 7 months. The maximum amount of intrusion reached 4.1 mm, and the minimum was 1.8 mm after 7 months of force application ([Figure 4](#) ). Although there was no significant difference between the 2 groups (*P* < .05), the amount of intrusion was slightly higher in the 4-month group.

The vital staining dyes labeled the alveolar bone parallel to its surface, and the other side showed no label, as shown in the controls in [Figure 5a](#). The experimental sample showed no labeling anywhere in the alveolar bone surface ([Figure 5b](#)). In both the 4-month and the 7-month groups, bone resorption was observed in the lingual or buccal side of the root when compared with the other side of the control samples ([Figure 6](#)). New Haversian system formation was observed in the cortical bone at the inferior margin of the mandible of intruded samples, while fewer were seen in the control samples, as shown in [Figure 5](#).

Mandibular canal remodeling and root resorption

Because dogs do not have a distinct bony structure surrounding the inferior alveolar neurovascular bundle, as the mandibular canal seen in humans, clear labeling in the bony structure was undetectable. In the samples in which the molars were intruded for 4 months, the root apex of fourth premolars reached the inferior alveolar artery and nerve. While the artery was always apart from the root surface, the inferior alveolar nerves were positioned more closely to the root surface and even touched the periodontal ligament in some cases. In the sections of the 7-month group, the root apex nearly reached the internal surface of the cortical bone at the inferior mandibular margin. The inferior alveolar neurovascular bundles repositioned inferiorly after 7 months of tooth movement, compared with unintruded controls ([Figures 5b and 6c](#)).

Although root resorption was observed in the 4-month group, it was mainly localized in the cementum. However, in the 7-month group, root resorption reached the dentin at one-third of the apical area of the roots ([Figures 6c and 7b](#)). Vital labeling samples ([Figure 5](#)), histological analysis ([Figure 6](#)), and CMR ([Figure 7](#)) showed modeling of cementum at the resorbed surface of the dentin or cementum in both groups ([Figures 5 through 7](#)).

The root lengths, as measured on radiographic films, are summarized in [Table 2](#). No statistically significant differences were observed in root length before or after the intrusion for 4-month or 7-month groups. The amount of root resorption was $0.1 \text{ mm} \pm 0.1 \text{ mm}$ (mean \pm SD) in the 7-month group.

Osseointegration of titanium bone screws and peri-miniplate soft tissues

The titanium miniplates in both the force-loaded samples and unloaded controls were quite stable clinically throughout the experimental period. When the force-loaded miniplates were compared with those of the control, the distance between the bone screw and the bone surface was apparently closer in the force-loaded samples than that of the unloaded controls ([Figures 8a and b](#)). Highly calcified bony structure containing Haversian systems attached to the bone screws in the force-loaded sample. Vital labeling dyes were incorporated in the newly deposited bone matrix attached to the screw surface in both the force loaded and unloaded vitally labeled samples.

The soft tissues surrounding the miniplates at the transmucosal area showed slight inflammatory changes, as shown in [Figure 8d](#). Three months after implantation surgery, the transmucosal miniplates were quite stable and were loaded with 100 g to 150 g of intrusive force. Seven months after implantation, invasion of blood vessels and monocytes was observed in the peri-implant alveolar mucosa. The stratified squamous epithelium adjacent to the miniplates developed well in the control epithelium, while it partially showed higher keratinization in the experimental groups.

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Experimental procedures

SAS itself has already been applied to clinical orthodontic treatment.^{1,2} In the present investigation, we demonstrated that titanium miniplates, with 3 monocortical bone screws fixed in the mandible, showed good histological stability and osseointegration under orthodontic force loading. As shown in the articles concerning the maintenance of undisturbed osseointegration of dental implants, only slight inflammatory changes were reported in the peri-implant soft tissue when oral hygiene was good.^{12,13} In the present experiment, we maintained good oral hygiene by oral rinsing and brushing around the transmucosal area of the titanium miniplates. Even though there were slight inflammatory changes, there was no bone resorption that decreased the stability of the implants. Meticulous oral hygiene is important in maintaining favorable soft tissue conditions around the miniplate for clinical orthodontic treatment with SAS.³ At the same time, the orthodontic miniplate, temporarily placed in a nonsubmerged manner and a one-stage procedure, was quite stable in the present study, in agreement with previous reports.¹⁴

The present experiment is the first report of the effect of excessive intrusion of molars on the roots of the teeth and the inferior alveolar neurovascular bundles. Clinically, radiographs of human mandibles show clear radiopaque lines around the inferior alveolar neurovascular bundles. However, in some cases, histological analyses of human mandibles showed no distinct bony structures of a mandibular canal, especially at the molar regions.¹⁵ While the inferior alveolar neurovascular bundles were not surrounded by distinct bony structures in the molar regions in dogs, it was clear that our results were informative enough for application in human cases.

While the amount of intrusion observed in the 4-month and 7-month groups were different at the 16th week, it was clear that there were

no statistically significant differences between them. It might be caused by the diversity of the magnitude of force applied, the individual difference in the experimental animals, or other unknown factors. We used 5 teeth in 3 different animals of both groups; however, increasing the number of animals may reduce these kinds of differences.

Molar intrusion and its influence on the inferior alveolar neurovascular bundles

We believe this is the first report that the amount of intrusion of mandibular molars reached 3.4 mm on the average even in an animal experiment. Southard et al¹⁶ reported that molar intrusion is possible using dental implants, but 3-dimensional control of molar intrusion was not accomplished with their system. Although slight inclination or side shift was observed in most of the experimental samples in the present study, the direction of tooth movement was nearly perpendicular to the occlusal plane, and intrusion into the mandibular body was well controlled. The mechanics employed in this study allowed for free inclination of the tooth axis, however, the intrusive force generated by the coil springs seemed to be exerted through the center of resistance of the experimental tooth and thus accomplished the desirable molar intrusion. In our previous clinical report,¹ since we used similar SAS mechanics for anterior open-bite patients, we achieved counterclockwise rotation of the mandibular occlusal plane that decreased the lower facial height.

As the molars were intruded, the inferior alveolar neurovascular bundles seemed to be repositioned and the inner surface of the cortical bone was remodeled to enlarge the marrow spaces for neurovascular bundles to avoid severe destruction of inferior alveolar arteries and nerves. In a previous study, investigators attempted surgical repositioning of the inferior alveolar nerve for dental implantation. Their results demonstrated a full and rapid recovery of lip sensation.¹⁷ Therefore, it could be considered that gradual repositioning of the inferior alveolar nerve accompanied by tooth movement should not affect the neurosensory function. This is the first histological observation that molar intrusion is possible at this level without any iatrogenic effects on the nerves or blood vessels. However, further investigation is needed to clarify the effect on neurosensory function.

Root resorption

Many reports have demonstrated that orthodontic tooth movement, including excessive intrusion of the anterior teeth, showed a different type of root resorption.¹⁸⁻²¹ Root rounding is one of the most common types of root resorption and is usually corrected by formation of cementum except in cases where roots are extensively resorbed.²²⁻²⁴ Additionally, the tooth support is not lost in this type of the root resorption.²⁵ In the present experiment, the amount of root resorption observed was 0.1 mm and was localized in the cementum after 4 months of applying intrusive force and reached the dentin after 7 months. Even with excessive intrusion, the amount of root resorption seen at the root apex of the molars was small enough and it had been repaired by cementum formation.²⁶ The results suggest that mechanical stress of tooth movement affects the alveolar bone and cellular cementum differently. Under the compressive force of tooth movement, the bone is resorbed whereas the cementum resists resorption and calcification is inhibited.²⁷ Therefore, root resorption and consequent cementum repair would not be a critical problem for root support after intrusive tooth movement using SAS.

Osseointegration and peri-miniplate mucosa

Endosseous implants have been extensively investigated to demonstrate that titanium implants can be integrated in the alveolar bone. Albrektsson et al²⁸ indicated that a titanium implant can be integrated directly on the bone surface without fibrous tissue intermediates. After this finding, many studies have been conducted and indicate that the osseointegration of various types of titanium implants can progress under masticatory functional and/or orthodontic mechanical loading.²⁹⁻³¹ In this study, we showed that osseointegration of titanium bone screws under orthodontic mechanical loading achieved a higher level of bone-to-implant contact than unloaded screws.³² Therefore, not only endosseous dental implants³³ but also monocortical screws can be stabilized by increased mineralized bone-to-implant contact in terms of osseointegration under biomechanical stress. Additionally, as Degasne et al,³⁴ Deporter et al,³⁵ and Martin et al³⁶ indicated in their in vivo and in vitro studies, sandblasted rough surfaces in our titanium miniplates would enhance SAS osseointegration by promoting cell proliferation and matrix deposition.

On the other hand, many studies^{12,13,37,38} have indicated that epithelial attachment to the endosseous dental implants depended on the superficial property of the miniplates. The mirror surfacing of our titanium miniplates could support epithelial attachment since histological analysis of the peri-miniplate mucosa showed only slight inflammatory changes. Additionally, this was also considered to be a result of careful plaque control and meticulous oral hygiene of the intraorally exposed portions of the miniplates.

CONCLUSION [Return to TOC](#)

In the present study, we demonstrated that (1) titanium miniplates could be maintained in the oral cavity without severe inflammation around the implantation site, (2) root resorption was minimal and was repaired by formation of new cementum, and (3) the inferior alveolar neurovascular bundle was not damaged since it seemed to reposition itself with tooth movement. Thus, we conclude that the SAS utilizing transmucosal titanium miniplates as a temporary immovable orthodontic anchorage can provide a new modality for molar intrusion without serious iatrogenic problems. Since the present study can only provide descriptive results from histological evaluation, further investigations will be needed to elucidate physiological changes after molar intrusion.

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TABLE 1. Number of Experimental and Control Molars

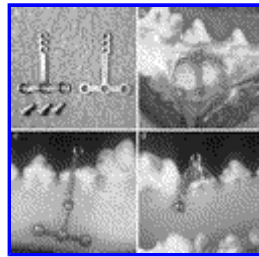
	Third Premolar	Fourth Premolar
Control group	12	2
4-mo group	0	5
7-mo group	0	5
Total number of teeth	12	12

TABLE 2. Amount of Root Resorption after Molar Intrusion (mm)^a

	4 mo (<i>n</i> = 5)		7 mo (<i>n</i> = 5)	
	Mean	SD	Mean	SD
Root length				
Before intrusion	7.1	0.4	7	0.3
After intrusion	7.1	0.4	6.9	0.2
Amount of root resorption	0	0.1	0.1	0.1

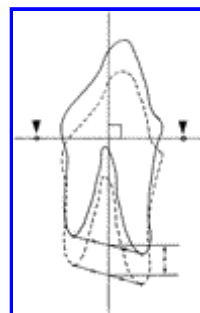
^a SD, standard deviation; N, number of specimens per group.

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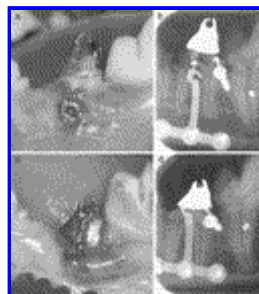
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FIGURE 1. SAS components and surgical procedures. Orthodontic titanium miniplates and bone screws were shown in (a) and the surgical procedures employed in this study were shown in (b), (c), and (d). Titanium miniplates were implanted on the buccal cortical bone of the mandible using titanium monocortical bone screws. Another bone screw was placed in the lingual alveolar bone between the fourth premolar and first molar.



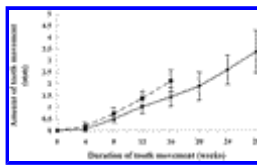
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FIGURE 2. The amount of intrusion was measured as indicated. Arrowheads indicate 2 metal reference points placed in the mandibular cortical bone



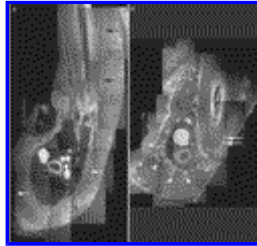
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FIGURE 3. Intrusion of the fourth premolars is shown in oral photographs and radiographic films. Oral photographs indicate the fourth premolar before and after intrusion (a and c). Standardized radiographic films indicate the fourth premolar before and after intrusion (b and d).



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FIGURE 4. Amount of intrusion of mandibular 4th premolars by using SAS. The amount of tooth movement reached 3.4 mm on average in the 7-month group. The solid line indicates the time course changes of the tooth movement in the 7-month group and the broken line indicates the 4-month group, with standard deviation bars. There were no statistically significant differences between the 4-month and 7-month groups.



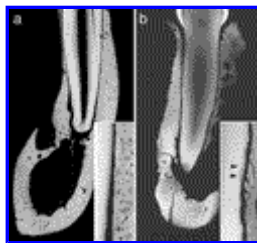
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FIGURE 5. Bone and cementum remodeling shown during intrusion of fourth premolars. Two colored vital staining sections are indicated, with (a) the control and (b) the 4-month group. White arrows indicate newly formed cementum labeled in the intruded molars. No labeling was observed on the surface of the alveolar bone in the intruded groups, while labeling was shown in the controls (black arrows). A newly formed Haversian system (arrowheads) was labeled with calcein and tetracycline in (b).



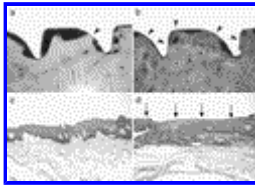
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FIGURE 6. The fourth premolar was intruded in the inferior border of the mandibular body. Photomicrograms of H&E-stained sections of control (a), the 4-month group (b), and 7-month group (c) are indicated. The inferior alveolar artery (arrows) and nerves (arrowheads) were apart from the roots in the intruded groups.



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FIGURE 7. CMR images of an unintruded fourth premolar (a and c) and 7-month group (b and d) indicate that the resorbed dentin surface of the 7-month group was repaired by cementum formation (d). Irregular surface of resorbed dentin was covered with newly formed cementum



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FIGURE 8. Osseointegration of SAS and transmucosal alveolar soft tissues. Contact microradiographs of bone screws and surrounding bones are indicated in (a) controls and (b) the 7-month group. Photomicrograms of alveolar mucosa at implantation site: (c) control and (d) transmucosal area of the miniplates in the 7-month group. Intruded groups showed close contact (arrowheads) between bone screw and bone. Blood vessels and monocyte invasion (asterisks) with slight inflammation were observed in the transmucosal area of the miniplates (arrows)

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