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Effects of soft tissue grafting prior to orthodontic treatment on preventing gingival recession in dogs

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ABSTRACT

Purpose: This study was conducted to assess the efficacy of prophylactic gingival grafting in the mandibular anterior labial area for preventing orthodontically induced gingival recession.
Methods: Eight mongrel dogs received gingival graft surgery at the first (I1) and third (I3) mandibular incisors on both sides based on the following group allocation: AT group (autogenous connective tissue graft on I1), AT-control group (contralateral side in the AT group), CM group (xenogeneic cross-linked collagen matrix graft on I3) and CM-control group (contralateral side in the CM group). At 4 weeks after surgery, 6 incisors were splinted and proclined for 4 weeks, followed by 16 weeks of retention. At 24 weeks after surgery, casts were made and compared with those made before surgery, and radiographic and histomorphometric analyses were performed.
Results: Despite the proclination of the incisal tip (by approximately 3 mm), labial gingival recession did not occur. The labial gingiva was thicker in the AT group (1.85±0.50 mm vs. 1.76±0.45 mm, $P>0.05$) and CM group (1.90±0.33 mm vs. 1.79±0.20 mm, $P>0.05$) than in their respective control groups.
Conclusions: The level of the labial gingival margin did not change following labial proclination of incisors in dogs. Both the AT and CM groups showed enhanced gingival thickness.

Keywords: Animal research; Collagen; Connective tissue; Gingival recession; Orthodontic tooth movement

INTRODUCTION

Gingival recession, which refers to apical dislocation of the gingival margin resulting in a denuded root surface, occurs for several reasons such as periodontal destruction caused by poor oral hygiene, mechanical or thermal trauma, and orthodontic tooth movement leading to partial alveolar bone deficiency [1-4]. Gingival recession may cause aesthetic problems [5] and hypersensitivity [6], and may also increase the susceptibility to caries [7,8]. Regardless of the mechanism underlying recession, it is more commonly observed in patients with a thinner gingiva [9-11].

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Orthodontic treatment does not always induce gingival recession, and there have been some previous reports of a weak correlation between the degree of tooth proclination and gingival recession [12-17]. However, other studies found a strong relationship between orthodontic tooth movement or inclination and recession of the marginal gingiva in procedures where the teeth are moved out of the alveolar bone housing. In particular, the mandibular incisors are likely to exhibit recession when performing camouflage treatment in skeletal class II patients or decompensation during presurgical correction in skeletal class III patients [16]. Because the mandibular incisors have a thin labial alveolar plate and an inadequate thickness and amount of keratinized gingiva [18], excessive proclination may lead to partial alveolar bone dehiscence, which causes gingival recession [9,16].

Prophylactic soft tissue augmentation prior to orthodontic treatment has been recommended in patients with an insufficient amount of attached gingiva or thin soft tissue [19-23]. There are several reasons for attempting to prevent gingival recession [24]. For example, thick gingival tissue contains a larger volume of collagen fibers and extracellular matrix, which resists collapse and contraction; furthermore, it also has increased vascularity, which enhances the oxygen supply, growth factor migration, and clearance of toxic products. Autogenous soft tissue (AT) grafts, such as free gingiva or subepithelial connective tissue grafts, have long been considered to be the gold standard for these purposes [25], but concerns have been raised that involving the donor site might increase patient discomfort [26]. This situation has led to the concept of using xenogeneic substitutes. Previous preclinical studies revealed that xenogeneic cross-linked collagen matrix (CM) grafting increased gingival thickness [27] and that the increase was not inferior to that achieved by AT grafting [28]. In a randomized controlled clinical trial [29], the increase in the gingival volume after CM grafting was shown to be comparable to that achieved when using AT grafting at implant sites. However, no previous research has evaluated the efficacy of CM and AT grafting performed for prophylactic purposes prior to orthodontic treatment.

Therefore, the aim of this preclinical study was to determine the preventive effect against gingival recession of labial gingival grafting performed prior to orthodontic treatment using AT or CM.

MATERIALS AND METHODS

This study was performed according to the checklist contained in the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines [30]. The selection and management of the experimental animals and the experimental protocol were approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Republic of Korea (approval No. 2018-0008).

Sample size calculation

The required sample size was estimated using a power calculation with a significance level (α) of 5% and power (1–β) of 90% in accordance with the criteria used by a previous study [28].

Experimental animals, housing and husbandry

Eight healthy male mongrel dogs with mean body weights of 25–30 kg and a mean age of 12 months were used for the experiments. They were raised under standard laboratory conditions with appropriate feeding and housed at room temperature (15°C–20°C) and humidity (>30%). The animals had sound periodontium with permanent dentition. To maintain acceptable oral hygiene, scaling and plaque control were conducted before surgery (Figure 1A and B).

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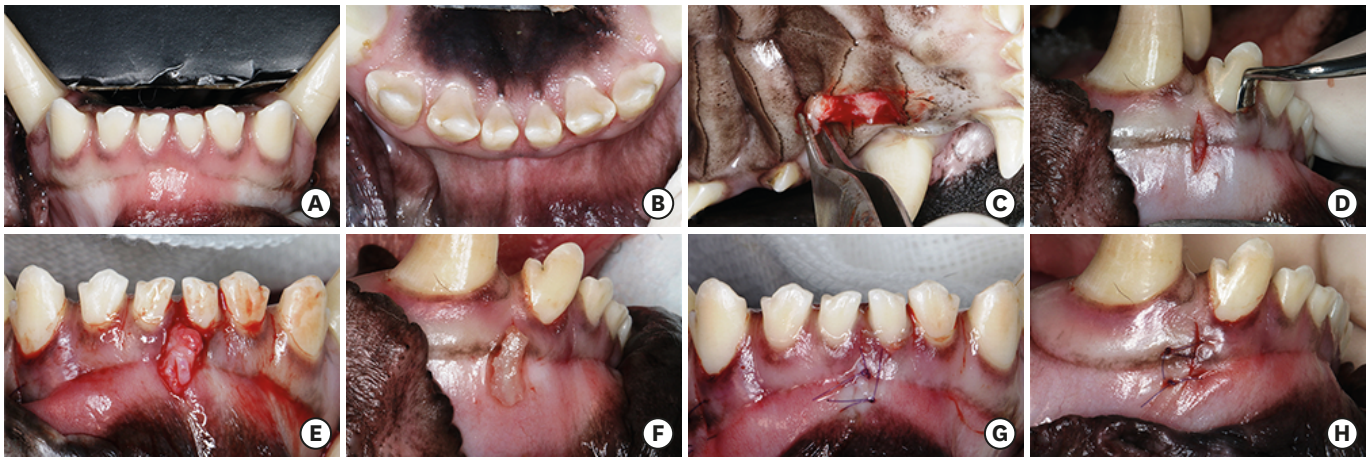

Figure 1. Clinical photographs of the surgical procedure: (A) labial view in the presurgical state, (B) occlusal view in the presurgical state, (C) harvesting of subepithelial connective tissue from the palate, (D) formation of a subperiosteal pouch on the labial gingiva, (E) grafting of subepithelial AT in the first incisor area (AT group), (F) grafting of xenogeneic cross-linked CM in the third incisor area (CM group), (G) primary closure in the AT group, and (H) primary closure in the CM group. AT: autogenous connective tissue, CM: collagen matrix.

Experimental procedures

Material preparations

Two different types of graft materials were prepared for soft-tissue augmentation: AT and CM (Collagen Graft®, Genoss, Suwon, Korea). The AT was obtained from the palate as subepithelial connective tissue (Figure 1C), and the CM was a double-layered matrix with chemically cross-linked type I collagen derived from bovine tendon. The AT and CM were grafted onto the areas of the mandibular first incisor (I1, AT group) and third incisor (I3, CM group), respectively, and the contralateral sides of the I1 and I3 areas were used as the controls (AT-control and CM-control groups, respectively), which did not receive any surgical intervention. The AT was trimmed to a length, width, and depth of 4 mm, 4 mm, and 2 mm, respectively; the corresponding dimensions for the CM were 6 mm, 6 mm, and 2 mm.

Surgical procedures

On the day of surgery, general anesthesia was induced by an intramuscular injection of medetomidine (0.75 mg/kg; Tomidin®, Provet Veterinary Products, Istanbul, Turkey) after applying inhalation anesthesia with isoflurane (Forane®, Choongwae Pharmaceutical, Seoul, Korea). Local infiltration anesthesia at the surgical site was performed with lidocaine (2% lidocaine HCl and 1:80,000 epinephrine; Kwang Myung Pharm, Seoul, Korea).

Subperiosteal pouches were prepared for graft surgery as described previously [27]. A crevicular incision was made in the I1 and I3 areas, and a vertical incision was made in the attached gingiva underneath the marginal gingiva on the I1 mesiolabial and I3 distolabial sides, to form a subperiosteal pouch (Figure 1D). An Orban interdental knife was carefully inserted into the incision line and proceeded to the gingival sulcus to form a gingival tunnel that allowed the graft materials to advance coronally (Figure 1E and F). Primary closure of the vertical incision was performed using resorbable silk material (Monosyn® 6-0 Glyconate Monofilament, B. Braun, Tuttlingen, Germany) (Figure 1G and H).

The dogs were given an antibiotic (20 mg/kg cefazolin sodium; Yuhan, Seoul, Korea) and an anti-inflammatory (0.2 mg/kg meloxicam; Mobic®, Boehringer Ingelheim, Ingelheim, Germany) for 7 days, and the surgical sites were cleaned daily by irrigation with 0.2%

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chlorhexidine (Hexamedine, Bukwang Pharmaceutical, Seoul, Korea) as post-surgical care. The suture materials were removed 7 days after surgery.

Orthodontic procedures

After 4 weeks of healing, the 6 mandibular incisors were splinted with 0.016-inch × 0.022-inch stainless steel (SS) wire and proclined using NiTi open coil springs (50 g force; Ormco, Glendora, CA, USA) with a 0.016-inch SS guide wire between the I3 and canine on both sides (Figure 2A and B). The 6 incisors were actively moved as a unit for 4 weeks, with examinations of the force every week, and thereafter the proclined position was maintained for 16 weeks (Figure 2C and D). The mandibular incisor area was cleaned each week by scaling and plaque control during the intervention period.

Sacrifice of experimental animals

The animals were sacrificed 24 weeks after surgery by injecting an overdose of 3% sodium pentobarbital. The mandible was dissected to obtain block specimens, after which micro-computed tomography (micro-CT) was performed and histological sections were fabricated.

Measurements

All measurements were made by 1 examiner (S.Y.H.) who was blinded to the surgical procedures and group allocations.

Cast analysis

Before (T0) and 24 weeks after (T1) surgery, dental models were fabricated by taking impressions of the mandibular dentition using prefabricated individual trays made from a self-cured acrylic resin (Formatray®; Kerr Manufacturing, Romulus, MI, USA) and polyvinylsiloxane impression materials (Aquasil Ultra LV® and Aquasil Ultra XLV®; Dentsply DeTrey, Konstanz, Germany), and dental stone (GC Fujirock® type 4; GC Corporation,

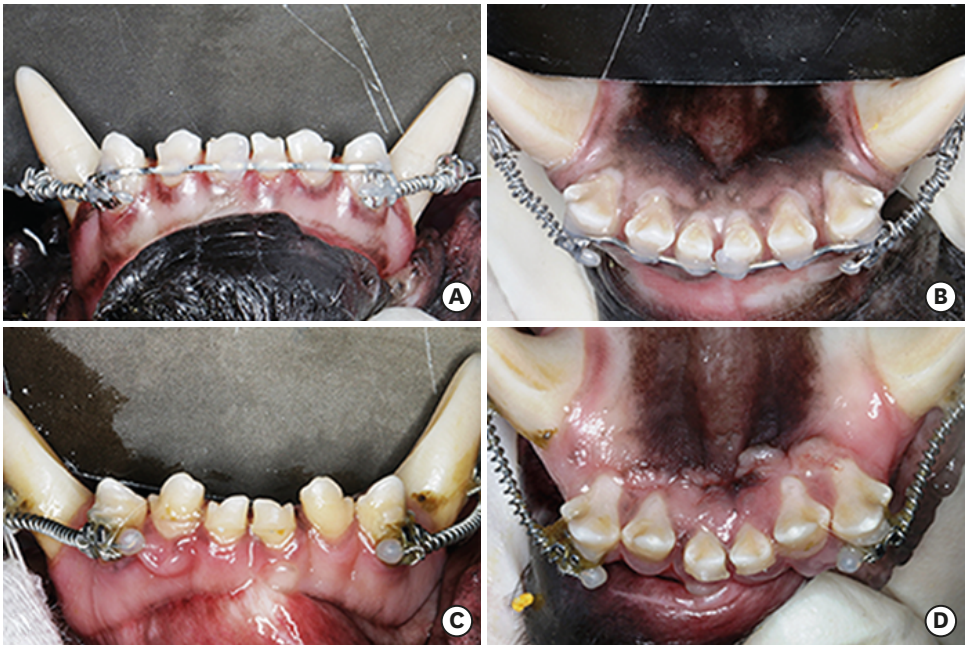

Figure 2. Labial and occlusal clinical photographs of the orthodontic treatment. (A, B) Stainless steel wires and NiTi open coil springs were applied to achieve active movement of the teeth at 4 weeks post-surgery. (C, D) The retention period, with the NiTi open coil springs in the inactivated state, was from 8 weeks to 24 weeks post-surgery.

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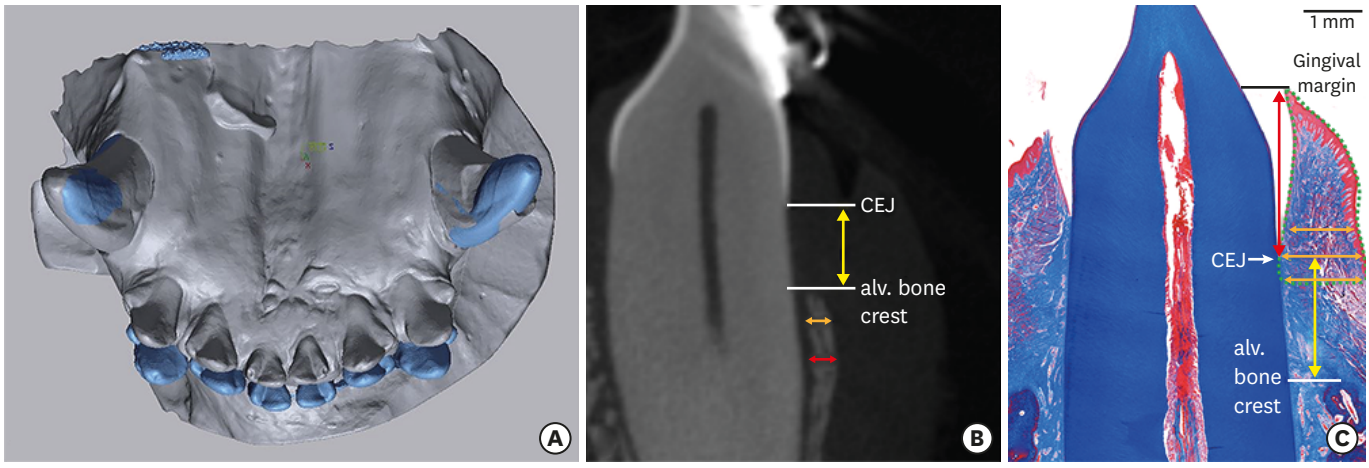

Figure 3. Cast, radiographic, and histomorphometric analyses. (A) A cast analysis was performed by superimposing pairs of scan files of cast models fabricated in the presurgical state (gray) and at 24 weeks post-surgery (blue). (B) Linear measurements (mm) made using micro–computed tomography (yellow arrow, vertical distance from the CEJ to the alveolar [alv.] bone crest; orange arrow, horizontal thickness of bone at 3 mm below the CEJ; red arrow, horizontal thickness of bone at 4 mm below the CEJ). (C) Linear histomorphometric measurements (mm) (red line, epithelium height; yellow line, connective-tissue attachment height; orange lines, horizontal thickness of the gingiva measured at the level of the CEJ and at 0.5 mm above and 0.5 mm below the CEJ; green-dotted closed line, gingiva area measured at 0.5 mm below the CEJ).
CEJ: cementoenamel junction.

Tokyo, Japan) was used to prepare dental casts. The two dental casts obtained at T0 and T1 were scanned (Imetric 3D, Courgenay, Switzerland) and superimposed on the basis of the positions of the posterior teeth that were not included in the orthodontic treatment (Geomagic Control software; 3D Systems, Rock Hill, SC, USA). The distances between the incisal tips at T0 and T1 were measured on the superimposed image (Figure 3A). The measurements were performed for I1 and I3 on both sides to assess the amount of anterior movement. The distances between the incisal tips of I3 and the adjacent canine on each side were also measured at each time point.

Radiographic analysis

After fixation in 10% neutral buffered formalin for 10 days, the block sections from the grafted and control sites were scanned using micro-CT (Skyscan 1076, Kontich, Belgium) with the following parameters: image resolution of 8.88 μm, 130 kV, 60 μA, 500 ms of exposure time after each 0.2° of rotation, and a 1.0-mm-thick aluminum filter. To perform the measurements, we used the profile function provided by the software to select a measurement point based on changes quantified in Hounsfield units, and all of the parameters were measured for I1 and I3 on both sides using OnDemand 3D® software (Cybermed, Seoul, Korea). To investigate the height of the alveolar bone level on the labial and lingual sides, the vertical distance between the cementoenamel junction (CEJ) and the alveolar bone crest was measured at the center-most cut of the cross-sectional image. Since the bone crest level was 2 mm below the CEJ in all specimens, the horizontal thickness of the labial bone was measured at 3 mm and 4 mm below the CEJ (Figure 3B).

Descriptive histology and histomorphometric analysis

After performing the micro-CT analysis, the specimens were trimmed and then dehydrated with ethanol. After being decalcified, the specimens were embedded in paraffin, and the I1 and I3 areas were cut parallel to the long axis in the labiolingual direction. The slices were stained with Masson trichrome, and the center-most section was chosen for subsequent analysis. The following parameters were measured using computer software (Case Viewer,

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3DHISTECH, Budapest, Hungary) (Figure 3C):

- Epithelium height (mm): the sum of the sulcus depth and the coronoapical length of the epithelial attachment, as measured from the gingival margin to the CEJ.
- Connective tissue (CT) attachment height (mm): the coronoapical length of the CT attachment, as measured from the CEJ to the alveolar bone crest.
- Gingival thickness (mm): the mean horizontal thickness of the labial gingiva measured at the level of the CEJ and at 0.5 mm above and 0.5 mm below the CEJ.
- Gingival area (mm^2): the area measured from 0.5 mm below the CEJ to the most coronal point of the marginal gingiva on the labial side.

Statistical analyses

All statistical analyses were performed using SPSS software (version 23, IBM, Armonk, NY, USA). The Wilcoxon signed-rank test was used to evaluate differences between each grafted group and its respective control group, with $P<0.05$ as the threshold for statistical significance.

RESULTS

Clinical outcomes

Although the 6 mandibular incisors exhibited excessive proclination, none of the sites showed gingival recession. There were no atypical complications after graft surgery (Figure 4A and B). Marginal gingival swelling was often observed during the period of active tooth movement (Figure 4C and D), but this swelling gradually decreased when the wires were removed during the retention period, and a healthy gingival condition was observed at 24 weeks post-surgery (Figure 4E and F).

Cast analysis

The space between the canine and I3 increased by 1.20±1.16 mm after orthodontic treatment. The anterior displacements of I1 and I3 were 2.55±0.94 mm and 2.85±1.13 mm, respectively (Table 1).

Micro-CT analysis

The vertical distances from the CEJ to the alveolar bone crest on the labial side in the AT group (2.72±0.29 mm) and the CM group (2.32±0.44 mm) did not differ significantly from those in their respective control groups (2.63±0.49 mm and 2.67±0.38 mm in the AT-control and CM-control groups, respectively) ($P>0.05$), and neither did the distances on the lingual side (2.19±0.54 mm in the AT group vs. 2.18±0.46 mm in the AT-control group; 1.80±0.19 mm in the CM group vs. 1.93±0.37 mm in the CM-control group) ($P>0.05$). When comparing the height of the alveolar bone crest on the labial side to that on the lingual side within each group, all labial measurements were larger than the lingual measurements; however, significant differences were only observed in the CM and CM-control groups ($P<0.05$). The horizontal thickness of the labial alveolar bone in the grafted groups did not differ significantly from that in their respective control groups at both 3 mm and 4 mm below the CEJ ($P>0.05$). All of the measurements are presented in Table 2.

Descriptive histology

No recession was evident below the CEJ in any of the teeth, and no infiltration of inflammatory cells was observed. The rete pegs were narrower and deeper in the AT and CM groups, while the AT-control and CM-control groups showed shallower morphology. No remnant of the matrix was seen in the CM group (Figure 5).

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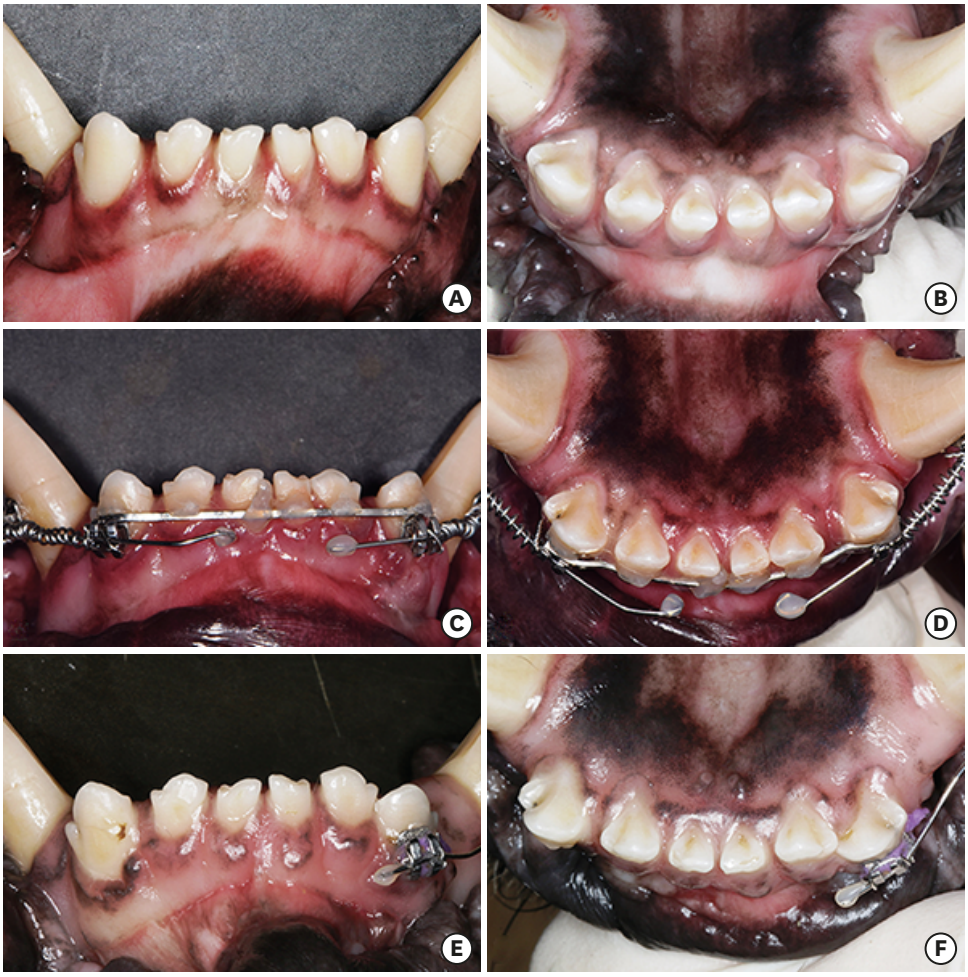

Figure 4. Labial and occlusal clinical photographs obtained during follow-up: (A, B) 1 month after surgery, (C, D) 2 weeks after initiating orthodontic treatment, and (E, F) 24 weeks after the surgery.

Table 1. Cast analysis after orthodontic treatment

	Space between canine and I3	Anterior displacement	
		I1	I3
Distance (mm)	1.20±1.16	2.55±0.94	2.85±1.13

Values are presented as mean±standard deviation. I1 and I3 are the mandibular first and third incisors, respectively.

Table 2. Results of radiographic measurements

Groups	Distance from CEJ to alveolar bone crest (mm)		Labial bone thickness at 3 mm under the CEJ (mm)	Labial bone thickness at 4 mm under the CEJ (mm)
	Labial	Lingual		
AT	2.72±0.29	2.19±0.54	0.56±0.16	1.39±0.71
AT-control	2.63±0.49	2.18±0.46	0.72±0.13	1.55±0.78
CM	2.32±0.44*	1.80±0.19	0.51±0.19	0.54±0.23
CM-control	2.67±0.38*	1.93±0.37	0.49±0.17	0.59±0.22

Values are presented as mean±standard deviation.
CEJ: cementoenamel junction, AT: autogenous connective tissue, CM: collagen matrix.
*Significantly different when compared to the distance measured on the lingual side within each group ($P<0.05$).

Histomorphometric analysis

The AT and CM groups showed shorter epithelia (−0.22 mm and −0.10 mm, respectively) and longer CT attachments (+0.35 mm and +0.05 mm, respectively) than the AT-control and CM-

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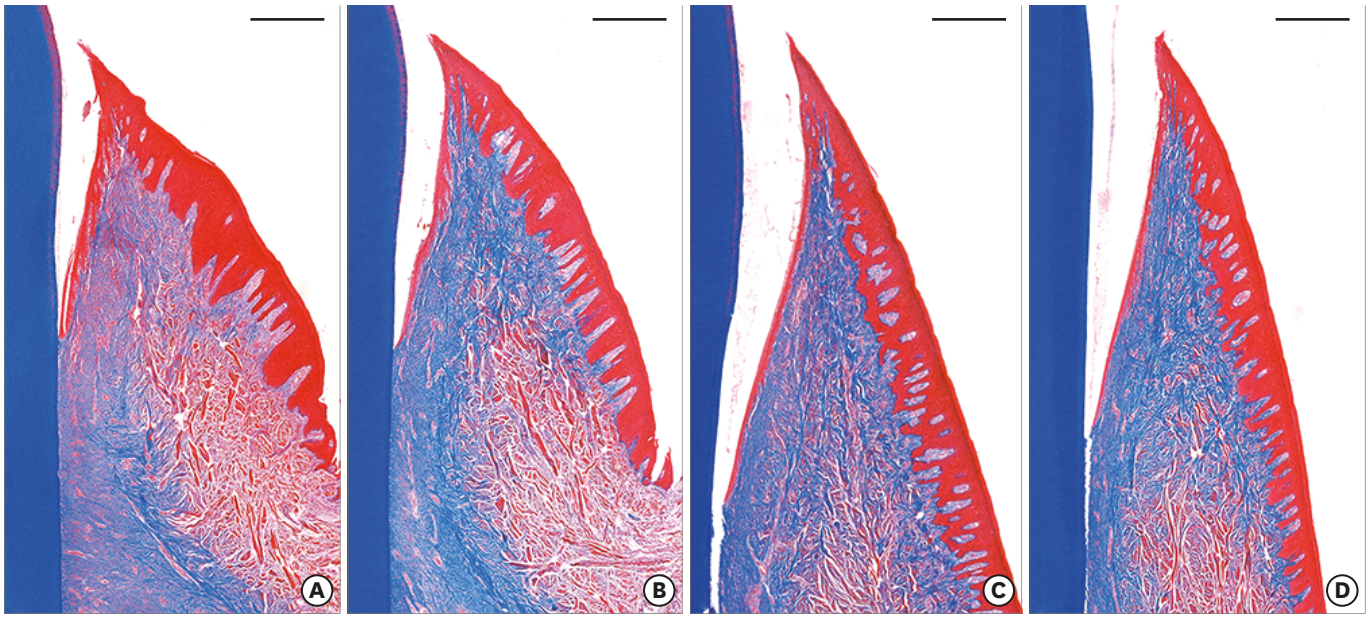

Figure 5. Descriptive histology (scale bar = 500 μm): (A) AT group, (B) AT-control group, (C) CM group, and (D) CM-control group.
AT: autogenous connective tissue, CM: collagen matrix.

Table 3. Results of histologic measurements

Groups	Epithelium height (mm)	CT attachment height (mm)	Gingival thickness (mm)	Area of gingiva (mm^2)
AT	2.94±0.23	2.21±0.24	1.85±0.50	4.44±1.88
AT-Control	3.16±0.37	1.86±0.28	1.76±0.45	3.93±1.61
CM	3.42±0.22	2.68±0.36	1.90±0.33	5.53±1.61
CM-Control	3.52±0.31	2.63±0.25	1.79±0.20	4.74±1.26

Values are presented as mean±standard deviation.
CT: connective tissue, AT: autogenous connective tissue, CM: collagen matrix.

control groups. The horizontal gingival thickness and the gingival area were both larger in the AT group (1.85±0.50 mm and 4.44±1.88 mm^2, respectively) and the CM group (1.90±0.33 mm and 5.53±1.61 mm^2, respectively) than in their corresponding control groups (1.76±0.45 mm and 3.93±1.61 mm^2 in the AT-control group, and 1.79±0.20 mm and 4.74±1.26 mm^2 in the CM-control group, respectively). However, none of the differences in these measured parameters were statistically significant (P>0.05). All of the measurements are presented in Table 3.

DISCUSSION

This preclinical study was designed to determine whether performing gingival grafting before moving the mandibular anterior teeth in dogs can prevent the gingival recession induced by orthodontic treatment. Despite labial proclination, labial gingival recession did not occur in any of the study groups. The thickness and area of the labial gingiva improved by 5.11% and 13.0% in the AT group and by 6.15% and 16.7% in the CM group, respectively, compared to the corresponding control groups.

There are a few possible reasons for the absence of gingival recession. First, gingival swelling owing to poor oral hygiene might have masked apical migration of the marginal gingiva. Even though plaque control was performed periodically, it was not easy to keep the periodontium in a healthy state. The dogs consistently presented with mild gingival swelling in the

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mandibular anterior area while the brackets and wires were attached, and Wennstrom et al. [2] similarly found less periodontal attachment loss and gingival recession than expected following orthodontic treatment in monkeys. However, inflammatory cell infiltration was not observed histologically since the specimens were investigated after a maintenance period of 16 weeks, at which time the gingival swelling had clinically resolved.

A second possible reason for the absence of gingival recession is that the age of the experimental animal may also be a critical factor contributing to the failure to induce change in the gingival level. Gingival recession increases with age in humans [31,32], and it is observed less frequently in adolescents than in older people, regardless of the application of orthodontic treatment, due to the faster turnover of cells in adolescents and their greater healing potential. It was found that proclination of the mandibular incisors in adolescents was not associated with gingival recession [33]. It was also similarly reported that patients younger than 16 years were less likely to develop recession [34]. The mongrel dogs used in the present experiments had a mean age of 12 months, which corresponds to adolescence in humans, and so it would have been more appropriate to use animals older than 12 months in order to induce as much gingival recession as possible.

Furthermore, the relatively short retention period following orthodontic movement could also help explain our findings. Generally, gingival recession occurs slowly after the completion of orthodontic treatment, rather than during orthodontic treatment itself. Since the prevalence of gingival recession steadily increased after orthodontic treatment [34], the follow-up period of 8 weeks after orthodontic treatment in the present experiment might have been insufficient to lead to recession of the labial gingiva. Lastly, the lack of gingival recession observed in this study could reflect the fact that the experimental model did not involve any additional factors that might induce gingival recession after orthodontic treatment, such as muscular stimulation or habitual toothbrushing [1].

In the micro-CT analysis, the mean alveolar bone level measured on the labial side was 2.32 to 2.72 mm, while that measured on the lingual side had a mean height of 1.80 to 2.19 mm. It was therefore possible to measure the horizontal thickness of the labial plate at 3 mm below the CEJ. The thickness of the labial bone did not differ significantly between the grafted groups and their respective control groups. These results could be readily predicted, since the amount of anterior movement of the teeth identified by superimposing the dental casts was statistically equivalent in all groups, which means that the orthodontic tooth movement in these experiments was well controlled on both sides. In the AT and AT-control groups, the bone was thicker at the 4-mm level than at the 3-mm level, while similar thicknesses were found at both levels in the CM and CM-control groups. This finding likely reflects differences in the anatomy of the I1 (AT and AT-control groups) and I3 (CM and CM-control groups) areas.

Some previous reports have described unabsorbed CM being encapsulated by fibrous tissue histologically even at 12 weeks after grafting [27,35,36], but no CM remnants were observed in the CM group in the present study, implying that the matrix had been fully degraded and remodeled. This finding is unsurprising since the specimens analyzed in these experiments were prepared at 24 weeks post-surgery, an interval twice as long as that in the previous studies.

Morphological changes in the rete pegs were seen in both the AT and CM groups. The rete pegs in both grafted groups became narrower and deeper than those in their respective control groups. It is well known that narrower and deeper rete pegs may reflect stronger

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integration between the epithelium and connective tissue, eventually enhancing the resistance to inappropriate forces applied to the gingiva [27,37]. It was suggested that narrower and deeper rete pegs represent a mature healing condition and furnish evidence that the grafted matrix has served as a scaffold to accelerate the healing process [38].

Since orthodontic movement did not result in gingival recession, the height of the epithelium and the CT attachment did not differ significantly between the AT and AT-control groups or between the CM and CM-control groups. As a result of soft tissue grafting, the AT and CM groups presented histologically evident increases in the thickness and area of the labial gingiva compared to the AT-control and CM-control groups, respectively. A statistical comparison was not performed between the AT and CM groups due to the differing surgical sites, but the thickness increased by approximately 0.1 mm in both groups. This amount is consistent with a previous report according to which comparable thicknesses were observed histologically [39], and a few other reports on volumetric assessments that also showed similar increases after grafting using either AT or CM [28,29]. These results imply that CM could be used as an alternative to AT grafting, which might also help to reduce surgical morbidity and patients' discomfort [29].

This study was subject to a few limitations. Firstly, as stated above, the level of the labial gingiva was preserved despite the presence of orthodontic proclination. Secondly, volumetric changes in the marginal gingiva and changes in the tooth position were not assessed at each time point because it was difficult to take fine impressions and to obtain accurate dental models owing to the presence of fixed orthodontic appliances. Furthermore, the change of the alveolar bone crest levels over time on the labial and lingual sides was not measured. Even though the alveolar bone crest height was estimated, it is difficult to say that these measurements represent alveolar bone dehiscence that occurred following orthodontic tooth movement, as the micro-CT scans were only taken after orthodontic treatment. It might have been better to obtain 2 sets of radiographs, before and after orthodontic treatment; however, this was not feasible since the micro-CT images could be only obtained after sacrificing the experimental animals. These problems could be addressed in future research by using an intraoral scanner to take optical impressions and taking serial cone-beam CT scans instead of micro-CT.

Within the limitations of the study, it can be concluded that CM might be potentially used as an alternative to AT in soft tissue augmentation, since both AT and CM grafts improved gingival thickness and area. Further studies are needed to overcome the limitations discussed above and to perform a thorough evaluation of the effects of gingival grafts in preventing orthodontically induced gingival recession.

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