The increase of keratinized and attached gingiva using collagen wound dressing in dogs: A clinical and histomorphometric comparative study

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ABSTRACT

Purpose: This study examined the width of keratinized gingiva and attached gingiva after an apically repositioned flap (APF group), APF combined with free gingival grafts (FGG group), and APF combined with collagen wound dressing (Collatape® group), both clinically and histomorphometrically.

Materials and Methods: The right and left maxillary canine areas of eight mongrel dogs were used (16 surgical sites). In the maxilla, the canine areas were used as experimental sites. Three different surgical techniques were performed on the sixteen canine areas (apically repositioned flap; APF group, APF combined with free gingival grafts; FGG group, and APF combined with collagen wound dressing; Collatape® group). After 6 weeks, clinical and histomorphometric evaluation were done.

Results: The FGG group showed more attached gingiva and a more favorable physiological appearance than the other groups. However, there was no significant difference in keratinized gingiva and attached gingiva among the three groups. According to the results of the histomorphometric examination, keratinized gingiva were formed in all groups. The FGG group showed thicker epithelium and connective tissue than the APF group. Collatape® group showed thicker connective tissue than the APF group.

Conclusion: The clinical and histomorphometric results suggested that APF combined with collagen wound dressing promotes more favorable healing of the keratinized and attached gingiva.

Key Words: Animal experimentation, Free tissue flaps, Membranes, Wound healing

Introduction

The attached gingiva (AG) consists of keratinized epithelium, dense connective tissue and periosteum, and plays an important role in protecting the periodontal structures [1]. For many years, the presence of an ‘adequate’ amount of keratinized gingiva was considered to be a keystone for the maintenance of periodontal health [2,3].

The presence of site-related conditions, e.g., gingival recession, thin periodontium and root prominence, combined with a reduced or missing amount of AG, may indicate a gingival augmentation procedure. Serino et al. [4] reported that sites with gingival recession should be considered susceptible to additional apical displacement of the soft tissue margin. The American Academy of Periodontology [5] suggested the following indications for gingival augmentation procedures: to prevent soft tissue damage in the presence of alveolar bone dehiscence during natural or orthodontic tooth eruption; to halt progressive marginal gingival recession; to improve plaque control and patient comfort around the teeth and implants; and to increase the insufficient dimensions of the gingiva in conjunction with fixed or removable prosthetic dentistry.

Since Friedman introduced the term ‘mucogingival surgery’ in the 1950’s, a range of procedures have been used to correct...
problems associated with the lack of AG [6]. One of the first surgical techniques designed to correct such problems was an apically repositioned flap (APF) [7]. This technique allowed surgeons to increase or preserve the area of AG by moving the tissue apically and exposing a variable band of crestal bone depending on how much AG was required [7]. Another technique to increase AG was a free gingival graft (FGG) [8] and free connective tissue graft [9,10]. Two of the advantages of these techniques are the availability of adequate donor tissue and ability to treat multiple teeth. The disadvantages include technical difficulty, postoperative discomfort, and poor continuity with adjacent tissue in color or shape.

Recently, many of the disadvantages of the classic procedure have been overcome by a modification of the procedure and the use of tissue engineering materials [7,11]. Collagen wound dressings have been used to stabilize blood clots, and protect the wound bed. Furthermore, they absorb blood and wound exudates and promote hemostasis, thus aiding wound healing while concurrently enhancing patient’s comfort [12].

The purpose of this study was to evaluate the width of keratinized gingiva and AG after an APF, APF combined with FGG, and APF combined with collagen wound dressing, clinically and histomorphometrically in dogs.

Materials and Methods

Surgical procedure

Eight mongrel dogs, approximately 1-year-old and weighing 17 to 19 kg each, were used in this experiment. The study protocol was approved by the Chosun University Dental Hospital Institutional Review Board (#CDMDIRB-0902-A29). Supragingival scaling was performed on all dogs prior to surgery. General anesthesia was induced by an injection of tiletamine-zolazepam (Zoletil 50®; Virbac, Carros, France; 5 to 10 mg/kg, intramuscular) and xylazine HCL (Rompun®; Bayer, Seoul, Korea; 0.15 mL/kg, intramuscular).

In both quadrants of the maxilla, the canine areas were used as experimental sites. Three different surgical techniques were performed on the sixteen canine areas (Table 1).

First, only an APF was performed (APF group). APF was performed according to the modified technique described by Carnio and Miller [7]. Before making the incisions, the level of crestal bone was probed to detect the presence of bone dehiscence. A periodontal probe or anesthetic needle can be used via the gingival sulcus. A horizontal beveled incision was made in the attached portion of the keratinized gingiva slightly apical to the alveolar crest. Notch was formed 3 mm under gingival margin with round bur. Mesial and distal extensions of the initial horizontal incision were then made (15 mm). Two vertical incisions were made on the mesial and distal ends connecting the horizontal incision (15 mm). These incisions extended beyond the mucogingival junction (MGJ). A split-thickness flap was elevated, moved apically, positioned at the desired level, and fixed with a periosteal horizontal suture using resorbable suture materials (Monosyn® 5-0; B. Braun Melsungen AG, Bethlehem, PA, USA). The size of the exposed periosteal bed was 15×15 mm (Fig. 1A).

Second, APF combined with a FGG was performed (FGG group). The APF procedure was performed in the same manner described for the first technique. The FGG was harvested from the palate, trimmed and shaped to fit the recipient site (Fig. 1B). The graft thickness was approximately 1.5 mm. The graft was fixed to the periosteal bed using a horizontal key suture. Pressure was applied to the recipient site for 3 minutes after suturing to ensure hemostasis and tissue adaptation.

Third, APF combined with collagen wound dressing (Collatape®; Zimmer Dental, Carlsbad, CA, USA) was performed (Collatape® group). Twofold Collatape® was used for the same thickness as the FGG. After preparing the recipient site, Collatape® was trimmed and shaped to fit the recipient site (Fig. 1C). Collatape® was fixed in a similar manner as described for the FGG method. Pressure was also applied for 3 minutes.

| Dog No. | Maxillary canine |
|---------|-----------------|
|         | Right           | Left            |
| Dog 1   | APF group       | FGG group       |
| Dog 2   | FGG group       | Collatape® group|
| Dog 3   | Collatape® group| APF group       |
| Dog 4   | APF group       | FGG group       |
| Dog 5   | FGG group       | Collatape® group|
| Dog 6   | Collatape® group| APF group       |
| Dog 7   | FGG group       | Collatape® group|
| Dog 8   | APF group       | Collatape® group|

APF group: apically repositioned flap only, FGG group: apically repositioned flap+free gingival graft, Collatape® group: apically repositioned flap+Collatape®.
Increase of keratinized and attached gingiva with collagen wound dressing

Postsurgical care

After surgery, each dog was injected with an antibacterial agent (Gentamicin 0.1mL/kg; Daesung, Gwangju, Korea) for seven days. Tooth cleaning with 0.2% chlorohexidine digluconate was performed three times per week for 4 weeks.

The sutures were removed two weeks after surgery.

Clinical measurements

The index was marked on the mid-buccal surface of the canine, 3 mm from the gingival margin. The probing depth (PD) was measured at three points (mesio-buccal, mid-buccal, and disto-buccal) to the nearest millimeter with a force-controlled probe (tip diameter: 0.45 mm; probing force: 20 g/pressure). The PDs at the three points were averaged for the purpose of analysis. The width of keratinized gingival (KG) at the mid-buccal point was measured from the MGJ to the free gingival margin. The width and thickness of the AG was calculated by subtracting the PD at the mid-buccal point from the width of the KG to the nearest millimeter (Fig. 1A, F).

Histologic and histomorphometric evaluation

Six weeks after APF, a soft tissue biopsy was harvested from

Fig. 1. Clinical procedure. (A) Apically repositioned flap (APF) was done. Recipient bed preparation was performed (15×15 mm) (APF group). (B) Free gingival graft (FGG) harvested from the palate (15×15×1.5 mm). Horizontal key suture to fix the graft on the recipient bed (FGG group). (C) Two fold collagen wound dressing (Collatape®) was similar thickness to the gingival graft (15×15×1.5 mm). Horizontal key suture to fix the Collatape® on the recipient bed (Collatape® group). (D-F) Six weeks healing after surgery. Smooth and physiological morphology of the attached gingiva was observed (D: APF group, E: FGG group, F: Collatape® group).

Fig. 2. Histomorphometric evaluation (H&E, ×40). Epi: thickness of epithelium, CT: thickness of connective tissue.
each dog under local anesthesia. The biopsy was performed in these areas. All the specimens were fixed in a 10% neutral buffered formalin solution for further descriptive histological analyses. After they were dehydrated in a graded ethanol series, the specimens were embedded in paraffin and serially sectioned in 5 µm thick sections. Each section was stained individually with H&E stain. The thickness of epithelium and connective tissue in coronal area of all sections was observed by using a optical microscope (LEICA DM750; Leica Microsystems, Wetzlar, Germany) equipped with a digital camera (LEICA ICC50 camera; Leica Microsystems) with ×40 and ×100 magnification and histomorphometric measurements was attained by calculating the average length of each section by measuring the length of the sections 10 times each using the i-SOLUTION Lite® processing and analysis program (IMT i-Solution Inc., Daejeon, Korea) on a personal computer (Fig. 2). The thickness of the section’s epithelium and connective tissue was measured perpendicularly to the surface of the tissue that attached to root.

**Statistical analysis**

A statistical software program (SPSS 16.0; SPSS Inc., Chicago,

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Fig. 3. Clinical parameters at the baseline and post-surgery. (A) Probing depth after surgery. (B) Width of keratinized gingiva after surgery. (C) Width of attached gingiva after surgery. (D) Shrinkage of keratinized gingiva after surgery.

APF group: apically repositioned flap only, FGG group: apically repositioned flap+free gingival graft, Collatape® group: apically repositioned flap+Collatape®.

*Statistically significant differences (p<0.05) by paired t-test.
IL, USA) was used for all statistical analyses. A paired t-test was performed to analyze the differences between the baseline and six weeks after surgery. An analysis of the variance (ANOVA) was performed to examine the difference in the continuous clinical parameters between the three surgical procedures. In addition, histomorphometric measurement was evaluated by

![Histological view](image)

Fig. 4. Histological view. (A, B) Apically repositioned flap (APF) only group. (C, D) APF combined with free gingival graft group (black arrow: gingival epithelium). (E, F) APF combined with Collatape® coverage (H&E; A, C, E: ×40, B, D, F: ×100).
ANOVA and post-mortem of Games-Howel. A p-value <0.05 was considered significant.

Results

Clinical findings

Healing of all groups was uneventful. The soft tissue grafts were fully integrated without any signs of necrosis (Fig. 1D-F).

Fig. 3 lists the preoperative and postoperative clinical measurements. Treatment with the three surgical procedures resulted in significant augmentation of the apico-coronal dimensions of the keratinized gingiva and AG (p<0.05) (Fig. 1).

In the APF group, the mean apico-coronal dimension of the keratinized gingiva was 12.85 mm (range, 7.2 to 16.2 mm) preoperatively and 20.40 mm (range, 17.0 to 21.9 mm) postoperatively. The mean apico-coronal dimension of the AG was 11.23 mm (range, 5.4 to 14.2 mm) preoperatively and 18.80 mm (range, 15.5 to 20.4 mm) postoperatively (Fig. 3B, C).

In the FGG group, the mean apico-coronal dimension of the keratinized gingiva was 14.30 mm (range, 13.1 to 15.3 mm) preoperatively and 22.23 mm (range, 19.7 to 26.5 mm) postoperatively. The mean apico-coronal dimension of the AG was 13.03 mm (range, 11.9 to 14.0 mm) preoperatively and 20.98 mm (range, 18.2 to 25.2 mm) postoperatively (Fig. 3B, C).

In the Collatape® group, the mean apico-coronal dimension of the keratinized gingiva was 13.13 mm (range, 10.8 to 15.1 mm) preoperatively and 20.43 mm (range, 19.8 to 21.5 mm) postoperatively. The mean apico-coronal dimension of the AG was 11.65 mm (range, 9.1 to 13.5 mm) preoperatively and 19.15 mm (range, 18.3 to 19.9 mm) postoperatively (Fig. 3B, C).

There was no significant difference in PD detected pre- and postoperatively in each procedure (Fig. 3A).

The average shrinkage of apico-coronal dimension in keratinized gingiva marked 25.5% (range, 4.95% to 38.63%) in the APF group, 23.9% (range, 5.69% to 33.33%) in the FGG group and 27.03% (range, 16.67% to 34.22%) in the Collatape® group, respectively (Fig. 3D).

Histologic and histomorphometric findings

According to the results of the histological examination, keratinized gingiva was formed in all groups (Fig. 4). The FGG group showed the thickest epithelium, and the APF group showed the thinnest epithelium and connective tissue in coronal area (Fig. 5).

The thickness of the keratinized gingiva was measured using the telescopic histology images. In the APF group, the average thickness of epithelium and entire connective tissue was 13.2 µm (range, 9.3 to 24.0 µm) and 1,002.7 µm (range, 706.7 to 1,440.0 µm), respectively. In the FGG group, the average thickness of epithelium and connective tissue was 30.8 µm (range, 12.0 to 49.3 µm) and 1,677.3 µm (range, 1,413.3 to 2,000.0 µm), respectively. In the Collatape® group, the average thickness of whole epithelium and keratinized layer was 19.2 µm (range, 10.7 to 30.7 µm) and 1,476 µm (range, 600.0 µm).

Fig. 5. Comparison of the histomorphometric parameters. (A) Width of epithelium after surgery. (B) Width of connective tissue after surgery. APF group: apically repositioned flap only, FGG group: apically repositioned flap+free gingival graft, Collatape® group: apically repositioned flap+Collatape®.

*Statistically significant differences (p<0.05) to APF group by Games-Howel.
to 2,066.7 µm), respectively (Fig. 5). There was statistically significant difference in the thickness of epithelium and connective tissue detected preoperatively and postoperatively between APF group and FGG group (p=0.039 and p<0.001). In addition, there was no significant difference in the thickness of epithelium and connective tissue detected preoperatively and postoperatively between Collatape® group and FGG group (p=0.201 and p=0.479) (Fig. 5).

Discussion

The aim of this study was to evaluate the changes in the amount of keratinized gingiva and AG and to examine tissue histomorphometrically after APF, APF combined FGG, and APF combined collagen wound dressing. The results showed that the amount of AG had increased six weeks after surgery in all procedures. The APF areas combined FGG showed a greater increase in AG and favorable physiological morphology than the other groups. However, there were no significant differences in amount of AG between the groups. In the APF areas combined collagen wound dressing, there was similar increase in the amount of AG to the only APF areas. It is because Collatape® could control bleeding, stabilize blood clots and protect the wound bed. For these reasons, the collagen wound dressing may act as a scaffold to disturb the apically positioned flap, making it move coronally, and protect the recipient bed. However, various collagen wound dressings are generally absorbed over 10 to 14 days. In addition, the collagen wound dressings are absorbed more rapidly if it is exposed directly to the oral environment [5]. Therefore, the collagen wound dressing could not act as a three-dimensional scaffold but promote healing with stabilization of blood clots.

The histological findings of the three gingiva specimens 6 weeks after surgery showed normal gingival tissue. Keratinized gingiva was formed in all groups. The specimens were composed of orthokeratinized or parakeratinized epithelium with low-formed rete ridges. In the other two groups, the specimens were composed of orthokeratinized epithelium with well-formed rete ridges [13]. The FGG group showed the thickest keratinized layer and the APF group showed the thinnest that.

According to several reports, 2.0 mm of AG is sufficient to maintain periodontal health [14,15], even in cases in which the subgingival restoration margins are placed [16,17]. In this study, modified APF surgery was performed to increase the AG [7]. According to some authors [18-20], the main factor determining the nature of the new tissues that develop over the exposed periosteum is the origin of the granulation cells that migrate over the wound. These cells migrate from the periosteal connective tissue, adjacent gingival and alveolar mucosa, periodontal ligament, and bone marrow spaces. The surgical wound created by APF is surrounded completely by keratinized tissue. This prevents the non-keratinized epithelial cells originating from the oral mucosa from proliferating onto the surgical area [7]. As a result, a predictable increase in the apico-coronal gingival dimension is possible. These results are in agreement with those reported elsewhere [7,21].

The shrinkage of keratinized tissue is a well-known clinical phenomenon that occurs during the healing process. The results of the present study showed that the apico-coronal dimensions of keratinized tissues were reduced by 25.5% in the APF group, 23.9% in the FGG group and 27.03% in the Collatape® group, respectively. Silva et al. [22], using FGG grafts, recently reported FGG shrinkage (length) of 25% and 22% in smokers and non-smokers, respectively, at 90 days [22]. When FGG is placed on a periosteal bed, FGG shrinkage is mostly attributable to loss of vestibular depth, caused by cicatricial contraction [23].

Augmentation of the keratinized tissue width and vestibular deepening with an autogenous free gingival graft is a predictable and effective method [24-26]. Although the incidence of complications is very low, discomfort and pain at the donor site are observed frequently [24]. The use of Collatape® eliminates the need for a secondary surgical site and provides an unlimited amount of donor tissue. However, it is difficult to conclude whether well-organized keratinized gingiva had been created.

In conclusion, the APF, APF combined with FGG, and APF combined with collagen wound dressing showed a successful increase in the keratinized gingiva and AG for a short period of time. The histomorphometric findings also showed that the keratinized layer of the APF combined collagen wound dressing was thicker than that of the APF group and thinner than that of the FGG group. These results suggested that APF combined with a collagen wound dressing promote healing of gingiva both clinically and histologically. However, further studies will
be needed to determine the influence of the collagen wound
dressing for long-term follow-up.

Acknowledgments

This study was supported by research fund from Chosun
University, 2013.

References

1. Carnio J, Camargo PM, Passanezi E: Increasing the apico-
coronal dimension of attached gingiva using the modified
apically repositioned flap technique: a case series with a
6-month follow-up. J Periodontol 78:1825-1830, 2007.
2. Nabers CL: Repositioning the attached gingiva. J Periodontol
25:38, 1954.
3. Carranza FA Jr, Carraro JJ: Mucogingival techniques in
periodontal surgery. J Periodontol 41:294-299, 1970.
4. Serino G, Wennström JL, Lindhe J, Eneroth L: The prevalence
and distribution of gingival recession in subjects with a high
standard of oral hygiene. J Clin Periodontol 21:57-63, 1994.
5. Wennström JL: Mucogingival therapy. Ann Periodontol 1:671-
701, 1996.
6. Newman MG, Takei HH, Klokkevold PR, Carranza FA: Carranza’s clinical periodontology: 10th ed. St. Louis:
Saunders, 2006. p.1005.
7. Carnio J, Miller PD Jr: Increasing the amount of attached
gingiva using a modified apically repositioned flap. J
Periodontol 70:1110-1117, 1999.
8. ten Bruggenkate CM, Krekeler G, van der Kwast WA,
Oosterbeek HS: Palatal mucosa grafts for oral implant devices.
Oral Surg Oral Med Oral Pathol 72:154-158, 1991.
9. Edel A: The use of a free connective tissue graft to increase the
width of attached gingiva. Oral Surg Oral Med Oral Pathol
39:341-346, 1975.
10. Edel A, Faccini JM: Histologic changes following the grafting
of connective tissue into human gingiva. Oral Surg Oral Med
Oral Pathol 43:190-195, 1977.
11. Seol KY, Kim SG, Kim HK, Moon SY, Kim BO, Ahn JM, Jang
HS, Kim HJ, Min JB, Lee BJ, Lim SC: Effects of decortication in
the treatment of bone defect around particulate dentin-coated
implants: an experimental pilot study. Oral Surg Oral Med Oral
Pathol Oral Radiol Endod 108:529-536, 2009.
12. Shankugam M, Kumar TS, Arun KV, Arun R, Karthik SJ:
Clinical and histological evaluation of two dressing materials in
the healing of palatal wounds. J Indian Soc Periodontol 14:241-
244, 2010.
13. Fehr C, Muhlemann HR: The surface of the free and attached
gingiva studied with the replica method. Oral Surg Oral Med
Oral Pathol 8:649-655, 1955.
14. Lang NP, Löe H: The relationship between the width of
keratinized gingiva and gingival health. J Periodontol 43:623-
627, 1972.
15. Wennström J, Lindhe J, Nyman S: Role of keratinized gingiva
for gingival health. Clinical and histologic study of normal and
regenerated gingival tissue in dogs. J Clin Periodontol 8:311-
328, 1981.
16. Maynard JG Jr, Wilson RD: Physiologic dimensions of the peri-
odontium significant to the restorative dentist. J Periodontol
50:170-174, 1979.
17. Nevins M: Attached gingiva--mucogingival therapy and
restorative dentistry. Int J Periodontics Restorative Dent 6:9-27,
1986.
18. Karring T, Cumming BR, Oliver RC, Löe H: The origin of granulation tissue and its impact on postoperative results of
mucogingival surgery. J Periodontol 46:577-585, 1975.
19. Karring T, Lang NP, Löe H: The role of gingival connective
tissue in determining epithelial differentiation. J Periodontal
Res 10:1-11, 1975.
20. Karring T, Ostergaard E, Löe H: Conservation of tissue
specificity after heterotopic transplantation of gingiva and
alveolar mucosa. J Periodontal Res 6:282-293, 1971.
21. Carnio J, Camargo PM, Passanezi E: Increasing the apico-
coronal dimension of attached gingiva using the modified
apically repositioned flap technique: a case series with a
6-month follow-up. J Periodontol 78:1825-1830, 2007.
22. Silva CO, Ribeiro Edell P, Sallum AW, Tatakis DN: Free gingival
grafts: graft shrinkage and donor-site healing in smokers and
non-smokers. J Periodontol 81:692-701, 2010.
23. James WC, McFall WT Jr: Placement of free gingival grafts
on denuded alveolar bone. Part I: clinical evaluations. J
Periodontol 49:283-290, 1978.
24. Egli U, Vollmer WH, Rateitschak KH: Follow-up studies of free
gingival grafts. J Clin Periodontol 2:98-104, 1975.
25. Han TJ, Takei HH, Carranza FA: The strip gingival autograft
procedure. Int J Periodontics Restorative Dent 13:180-187,
1993.
26. Soileau KM, Brannon RB: A histologic evaluation of various
stages of palatal healing following subepithelial connective
tissue grafting procedures: a comparison of eight cases. J
Periodontol 77:1267-1273, 2006.