Original Article

Evaluation of Color and Width of Attached Gingiva Gain in Two Surgical Techniques: Free Gingival Graft and Connective Tissue Graft Covered By Thin Mucosal Flap, a Clinical Trial

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ABSTRACT

Statement of the Problem: The periodontal health and marginal stability of gingiva can be negatively affected by a number of dental conditions in association with deficiency of attached gingiva.

Purpose: This study aimed to compare the color and width of tissue grafted by two surgical techniques of keratinized gingival augmentation, namely free gingival graft(FGG) and connective tissue graft (CTG) covered by thin mucosal flap .

Materials and Method: This clinical trial was performed on 15 adult individuals. The patients showed less than 2mm keratinized gingiva on two different recipient sides. One side was to be treated with CTG as the test group and the other side to be treated with FGG as the control group. The amount of keratinized gingiva before the surgery, size of grafted tissue during the surgery and 6 month after the surgery was documented. Six months after healing, the test and control sides were compared in terms of the width of generated gingiva on both sides, and the color match of the grafted areas with the surrounding gingiva or mucosa. The color of the grafted areas was determined and compared by using both professional evaluation and digital evaluation.

Results: In digital evaluation, ΔE (which shows color mismatch) was higher in FGG. In professional evaluation, visual analogue scale (VAS) was used by two blinded periodontists. The mean VAS in FGG was less than CTG. The mean increase of gingival width was higher in CTG. The increased width in CTG technique was more than that in FGG technique. This difference was statistically, but not clinically, significant.

Conclusion: Higher ΔE in control side and higher mean VAS CTG both showed better color adaptation of CTG side. FGG can be used in case of increasing keratinized gingiva, vestibular depth, and in patients with low smile line without esthetic concerns. However, using connective tissue in the underlying thin mucosal layer is preferred for gingival augmentation if there are adequate vestibule depth and esthetic concerns, like in maxillary canine.

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Introduction

The role of a strip of keratinized gingiva in maintaining periodontal health through preventing gingivitis and stabilizing the gingival margin has long been discussed [1-2]. Some experimental studies support the hypothesis that the periodontal health can be maintained through optimal plaque control in areas with low or no attached gingiva. On the contrary, other investigations reported clinical inflammation in all areas with less than 2mm keratinized gingiva, despite the fact that all the dental surfaces were plaque-free [1-2].

The above-mentioned supporters believe that aug-

mentation of keratinized gingiva may be unnecessary if measures are taken for optimal infection control. Yet, this might be impossible for a majority of the patients; thus, gingival augmentation in areas with gingival recession would prevent further destruction and recession. Moreover, evidence show that the areas with small amount of keratinized gingiva, particularly thin gingival biotype, are more likely to experience recessions compared with the areas protected with thick and wide gingiva [3].

A number of dental conditions, in association with loss or deficiency of attached gingiva, can negatively affect the periodontal health and marginal stability. These conditions are gingival recession, thin biotype, buccolingual displacement, root prominence, shallow vestibule depth, frenulum stretching, subgingival restorations, orthodontic treatments, as well as pain, and discomfort during oral hygiene performance [1, 4-6].

Based on the recommendation of the 1996 World Workshop in Periodontics, in case of alveolar bone dehiscence, no matter during normal growth or orthodontic treatment, gingival augmentation can stop the progression of gingival recession, control the plaque formation, and improve the patient discomfort around the tooth and implant [1, 6]. Among the gingival augmentation techniques including pedicle graft, free graft, and allograft, the most commonly used method is the free gingival graft (FGG) which is considered as the gold standard [1, 6].

In treating the mucogingival problems such as deficiency of keratinized gingiva and gingival recession, FGG can provide adequate amount of attached gingiva and cover the exposed root. Moreover, root coverage treatments with FGG are more predictable. They are the best option when the gingival recession is associated with inadequate vestibule depth or for the teeth, which require root coverage before receiving subgingival restoration.

In gingival augmentation, FGG increases the vestibule depth more predictably than other methods. However, FGG has unavoidable limitations such as the open wound in the palatal donor site and the wound of the grafted tissue in the recipient site that can cause hemorrhage and postoperative discomfort [7-8].

Another problem with free graft is the color discrepancy between the area treated with the graft and the adjacent gingiva, which result in keloid formation in the graft margin. Since the donor site is mostly the palate that contains lipid contents, color difference is so observable that the grafted area looks lighter in color even in a long time after initial healing [9].

Currently, the mucogingival treatments aim to meet not only the biological, but also aesthetic needs. Thus, it is preferred to use graft techniques that offer higher color match and better aesthetic such as subepithelial connective tissue graft (CTG) [7, 10]. According to the previous findings, the specificity of the grafted epithelium is determined by the underlying connective tissue [11]. The gingival connective tissue is able to induce the generation of keratinized epithelium. In other words, placing a keratinized tissue under a non-keratinized mucosa changes the surface epithelium to keratinized tissue overtime [11].

Previous studies showed that the result of root coverage by using FGG would not be aesthetically successful [7, 9-10, 12-13]. Hence, the present study was designed to use a novel gingival augmentation technique that would not only increase the amount of gingiva and alter the location of mucogingival junction, but also improve the grafted tissue aesthetically so that it would look more like the adjacent tissues in color. This study also compared the alterations of mucogingival junction location between the FGG and CTG techniques.

Materials and Method

This clinical trial was done on 15 healthy nonsmoker adults with good oral hygiene. The sample size was determined based on the advice of statistician according to the lack of studies with similar method and the limited number of qualified subjects. The study was approved by Ethics Committee of Shiraz University (IR.SUMS.REC.1396.149) and it was registered in Iranian Registry of Clinical Trials (IRCT: IRCT20171031037120N2). Patients were selected from Shiraz School of Dentistry; explained about their contribution and the treatment plan, and signed informed consent form if willing to participate. The exclusion criteria were smoking, pregnancy, systemic diseases, and use of antibiotics within the six preceding months, and lack of postoperative cooperation in maintaining oral hygiene or attending the follow-up sessions.

For the split mouth surgery, the two recipient areas

on the two sides as well as the surrounding area of each tooth (incisor, canine, or premolar), the two adjacent distal and mesial teeth had to be free of gingivitis and periodontitis (<15% plaque index and gingival index), bone resorption (evaluated by radiography), and <3mm pocket depth. There was <2mm keratinized gingiva on the buccal or labial side of the tooth, and adequate vestibule depth on the desired site .Vestibular depth should have been more than the apico-corocal size of the graft. One side was to be treated with CTG as the test group and the other side to be treated with FGG as the control group. The selection of control or test for each side was done randomly.

The donor site was the hard palate with available palate arch and at least 5 mm soft tissue on the bone. All patients were trained about oral hygiene after cause related therapy .The initial measurements were done on the buccal side in midbuccal area one month after cause related therapy.

Surgical technique on the test side

Two vertical partial thickness incisions (with very shallow incision) were created on the mucogingival junction extending towards the depth of vestibule. The distance between the two vertical incisions was slightly more than the width of a teeth and at most 12 mm. Then, a probe was horizontally moved to undermine the very thin surface flap (Figure 1). The third incision, which connected the two vertical incisions, was created on the mucogingival junction in order to preserve the existing keratinized gingiva.

In case of absence width of keratinized gingiva, the horizontal incision was created on the gingival margin and the vertical incision was extended upon that. These partial thickness incisions were created within the surface epithelium by using blade number 15. The partial thickness flap with the minimum thickness was raised to detach the epithelium from the underlying connective tissue, though it is impossible to keep only epithelium. Having raised the surface tissue while it was still attached on the apical area, the four sides of the created wound was deeply cut up to the bone surface. By using periosteal elevator, the connective tissue was removed along with the periosteum. In case of fenestration or dehiscence, the periosteum was preserved over the tooth root.

In the donor site (palatal tissue), a graded probe was used to confirm the presence of adequately thick tissue. Then, one horizontal incision as long as the bed area and two vertical incisions of 1-mm depth and 5-6 mm length were created on both sides. Then, 1-mm thick surface tissue, which was attached on one side, was detached from the underlying tissue by use of surgery forceps and a blade. The flap was pushed away, the four dimensions of the underlying wound was cut by The graft tissue was rinsed with sterile saline solution, placed over the prepared clean clot less bed, and sutured to the surrounding periosteum by using Vicryl suture. The thin epithelial flap was placed over it and sutured to the surrounding tissue so that it thoroughly covered the wound surface including the connective tissue.

Having controlled the hemorrhage in the underlying tissues, the palatal flap was returned to the place and sutured to the underlying tissues, so that the wound was thoroughly covered. Both areas were gently pressed with wet gauze for a few minutes, and after ensuring that there was no bleeding, the area was covered with Coe-Pak dressing.

Surgical technique on the control side

In order to treat the control side with FGG, on the recipient site, one partial thickness incision was created on the mucogingival junction and two releasing incisions on both sides (Figure 2).

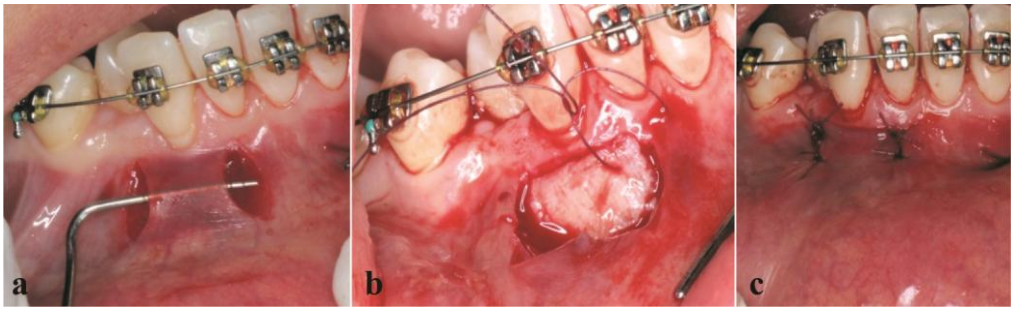

Figure 1: Connective tissue graft covered by thin mucosal flap

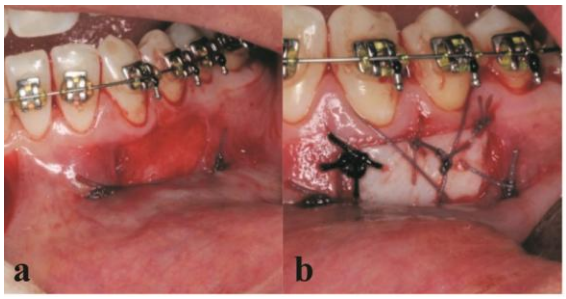

Figure 2: Free gingival graft

From the palate (donor site), a tissue of 1-2 mm thickness with the same length as the recipient site and a width of 5-6 mm was prepared, sutured, and fixed on the bed.

Postsurgical recommendations

Amoxicillin 500 mg (every 8 hours) was prescribed for 7 days, and oral acetaminophen codeine was prescribed for every 4 hours in case of pain. The patients were trained about soft diet, cold compress for the first 48 hours, plaque control in other areas, and use of Chlorhexidine mouthwash. They referred after 10 days for removing their wound dressings and sutures, and they were trained about plaque control in the operated areas. The next follow-up session was fixed for 6 months after the surgery.

Measurements and comparisons

Six months after healing, the test and control sides were compared in terms of the percent of generated gingiva on both sides, and the color difference of the grafted areas with the surrounding gingiva or mucosa (Figure 3). The apico-coronal size of the graft prepared for each area was calculated. Considering that the grafted tissue on both sides would be attached, the percentage of augmented tissue on each side was calculated. To do so, the amount of gingiva remaining after the surgery (distance between gingival margin and mucogingival junction) was divided by the width of the graft prepared for each side. The result shows how much the grafted tissue has remained and how much the location of mucogingival junction has shifted apically. Moreover, the rate of shrinkage would be calculated. These were calculated for both the test and control sides.

The color of the grafted areas was determined and compared by using both professional evaluation and digital evaluation [14]. The professional evaluation was done by two blinded examiners through Visual Analog Scale (VAS).

Figure 3: Right: free gingival graft and left: connective tissue graft after healing period

Actually, VAS is a scale for measurement of subjective features, which are difficult to measure. The test and control areas were assessed for color matching of the grafted tissue with the adjacent gingiva. The results were esthetically graded in three groups of Good [7-9], Moderate [4-6] and Poor [1-3, 14]. Kappa statistics were calculated to determine inter-examiner reliability.

Evaluation of color matching that required digital images has been taken with the same camera in similar lighting conditions (standardized photographs) [12]. To take standard photographs, a Canon camera (model; manufacturer country) was used with specific settings. The patient's chin was placed at 30-cm distance from the lens. Lip retractor was used to allow uniform lighting of the area. Photos of the two sides were taken in identical lighting conditions (at the same time and place) at an angle of 45° to prevent light reflex [15]. The photos were transferred to computer for analyses and comparisons by use of Adobe Photoshop software (CS6, 64 Bit, manufacturer country). There in, the eyedropper tool was used to pick color from the grafted area on each

side and another color drop from the mucosa or gingiva around the graft (background) to be used as a reference. The CIE (LAB) and LUT (RGB) color indices were used to compare the color of the two areas and the background color.

This study used digital images, which could be transferred to software, which allowed measurement and comparison with color indices. Color analysis can be easier to first decompose and then analyze the color rather than overall analysis. This can be achieved through different channels like analyzing each color into three main color components (Red, Blue, and Green).

The three-dimensional Lab system can also be used in which L represents the lightness (0-100), a is the degree of green (a-) or red (a+) ranging from -120 to +120, and b is the degree of blue (b-) or yellow (b+) ranging from -120 to +120.

This system allows the calculation of ΔE (Euclidean Distance) which is a criterion to compare the color between two areas, and color matching between two areas (test or control) and the adjacent gingiva. [16] CIE (Lab) system can help determining the graft color and the background area, and then determining the standard deviation and comparison to find out the difference or similarity of color in each grafting method in comparison with the healthy surrounding gingiva.

Statistical analyses

The quantitative and qualitative data were described as mean, standard deviation, and frequency. Repeated measures ANOVA were used to evaluate the changes over time.

Results

According to (Table 1, 2 and 3) higher ΔE, which represents the color difference between the grafted area and the surrounding tissues, was observed in FGG side than CTG. Moreover, the mean $VAS_{1,2}$ (blinded examiners 1, 2) in FGG (2.4) was lower than that in CTG (6.9). Comparing the two techniques regarding the amount of augmented tissue revealed that the mean increase of gingival width was 82% in FGG (18% shrinkage) and 85% in CTG side (15% shrinkage); i.e., the shrinkage was slightly lower in the side treated with CTG technique. However, in both sides, test and control, the width of attached gingiva was increased.

Discussion

The concept of covering CT with thin layer of epithelium was first carried out by Raoofi [17] that presented the inductive effect of keratinized connective tissue on overlying alveolar mucosa by underlying CT. CT could induce overlying epithelium to attached and functional gingiva [17]. This technique was also used by Kiani *et al.* [18] that by histological examination confirmed CT could transform overlying non-keratinized epithelium into masticatory and keratinized gingiva.

The current study aimed to compare the color and width of tissue grafted through two surgical techniques of keratinized gingival augmentation. The results revealed that both techniques increased the gingival tissue and moved the mucogingival line to a more apical location. However, the increased width in CTG technique (85%) was higher than that in FGG technique (82%). The difference was statistically, but not clinically, significant. The two main factors that affect the shrinkage are the graft thickness and the recipient bed preparation. Optimal shrinkage in FGG is 1-2mm; the less the thick ness is, the higher the shrinkage would be.

Table 1: Clinical characteristic difference between free gingival graft (FGG) and connective tissue graft (CTG)

	Att gain 1	Att gain 2	Vas 1 Surg 1	Vas 1 Surg 2	Vas 2 Surg 1	Vas 2 Surg 2	Delta E1	Delta E2	Mean Vas 1	Mean Vas 2
N	15	15	15	15	15	15	15	15	15	15
Median	.8300	.8500	3.00	7.00	2.00	7.00	20.6155	7.8102	2.5000	7.0000
Mean	.8253	.8520	2.60	6.87	2.27	7.00	20.4662	9.0602	2.4333	6.9333
Std.Deviation	.02031	.01612	.737	1.060	.799	1.000	10.74718	4.40539	.59362	.75277

Att gain 1: width of gingiva gain in FGG technique
Att gain 2: width of gingiva gain in CTG technique
Vas 1 Surg 1: visual analogue scale , examiner 1 consider for FGG
Vas 1 Surg 2: visual analogue scale , examiner 1 consider for CTG
Vas 2 Surg 1: visual analogue scale , examiner 2 consider for FGG
Vas 2 Surg 2: visual analogue scale ,examiner 2 consider for CTG
Mean vas 1: visual analog scale for FGG
Mean vas 2: visual analog scale t for CTG
Delta E1: the difference between FGG and the surrounding area
Delta E2: the difference between CTG and the surrounding area

Table 2: Statistical analysis to compare FGG and CTG

Test Statistics[a]					
	Att gain 2- Att gain 1	**vas2surg2- vas1surg1**	**vas2surg2- vas2surg1**	**DeltaE2- DeltaE1**	**MeanVas2- MeanVas1**
Z	-3.097	-3.436	-3.475	-2.953[c]	-3.454
Asymp. Sig. (2- tailed)	. 002	.001	.001	.003	.001

a. Wilcoxon Signed Ranks Test
c. Based on positive ranks

Therefore, it was highly tried to have similar tissue thickness in both sides (test and control) [19]. According to the previous studies [18-20], placing the graft on the periosteal bed increased the shrinkage compared with being placed on denuded bone. Placing the graft on denuded bone or creating fenestration in the apical periosteum, increases the dimensional stability; in other words, it decreases the mobility and postoperative shrinkage. However, the fenestration created in periosteum seems to have limited role in the dimensional stability of FGG. In the present study, neither periosteum omission nor fenestration was considered in the apical periosteum on the FGG side in order not to jeopardize the local blood supply. Meanwhile in the CTG side, there was no concern of the local blood supply since the area was covered with a thin surface layer.

The shrinkage phenomenon generally occurs during the healing process of gingival graft surgery. The current findings showed that shrinkage occurred both in FGG with the tissue placed on periosteal bed and in CTG with tissue placed on denuded bone and covered with a thin mucosal flap. However, it was lower when the graft was placed on denuded bone.

This study also aimed to evaluate and compare the FGG and CTG techniques regarding the color matching of the grafted gingiva and the adjacent tissue. Two blinded examiners performed the assessments by using VAS. The mean VAS of the CTG side was 6.9, which stands within the range of moderate-good color matching. However, this value was 2.4 in FGG side, which was within the range of poor-moderate group. Therefore, the blinded examiners reported better color match in CTG group.

The second method of color matching assessment was digital analysis, which compared the standard deviation of the grafted area from the adjacent area in the two groups. The results revealed the standard deviation to be lower in CTG group. This smaller standard deviation confirmed that the area grafted through CTG method was less different in color from the adjacent gingiva; in other words, color matching of the grafted tissue and the adjacent gingiva was better in CTG group than FGG group.

Donn *et al.* [13], in study of comparing FGG and CTG for root coverage procedure, observed color mismatch and pale appearance in sites treated with FGG and better color match and esthetic in sites of CTG which was concordance with our study. In a systematic review done on 2014, efficacy of soft tissue augmentation techniques around dental implant was compared. They concluded that FGG and sub epithelial connective tissue graft were the most successful techniques in increasing keratinized tissue and sub epithelial connective tissue graft was the best in esthetic [21].

Studies showed the importance of 2mm of keratinized and attached gingiva around dental implants and compared different options for its augmentation. They concluded that FGG should be considered as a "rescue" only in situations that esthetic is not an issue like low smile line patients due to the disadvantageous such as unaesthetic shade and color. However, CTG, as an alternative, can provide better volume and can be blend

Table 3: Difference between FGG and CTG

Variable	**Surg1 : FGG**	**Surg2: CTG**	***p* Value***
ΔE	20.61 (20.47 ± 10.75)	7.81 (9.06 ± 4.40)	0.003
Vas 1	3 (2.6 ± 0.73)	7 (6.87±1.06)	0.001
Vas 2	2 (2.27±0.79)	7 (7±1)	0.001
Average Vas	2.5 (2.43±0.59)	7(6.9±0.75)	0.001
Attachment gain (%)	83(82 ± 2)	85(85±1)	0.002

- "Wilcoxon-signed- ranks" test
- The value in the table are: median (mean ±SD)

* *p* Value<0.05 :significant

better with adjacent tissue [22-23].

The present study was superior to all similar investigations since surgical technique was the only variable. Regarding the split mouth surgery, all other intervening factors were omitted including the patient's condition, plaque control, tissue healing ability, the color of donor site, the background color of individual's gingiva. Moreover, not only we used connective tissue to increase esthetic, we tried to cover it with thin layer of recipient flap.

A potential factor, which might affect the results, is the vestibule depth. The constant and complete connection between the underlying connective tissue and the thin mucosal flap is the requisite for changing the nature of thin mucosal flap, augmentation of the gingiva, and altering the location of mucogingival junction. Inadequate vestibule depth disconnects the link and negatively affects the outcome. Another limitation of this study is the absence of comparing the buccolingual dimension of two sides.

Conclusion

CTG provides better esthetic and color adaptation. Both CTG and FGG techniques increase attached and keratinized gingiva but FGG seems more predictable in generating attached and keratinized tissue when the vestibule is not adequately deep and there is the simultaneous need for augmentation of keratinized gingiva and increasing of the vestibule depth. Likewise, it is feasible in patients with low smile line without esthetic concerns. However, use of connective tissue in the underlying thin mucosal layer is preferred for gingival augmentation if there are adequate vestibule depth and esthetic concerns.

Conflict of Interest

None declared.

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