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RESEARCH

Aesthetic evaluation of free gingival graft applied by partial de-epithelialization and free gingival graft applied by conventional method: a randomized controlled clinical study

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Abstract

Objective The aim of this clinical study was to compare the partially de-epithelialized free gingival grafts (d-FGGs) with the conventional free gingival grafts (FGGs) aesthetically using photographic analysis and Visual Analogue Scale (VAS).

Materials and methods A total of 30 defects were treated in 15 patients with attached gingiva insufficiency. In the split-mouth study, d-FGGs were applied on one side (test group), while FGGs were applied on the contralateral side (control group). Results of clinical periodontal parameters were evaluated in 6-month follow-up, and aesthetic evaluation results were evaluated in 1-, 3-, and 6-month follow-ups.

Results Among the periodontal clinical parameters examined, only the keratinized tissue (*KT*) width was found to be statistically significantly higher in the test group compared to the control group. In the photo analysis evaluation, the ΔE value at the 3rd month was statistically significantly higher in the test group. In VAS evaluation, there was no significant difference between the two groups in the time periods examined.

Conclusion Photo analysis is an objective, sensitive, reproducible, and safe method that can be used in dentistry and medicine by detecting the slightest changes and giving more detailed and superior results than visual evaluation. The d-FGG application could provide acceptable aesthetic results by providing an appearance compatible with the gingival contour and gingiva in the adjacent region in creating keratinized gingiva.

Clinical relevance While the free gingival graft has color differences with the recipient area, the deep-thelialized gingival graft has a compatible appearance with the recipient area. Partially, de-epithelialized free gingival graft is a method with acceptable aesthetic results.

Trial registration U.S. National Institutes of Health Clinical Trials: NCT04970524

Keywords Cosmetic periodontal plastic surgery · Treatment planning · Connective tissue graft(s)

Introduction

To maintain a healthy gingival margin, at least 2-mm-wide attached gingiva is needed [1]. If the width of the attached gingiva is less than 2 mm, the mobility of the gingival edge increases and subgingival plaque accumulation becomes easier [2]. Insufficient attached gingiva and shallow vestibular depth negatively affect the maintenance of oral hygiene procedures [3, 4].

Free gingival grafting operations have been developed to cover the exposed root surface and to create keratinized tissue in the treatment of gingival recessions and shallow attached gingiva. Since the vestibular depth and keratinized tissue increase after free gingival grafting applications, oral hygiene maintainace of the patient become easier [5, 6].

The color, consistency, structure, and keratinization level of FGG bear the characteristics of the region from which it is taken. During the healing phase of the graft placed in the recipient area, the overlying epithelium becomes necrotic and the new epithelium proliferates from neighboring tissues. Despite this, the FGG retains its own characteristics. This event shows that the connective tissue in the FGG

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determines the structure of the new epithelium formed on its genetic properties [7].

In the treatment of gingival recessions, it has been shown that d-FGG can be applied in cases where the thickness of the mucosal tissue in the hard palate is not sufficient for connective tissue graft (< 2.5 mm) and when a large graft is needed mesio-distally and apico-coronally [8]. It has been suggested by some studies that the aesthetic success of d-FGG is higher than conventional FGG [9]. In the treatment of gingival recessions, d-FGG applications are preferred in order to provide complete root surface coverage, to achieve a suitable aesthetic appearance in terms of color and structure, and to ensure proper realignment of the mucogingival border. [10]

When making aesthetic comparison, one of the parameters first perceived by the human eye is color. Various color systems have been developed to explain the color principle, to use it in practical applications and to express color numerically. "Commission International de l'Eclairage (CIE) / International Commission on Illumination" color system is one of the most widely used color systems [11, 12].

Gingival color varies in different individuals and in different parts of the mucosa [13, 14]. The color of a clinically normal gingiva may change with the thickness of the mucosa [15], pigmentation [16], hormonal effects [17], vascularity [18], and the degree of keratinization [19].

In our study, we expected d-FGG application to areas with shallow vestibular depth to have a more aesthetic appearance than the conventional FGG method. In the literature review, we did not find any study evaluating the aesthetic color match of d-FGG application and conventional FGG application in terms of photographic analysis. In the light of these findings, the aim of the study was to compare different types of free gingival grafts in terms of aesthetics, as we think that the application of d-FGG to the region with gingival recession or shallow vestibule depth will have a more aesthetic appearance than conventional FGG.

Material and methods

The materials and methods of our study were approved by the Izmir Katip Çelebi University, Faculty of Medicine, Clinical Research Local Ethics Committee at the meeting dated April 25, 2019, with the protocol numbered 44. The study was conducted on a total of 30 defects in 15 patients, 10 females and 5 males, with attached gingiva of less than 2 mm in their right and left mandibular teeth, aged between 26 and 56 (mean age: 41.13 ± 8.21), who applied to Izmir Katip Çelebi University Faculty of Dentistry Department of Periodontology between 2019 and 2020. All participants met the study inclusion criteria: The absence of a known systemic disease; not using any medication that may affect the periodontal tissues or prolong the bleeding time; having not previously operated on the palate area, which is both the recipient area and the donor area; the depth of the probing sulcus of all teeth < 3 mm; all mouth plaque index and gingival index scores < 1; the teeth in the operation area are vital; there is no decay and/or restoration on the root surfaces of the teeth in the operation area; and there is a lack of attached gingiva in the opposite jaws. Pregnant or lactating women, smokers, individuals under the age of 18 were not included in the study.

Study design

The study is a split-mouth, double-blind randomized controlled clinical trial comparing d-FGG with conventional FGG for the treatment of attached gingiva. Treatment results and oral hygiene habits were evaluated in the first month following phase 1 treatment in patients who met the selection criteria. Patients who met the selection criteria and had good oral hygiene were included in the study. The time sequence of the study protocol is shown in Fig. 1.

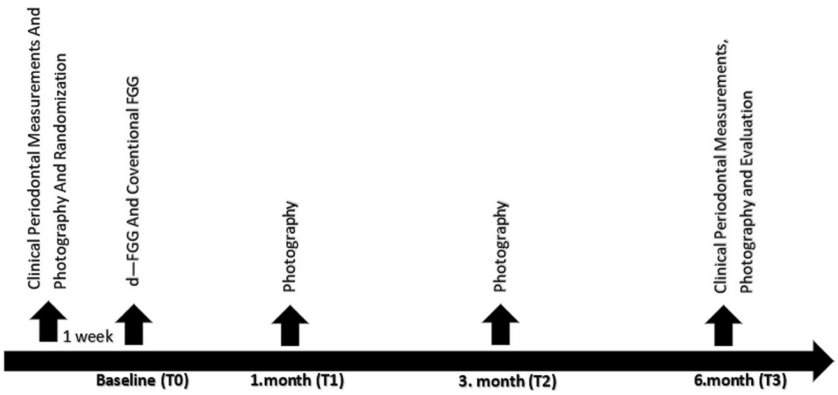

Fig. 1 Timeline of study design

Sample size

Sample size was calculated with an expected parameter estimate based on a previous randomized comparative study [20]. Assuming a mean VAS color score of 6.2 in the control group and a 7.27 VAS color score in the test group with a pooled standard deviation of 1.8, the minimum sample size should be approximately 15 patients, within 95% confidence and 80% strength per group.

Randomization

Patients' areas to be treated were assigned to one of the two treatment groups using a computer-generated randomization table. Allocation concealment was achieved using a sealed coded opaque envelope containing the treatment of the subject. The envelope was opened during the surgery immediately before the graft harvesting as described by Zucchelli et al. [21].

Clinical measurements

A single blind and calibrated examiner (H.O.A), who was masked from the treatment allocation, performed all clinical measurements and taking all pictures. A calibration exercise was carried out to assess the intra-examiner reproducibility.

Probing pocket depths, keratinized gingival width, gingival thickness, and gingival recession depth in the teeth in the operation area were measured before the operation and 6 months after the operation using a periodontal probe (PCP-UNC15; Hu-Friedy, Chicago, IL, USA). All clinical measurements made were recorded in the patient follow-up form specially prepared by the researcher. The following clinical parameters were recorded 1 week before surgery and 6 months after surgery.

PD-Buc: Probing depth at the central buccal site.
KT: Keratinized tissue measured from the most apical point of the gingival margin to the mucogingival junction (MGJ) at the middle buccal point.
RD: Recession depth at the central buccal site measured from cemento-enamel junction (CEJ) to the gingival margin.
GT: Gingival thickness was determined at a three-point 1-mm apical to the gingival margin with a short anesthetic needle that was inserted perpendicular through gingiva until the bone was touched and a silicon stopper that was adjusted flushing with the surface and fixed with cyanoacrylate adhesive, and penetration depth was then measured [22]. The average of these values was recorded.

Standardization of photographs for aesthetic evaluation

As suggested by Smith et al., standard red and white discs with known CIELab color values, which are not affected by saliva on the teeth, were placed on the vestibule faces of the crowns of the relevant teeth in the areas to be operated [23]. The patient's chin was positioned 30 cm from the lens. The lip retractor was placed in the mouth at a pressure that would not interfere with the blood flow of the superficial veins in the gum. The surface of the mucosa was dried and cleaned with an air blow or gauze pad. In order to avoid the light reflex, the photos of the two sides were taken in the same light conditions (at the same time and place) at a 45° angle. The photographs were taken by the same researcher each time at "1.5" magnification with a Canon brand Macro ring lite mr-14 ex macro lens digital camera. The photos were taken at the beginning, 1st month, 3rd month, and 6th month in the same way.

Surgical procedure

All surgeries were performed at the same time by the same periodontist (Y.N). All surgical procedures of the areas of the patients participating in the study with attached gingiva of less than 2 mm were performed as suggested by Sullivan and Atkins [24]. In both FGG methods, the graft thickness was taken as 1.5 mm. In the test group, in addition to these procedures, the epithelial layer of the graft outside the mouth was partially de-epithelialized with a scalpel numbered 15 with 2 vertical and 1 horizontal columns parallel to each other (in the form of the letter T facing each other) (Figs. 2 and 3). In order not to affect the results, care was taken to have the same graft dimensions by measuring with the help of caliper and root canal tool in two graft types applied to the same patient.

Photo color analysis and visual aesthetic evaluation

Color harmony evaluation was made in 2 different ways, both by photo analysis of a another blind researcher (A.G) using computer software and by visual evaluation of the same blind researcher at the postoperative 1st, 3rd, and 6th months.

For color analysis of digital photographs, standard red and white reference discs with known CIELab color values were placed on the vestibule surfaces of the relevant teeth in the operation area to provide a certain parameter. The red and white disks in each photo gave the same pixel value when the photos were checked for calibration and analysis of the image. After that, Adobe Photoshop CS6 (Adobe Systems, San Jose, CA, USA) CIELab color parameters were evaluated with all digital photographs

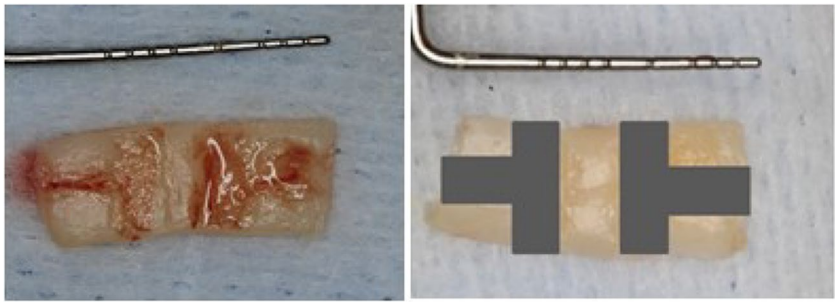

Fig. 2 d-FGG and clearly demarcation of the de-epithelialized portion

computer software. Here, the eyedropper tool with a radius of 50 was used to select color in both operation areas as suggested by Kaki et al. [25] to ensure reliability,colors were selected from different parts of the operation area with the dropper tool three times, and the CIELab values of these colors were averaged (Fig. 4). The CIELab parameters of the initial, 1st, 3rd, and 6th month photographs of the test and control groups were calculated and compared according to the CIELab color parameters on the standard cards, and ΔE values were calculated. Then, the statistical analysis of the obtained data was made in the analyzed time periods and between groups.

The visual color match evaluation criteria were based on the degree of color matching compared to adjacent tissue and the tissue contour of the surgical site. The evaluator scored the photographs twice, at two different times, using the Likert scale criteria given below. The weighted average of the scores was then calculated: Texture color matching analysis: (1) color exactly similar to the photo before surgery, (2) slightly lighter color than the pre-operative photo, (3) much more incompatible light color, (4) extremely bad color mismatch; tissue contour fit analysis: (1) tissue exactly similar to the pre-operative photo, (2) mild tissue irregularities, (3) tissue irregularities that differ significantly from the adjacent tissue in the surgical site, (4) severe tissue irregularities.

Initial and post-op photographs of the test and control groups are shown in Figs. 5 and 6.

Statistical analysis

Statistical analysis was performed using SPSS version 17 for Windows (SPSS Inc. Chicago, USA). The Shapiro–Wilk test was used to examine whether all data were normally distributed. As a result of these statistical tests, it was determined that the data were not distributed normally. Comparison of the changes in *PD*, *KT*, *GT*, and *RD* values at baseline and 6 months after surgery between the time periods and between groups was analyzed using the Wilcoxon signed-rank test. Statistical analyses of different time points in each group for photo analysis parameters and VAS values were performed using the Friedman test. Pairwise comparison of photo analysis parameters and VAS values between groups was analyzed using the Wilcoxon signed-rank test. Results were represented as mean ± standard deviation (SD) and median, and a p value of < 0.05 was considered statistically significant.

Results

The study was completed with a total of 15 patients, 10 females and 5 males (mean age: 41.13 ± 8.21), aged between 26 and 56; no patients were excluded from the study.

Clinical parameters

In the period of time examined (T0–T3), the amount of change of clinical parameters is given in Table 1.

The change amounts of *KT* values between the baseline and post-op 6th month in the control and test groups were 3.28 ± 0.96 mm and 3.64 ± 0.86 mm, respectively, and the difference was statistically significant ($p < 0.05$).

The changes in *PD*, *GT*, and *RD* values between baseline and postoperative 6 months in the control and test groups were 0.66 ± 0.53 mm and 0.64 ± 0.38 mm for *PD* values, 1.7 ± 0.32 mm and 1.68 ± 0.38 mm for *GT*, and 0.57 ± 0.53 mm and 0.53 ± 0.52 mm for *RD* values, respectively. In the comparison between the control and test

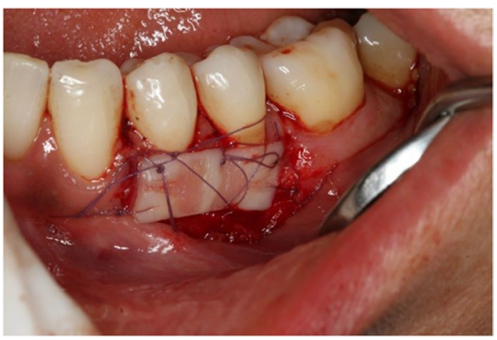

Fig. 3 Placement of the d-FGG in the recipient site. 6.0 vicril suture material was used to standardize healing in all graft operations

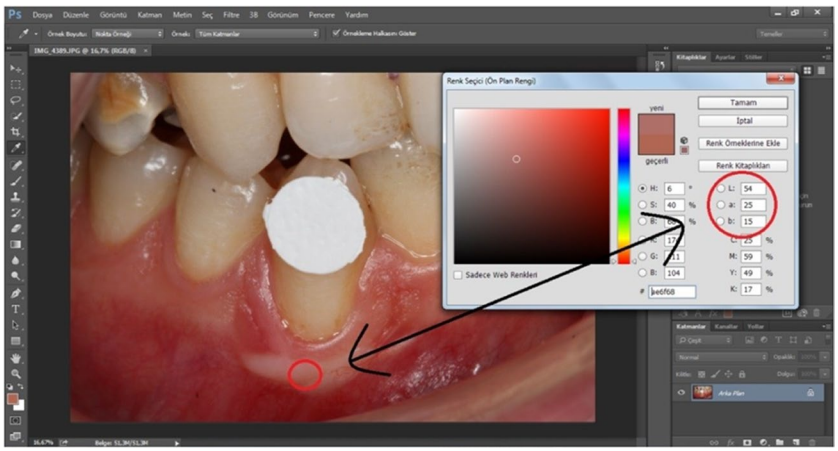

Fig. 4 Calculation of color values of operation regions using Adobe Photoshop CS6 program

groups, there was not a statistically significant difference in the amount of change in *PD*,*GT* and *RD* values ($p > 0.05$).

The clinical data of the examined time periods (T0–T3) are given in Table 2.

In the T3 period, *KT* and *PD* values were found to be significantly higher in the test group ($p < 0.05$).

In control and test groups, the *PD* values between the baseline and post-op 6th month were 2.11 ± 0.5 mm and 1.38 ± 0.4 mm, and 2.13 ± 0.5 mm and 1.49 ± 0.4 mm, respectively, and the difference both intra-group and inter-groups was found statistically significant ($p < 0.05$). In control and test groups, the *KT* values between the baseline and post-op 6th month were 0.78 ± 0.57 mm and 4.1 ± 1.11 mm, and 0.78 ± 0.54 mm and 4.42 ± 1.1 mm, respectively, and the difference both intra group and inter groups was found statistically significant ($p < 0.05$).

Photo analysis and visual aesthetic evaluation

The data of *LAB* values are given in Table 4.

When the data are analyzed in terms of *L* value, The *L* value in T1 and T2 was higher in the test group than the control group, and a statistically significant difference was found between these two groups when the test and control groups were compared in T1 and T2 ($p < 0.05$). In T0 and T3, no statistically significant difference was found between the control and test groups ($p > 0.05$).

When the data is analyzed in terms of *A* and *B* values, at T2, the *A* value was higher in the test group compared to the control group, while the *B* value was higher in the control group compared to the test group. A significant difference was found in both the *B* and *A* values in the test and control groups at T2 ($p < 0.05$), but no significant difference was found in other time intervals (T0, T1, T3) ($p > 0.05$).

ΔE (difference between any two CIElab values) data and visual evaluation values are given in Table 3.

According to this, the ΔE value at T2 was higher in the test group than in the control group. A statistically significant difference was found between the test and control groups ($p < 0.05$). There was no statistically significant difference between the control and test groups in T1 and T3 ($p > 0.05$). There was no statistically significant difference between the test and control groups in the time periods observed in both VAS color and VAS tissue values ($p > 0.05$).

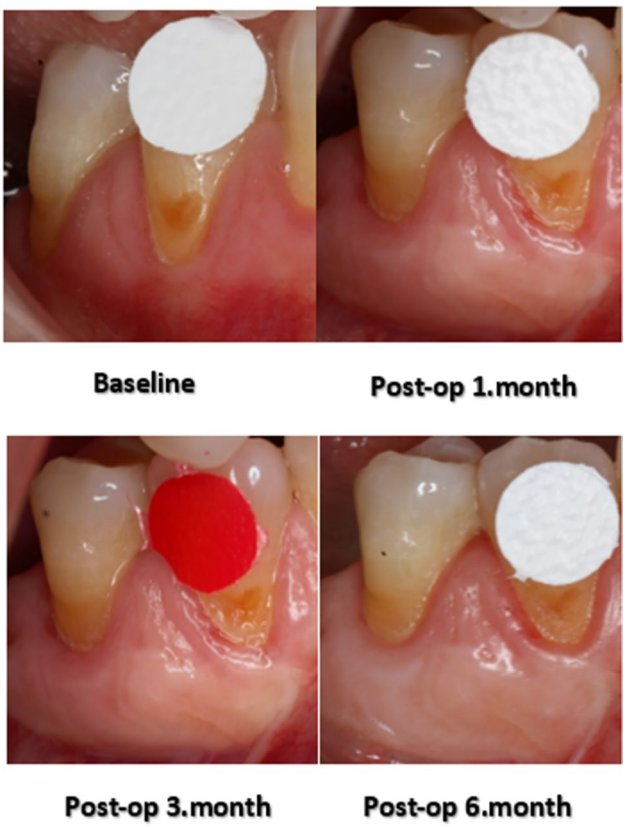

Fig. 5 Initial, post-op 1st, 3rd, and 6th month photos of the test group

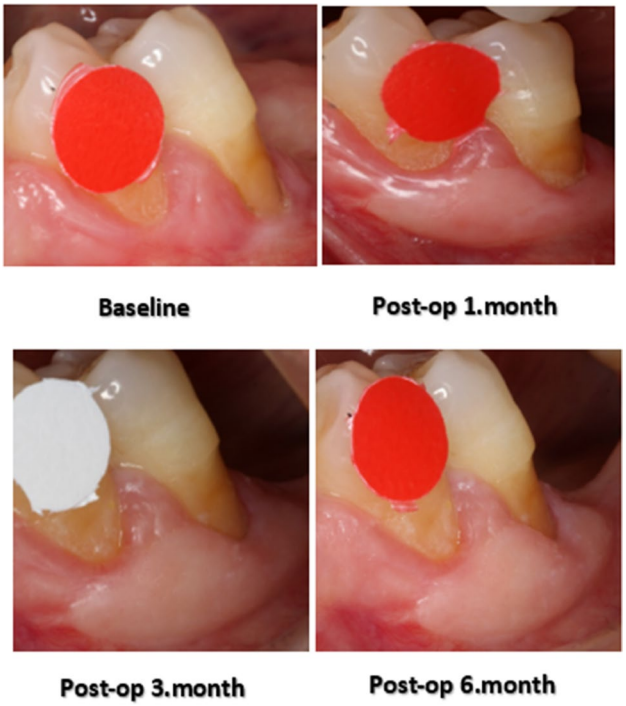

Fig. 6 Initial, post-op 1st, 3rd, and 6th month photos of the control group

Discussion

Many studies have concluded that modification applications with FGG, connective tissue graft, and d-FGG should be used to increase the amount of keratinized gingiva and vestibular sulcus depth and to eliminate the gingival-mucosal incompatibility [9, 21, 26, 27]. When the results obtained from our study were analyzed, a statistically significant increase was found in both groups in the 6th month compared to the baseline ($p < 0.05$). However, a statistically significant higher increase was found in the *KT* value in the test group ($p < 0.05$). According to the results of our study, it can be interpreted that this difference occurred because the graft in the control group contracted more. Grafts placed on the bone surface and grafts placed on the periosteum can contract at different rates [28, 29]. Since the graft is fed through the plasmatic circulation during the early healing period, it was thought that the graft size and shrinkage may change. While it causes excessive shrinkage in thin grafts, the fact that the graft is too thick makes adaptation difficult in the recipient bed and interrupts the blood supply and vascularization process. The researchers, who stated that the ideal thickness for this was 1–1.5 mm, stated that the thicker or thinner grafts had more shrinkage [30]. In our study, graft thicknesses were taken as 1.5 mm in both FGG methods. However, de-epithelialization was performed from a certain region of the graft (t-shaped) in the test group. The thickness of the regions where de-epithelialization was applied was measured with a caliper. The thickness difference of the deepitelized regions (t-shaped) in the test and control groups was 300–400 Å [31]. In addition, since we did not include a certain gingival recession defect in the study, FGGs were placed on the bone surface in some patients and on the periosteum in some patients due to the presence of malposition. For these reasons, there may be a significant difference in the *KT* value in the test and control groups.

Having a sufficiently thick keratinized tissue is very important for both natural teeth and dental implants. One of the main indications for FGG is to reconstruct adequate keratinized tissue width and gingival thickness in the presence of mucogingival defects [32]. It is known that partially epithelialized FGG, introduced by Cortellini et al., increases gingival thickness [9]. This research showed that both FGG methods significantly increased *GT* over the examined time periods, which is in line with previous studies.

One of the results of the researches is that the width of the keratinized tissue increases after both FGG and connective tissue graft procedures, while the probing pocket depths are not more than 2 mm [33]. In our study, *PD* amounts decreased after the operations in both FGG techniques in accordance with previous studies and *PD* values were not obtained more than 2 mm in the 6th month. The amount of change in the *PD* value between the T0–T3 periods was not found to be statistically significant in the comparison between the groups ($p > 0.05$).

According to Cairo et al., the best root closure results in RD1 gingival recessions without interproximal attachment loss [34]. In our study, since root surface coverage was not targeted in two different FGG techniques, all gingival recession defect types (RD1, RD2, RD2) were included in

Table 1 The amount of change of clinical parameters data between the beginning and the 6th month

Measurement	Groups	*N*	T0–T3 change amounts		
			Mean ± SS	Median	Max–min
PD (mm)	Control	15	0.66 ± 0.53	0.48	1.99–0
	Test	15	0.64 ± 0.38	0.5	1.33–0
	P value	15	> 0.05		
KT (mm)	Control	15	3.28 ± 0.96	3.25	5–1.5
	Test	15	3.64 ± 0.86	3.75	5–2
	P value	15	**< 0.05**		
RD (mm)	Control	15	0.57 ± 0.53	0.5	2–0
	Test	15	0.53 ± 0.52	0.5	1.5–0
	P value	15	> 0.05		
GT (mm)	Control	15	1.7 ± 0.32	1.42	2–1.2
	Test	15	1.68 ± 0.38	1.48	2.2–1.42
	P value	15	> 0.05		

Bold entries, indicate statistically significant difference

Table 2 Data of periodontal clinical parameters of operation areas

Measurement	Groups	*N*	Baseline (T0)			Post-op 6th month (T3)		
			Mean ± SS	Median	Max–min	Mean ± SS	Median	Max–min
PD	Control	15	2.11 ± 0.5	2.14	3–1.3	1.38 ± 0.4[a]	1.33	2.27–0.33
	Test	15	2.13 ± 0.5	2.12	3.3–1.3	1.49 ± 0.4[a]	1.49	2.16–0.66
	P	**< 0.05**				**< 0.05**		
KT	Control	15	0.78 ± 0.57	0.5	2.5–0	4.1 ± 1.11[a]	4	6–2.5
	Test	15	078 ± 0.54	1	2–0	4.42 ± 1.1[a]	4	7–3
	P	**< 0.05**				**< 0.05**		
RD	Control	15	2.6 ± 1.2	2.5	5–0	2.03 ± 0.94	2	3.5–0
	Test	15	2.67 ± 1.5	3	6–0	2.14 ± 1.14	2.5	5.5–0
	P	> 0.05				> 0.05		
GT	Control	15	1 ± 0.52	1	1.5–0.5	2.68 ± 0.4[a]	2.8	3.2–2
	Test	15	1 ± 0.5	1	1.5–0.5	2.62 ± 0.6[a]	2.6	3.5–1.8
	P	> 0.05				> 0.05		

Bold entries, indicate statistically significant difference.

[a]Statistically different from T0 (comparison between the time periods examined)

the study according to the current classification of Cairo. It was first shown by Sullivan and Atkins that FGG can heal when positioned on the bare root surface and even in some cases can provide root surface closure [24]. Contrary to this view, FGG is basically a periodontal plastic surgery technique applied to increase the width of the attached gingiva, according to Rateitschak et al. When it is applied to cover open root surfaces, as a part of the graft remains on the avascular root surface, achieving complete closure with this technique is difficult to predict the results and the chance of success is low [35]. In accordance with these studies, the depth of withdrawal decreased after the FGG procedures in both groups. However, no statistically significant difference was found in the data analyzed both within and between groups ($p < 0.05$). The movement of the gum edge towards the coronal during the healing period after graft operations is called "creeping attachment" and was first described by Goldman et al. Creeping attachment can continue from the 1st month to the 1st year, and it has been reported that the amount of creeping attachment obtained can be maintained up to the 5th year [30, 36]. This insignificant increase in both groups can be explained by the formation of creeping attachments.

According to studies examining gingival color, it was concluded that brightness (*L*) is the parameter that causes higher unacceptable color changes compared to *A* and *B* values (Table 4) [37]. While the *L* parameter indicates the whiteness of the gingiva, the *A* parameter refers to the rash. Most of the studies have associated the *L* parameter with the degree of keratinization and the *A* value with the degree of vascularization [23]. In our study, the main parameters evaluated among the CIELab parameters were *L* and *A*

Table 3 Data for Δ*E* value in photo analysis and visual evaluation

		Vas color			Vas tissue			Δ*E*		
Groups		Control	Test	*P*	Control	Test	*P*	Control	Test	*P*
1st month (T1) (Δ*E*1)	Mean ± SD	1.57 ± 0.64	1.35 ± 0.63		1.85 ± 0.77	1.85 ± 0.66		9.6 ± 4.7	9.1 ± 7.4[b]	
	Median	1.5	1	> 0.05	2	2	> 0.05	8.6	7.1	> 0.05
	Max–min	3–1	3–1		3–1	3–1		19.4–3.1	29.7–1.1	
3th month (T2) (Δ*E*2)	Mean ± SD	2 ± 0.85	2.16 ± 0.71[a]		2.08 ± 0.9	2.41 ± 0.79		10 ± 4.9	13 ± 6.7	
	Median	2	2	> 0.05	2	3	> 0.05	9	15.6	**< 0.05**
	Max–min	3–1	3–1		3–1	3–1		19.1–3.9	24.6–4.3	
6th month (T3) (Δ*E*3)	Mean ± SD	2.09 ± 0.83	2.5 ± 0.79[a]		2 ± 0.77	2.36 ± 0.5		10.4 ± 5.4	10.6 ± 5.2	
	Median	2.09	2.5	> 0.05	2	2	> 0.05	9.3	9.7	> 0.05
	Max–min	3–1	4–1		3–1	3–2		21.7–3.3	21.6–4.7	

"Bold" entries, indicate statistically significant difference

[a]It is statistically significantly different from T1 (comparison between time periods examined)

[b]It is statistically significantly different from T2 (comparison between time periods examined)

Table 4 Data of *LAB* values in photo analysis

		L			A			B		
		Control	Test	*P*	Control	Test	*P*	Control	Test	*P*
T0	Mean ± SD	51.88 ± 6.89[b]	51.43 ± 3.45	> 0.05	31.62 ± 5.12[b]	31.17 ± 4.84	> 0.05	20.77 ± 3.35	20.18 ± 3.13	> 0.05
	median	52.2	52.2		31.8	32.5		21.7	20.4	
	Max–min	63.8–39.3	56–44.3		41.9–23.2	36.8–17.6		25.7–14.4	26.1–14.5	
T1	Mean ± SD	54.2 ± 4.81[b]	57.04 ± 5.5[a]	**< 0.05**	29.08 ± 3.35[a b]	27.07 ± 4.75[a]	> 0.05	19.76 ± 3.05	18.81 ± 2.88	> 0.05
	Median	55.6	56.1		29.1	27.5		19	18.1	
	Max–min	61.3–45.1	74–51.6		33.8–22.1	36–18.9		28.8–15.9	24.2–15.1	
T2	Mean ± SD	53.31 ± 6.54[b]	58.15 ± 5.82[a]	**< 0.05**	28.01 ± 3.56 [a b]	24.74 ± 4.52[a]	**< 0.05**	20.88 ± 5.45[b]	18.22 ± 3.22[a]	< 0.05
	Median	55.6	59.5		27.3	27.3		18.7	17.9	
	Max–min	62.5–42.8	71–49.1		33.5–23	32–16.1		35.4–14.1	23.2–12.2	
T3	Mean ± SD	57.56 ± 3.03	57.95 ± 4.48[a]	> 0.05	25.37 ± 3.7 [a]	25.84 ± 3.91[a]	> 0.05	18.68 ± 2.38 [a]	17.7 ± 3.39 [a]	> 0.05
	Median	58.1	57.7		25.1	26.7		18	17.4	
	Max–min	62.8–51.9	67.7–51.1		32.7–18	31.3–17.5		24.6–15.9	23.4–10.9	

Bold entries, indicate statistically significant difference

[a]It is statistically significantly different from T0

[b]It is statistically significantly different from T3 (comparison between time periods examined)

parameters. Accordingly, in the test group, the *L* value at T2 was the highest and the *A* value was the lowest, and the Δ*E* value was found to be statistically significant only at T2 between the test and control groups. In other words, it can be concluded that the d-FGG appeared much whiter than conventional FGG at the 3rd month. However, in the 6th month, the values of the CIELab parameters of the control and test groups were found to be close to each other again.

We think that the reason for this color difference in the 3rd month in our study is due to the change of d-FGG in the healing process. When we consider the *L* and *A* parameters, we have two views regarding the occurrence of this difference in the healing process of d-FGG. Our first opinion is that, in the test group, the connective tissue of the graft may have been damaged during the de-epithelization procedure applied to the graft. Therefore, during the differentiation period of the graft applied to the recipient bed, hyperkeratinization may have occurred in the epithelial layer in the 1st and 3rd months. Due to the thickening of the keratin layer in hyperkeratinization, the distance to the vascular area may be increased. For this reason, the tissue may have appeared whiter in the 1st and 3rd months. Since the differentiation process of the de-epithelialized graft ends in the 6th month, the epithelial layer of the graft may be completely organized and the hyperkeratotic appearance may disappear. Our second opinion is that the epithelial layer above the FGG becomes necrotic during the healing process, then a new epithelial layer is formed with the genetic information of the connective tissue [38–41]. Since the epithelial amount of FGG we de-epithelized is less than conventional FGG, we think that the amount of epithelium that will be necrotic during the healing process of the graft has decreased. Thus, d-FGG may have entered the differentiation process earlier than conventional FGG, so d-FGG may have displayed a whiter appearance at the 3rd month.

In our study, VAS color values were compatible with the Δ*E* value. However, unlike the Δ*E* value, there was no statistically significant difference between the test and control groups at 3 months ($p > 0.05$). Johnston and Kao reported a clinically acceptable threshold of Δ*E* 3.7 for visual perception for color determination of prosthetic restorations in the CIELab system [42]. In fact, other studies have found this *E* threshold value to be higher at "4.6" and "6.8" [25, 43]. In our study, the reason for the lack of statistically significant difference in VAS color value in the 3rd month may be that the Δ*E* parameter in the follow-up periods was lower than the above values. Thus, we can say that the Δ*E* value we found is not perceptible. In a study conducted by Bayındır et al. to determine the most effective gingival color scale, they measured gingival color spectrometry on 122 patients in a specific population and calculated a threshold Δ*E* value. According to this study, the Δ*E* threshold value for the gingiva was determined at "2.75" [43]. The results we obtained were consistent with the Δ*E* threshold value of' "2.75" suggested by Bayındır and his friends. Another reason for this difference may be that the VAS color value is calculated according to the adjacent tissues, and the Δ*E* value is calculated according to the color of the initial tissue.

Our study has some limitations. One of these limitations is that the number of participants is 15. Although photo analysis is a sensitive method, the small sample size may have affected our results. Therefore, we think that the more accurate results can be obtained with the increase in the number of samples. Another limitation is that instead of

including a specific gingival recession defect in our study, all gingival recession defects classified by Miller and Cairo were included in our study. The application of the grafts to different gingival recession defects may disrupt the standardization and cause differences in the healing of the graft. Therefore, it may have affected the results of our study. In our study, photos were tried to be taken with a standard method as much as possible. However, the lighting conditions that we cannot prevent can be considered as another limitation that affects our primary results.

Conclusions

Within all limitations, d-FGG is a preferred method in mucogingival surgeries, as it has a high clinical success on the periodontal parameters examined and exhibits an aesthetically acceptable color match. It has been understood that photographic analysis detects the slightest changes and gives more detailed and superior results than visual evaluation.

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Author contribution Yonca Naziker contributed to graft operations, data interpretation, supervision, and article writing. Abdullah Seçkin Ertuğrul contributed to the research design, statistical analysis, data interpretation, and article writing.

Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Research involving human participants The protocol of the study was approved by the Izmir Katip Çelebi University, Faculty of Medicine, Clinical Research Local Ethics Committee, at the meeting dated April 25, 2019, with the protocol numbered 44. (U.S. National Institutes of Health Clinical Trials: NCT04970524).

Informed consent After giving verbal and written information about the purpose and method of the study to all patients, written informed consent was obtained for participation.

Conflict of interest The authors declare no competing interests.

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