

Research Article

## COMPARISON OF MICROSURGICAL AND CONVENTIONAL TECHNIQUES FOR AUGMENTATION OF KERATINIZED MUCOSA AROUND DENTAL IMPLANTS

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### ABSTRACT

**Objective:** This study aims to compare the clinical outcomes of periodontal microsurgery with conventional periodontal surgery in keratinized mucosa augmentation around the implant.

**Materials and Methods:** Ten patients with at least 2 implants with a keratinized mucosal width of < 2 mm were included. Free gingival graft (FGG) was applied in the test group with the microsurgery technique whereas in the control group with the conventional periodontal surgery technique. Keratinized mucosa width (KMW) and palatal mucosal thickness (MT) were measured at baseline and the 3<sup>rd</sup> month. Graft shrinkage (GS) was evaluated at the 3<sup>rd</sup> month. Donor site wound healing was evaluated at the 10<sup>th</sup> day and 3<sup>rd</sup> month. Feedback forms were collected on the 10<sup>th</sup> day.

**Results:** KMW values increased significantly in both groups at the 3<sup>rd</sup> month compared to baseline. The percentage of GS was lower in the test group at the 3<sup>rd</sup> month, but this difference was not significant. MT values in the test group were closer to the baseline values at the 3<sup>rd</sup> month compared to the control group. Wound healing scores were similar in both groups at the 10<sup>th</sup> day and 3<sup>rd</sup> month. Postoperative pain levels decreased more rapidly in the test group.

**Conclusion:** The microsurgical technique provides a faster increase in palatal MT and lower GS during the healing period and an earlier reduction in postoperative pain levels.

**Keywords:** Dental implant, free gingival graft, graft shrinkage, keratinized mucosa width, microsurgery.

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## INTRODUCTION

The keratinized mucosa is an important criterion to be evaluated for the health of soft tissues for dental implant. Insufficiency of keratinized mucosa around dental implants is associated with plaque accumulation around the implant, soft tissue inflammation, mucosal recession, attachment and bone loss. Keratinized mucosa is essential for the long-term health of peri-implant tissue and provides significant advantages in terms of patient comfort and plaque control (1).

There are various methods such as using free gingival graft (FGG), connective tissue graft, apically positioned flap, enamel matrix derivatives and acellular dermal matrix to increase the width of the keratinized mucosa (2). FGG is the gold standard in keratinized mucosa augmentation due to its simplicity of application technique and successful and predictable results, but one of its most important disadvantages is graft shrinkage (GS) (3).

Microsurgery refers to surgical procedures using smaller instruments and suture materials through magnification systems. With the developments in the fields of medicine and dentistry, treatment with microsurgery has started to be used more and more. Studies have shown that with the use of microsurgery in periodontology, diagnosis and treatment efficiency is increased, primary wound closure is provided and superior clinical results are obtained (4,5).

The results of periodontal microsurgery in the formation of peri-implant keratinized mucosa have been demonstrated in a limited number of clinical studies (6). The effect of periodontal microsurgery on GS and the level of pain reported by patients in keratinized mucosa augmentation with a peri-implant FGG has not been proven. In addition, there is no study in the literature comparing periodontal microsurgery with conventional periodontal surgery in split mouth design in peri-implant keratinized mucosa augmentation with FGG. Therefore, this study aims to compare the clinical results of FGG operation performed with periodontal microsurgery and conventional periodontal surgery methods around the implant and to compare the postoperative pain. The hypothesis of our study was that "Periodontal microsurgery method in soft tissue augmentation with FGG accelerates wound healing, reduces GS and postoperative pain compared to conventional periodontal surgery method."

## MATERIALS AND METHODS

### *Study population*

A total of 10 patients (7 females, 3 males, aged 41-53 years, mean $\pm$ Sd = 47,5 $\pm$ 3,69) who applied to Kirikkale University Faculty of Dentistry for the control of their dental implants were included. 20 defects were randomly divided into periodontal microsurgery (test) and conventional periodontal surgery (control) groups. Whole mouth plaque index and whole mouth gingival index <15% and no other signs of inflammation, individuals with at least 2 different peri-implant sites with keratinized mucosa <2 mm in the buccal region, implants consisting of fixed prosthetic restoration that have been in function for at least 6 months, individuals who have not undergone any surgical intervention in the relevant region and soft tissue thickness of at least 3 mm in the palate and no torus were determined as inclusion criteria. Exclusion criteria were as follows: (1)

individuals with systemic disease that were either contraindications to periodontal surgeries or could interfere with the wound healing process, such as diabetes, as well as those with a history of high-dose steroid treatment, anti-coagulation medication use, radiotherapy, or other conditions (2) smokers (3) pregnant or lactating individuals (4) use of any drug that may have an effect on periodontal tissues. The study was conducted in accordance with the ethical rules of the Declaration of Helsinki and it was approved by Kirikkale University Clinical Research Ethics Committee (No: 16/08 Date: 01.08.2019). Written and verbal consent was obtained from all participants before starting the study.

### *Preoperative procedures*

A plaster model was obtained by taking a silicone impression (Optosil, Bayer Dental, Germany) from the patients and the stent was prepared. T-shaped grooves were prepared on the stent at the mesio-distal midpoint of the relevant implant site and measurements were made from a fixed reference point at the beginning and control sessions. In the evaluation of the recipient site, probing depth (PD), clinical attachment level (CAL), modified plaque index (mPI) (7), modified gingival index (mGI) (8), modified sulcus bleeding index (mSBI) (7) and keratinized mucosa width (KMW) parameters were used. A standardized, color-coded, pressure-calibrated (20-25 g) plastic periodontal probe (Click-Probe ® blue, Kerr GmbH, Biberach, Germany) was used during the measurements.

In order to determine the width of the keratinized mucosa, the operation area was stained with Lugol's solution (Aromel Kimya Medikal, Konya, Turkey) containing iodine and potassium iodide and the alveolar mucosa was determined. The distance between the standard placed probe on the prepared stent and the mucosal edge and mucogingival junction was recorded. The distance was measured with a digital precision caliper (Leo Digital Caliper Asya Ticaret/Istanbul, Turkey).

Mucosal thickness (MT) and wound healing (WH) were evaluated in the evaluation of the donor site. MT was measured by transgingival probing. After local anesthesia was given to the area where the FGG was to be taken, the size 25 endodontic reamer was placed vertically from 5 mm apical to the midpalatal line of the first premolar tooth. When the hard tissue contact was taken, the stopper placed on the reamer was fixed by contacting the tissue. In this way, the value between the outer surface of the bone and the mucosa was determined as the MT and was measured and recorded with the help of a digital precision caliper (Leo Digital Caliper Asya Ticaret/Istanbul). Wound healing at the donor site was evaluated according to "Landry's Wound Healing Index" (9).

### *Surgical procedure*

In the test group, all the procedures were completed under  $\times 3.0$  magnification (Keeler 3.0X Galilean Standard Loupe) with the equipment designed for microsurgery including blades (HUF.MIM64, Hu-Friedy, Chicago, IL, USA), needle holder, scissors, and tissue forceps (Hu-Friedy, Chicago, IL, USA). In the conventional periodontal surgery group, the #15 and 15C blades (Hu-Friedy, Chicago, IL, USA.) and the standart equipments (Hu-Friedy, Chicago, IL, USA) were used without any magnification.

After local anesthesia, a horizontal incision was made with a scalpel at the mucogingival junction line. Oblique incisions were made at the mesial and distal corners of the end of the incision line to obtain the graft

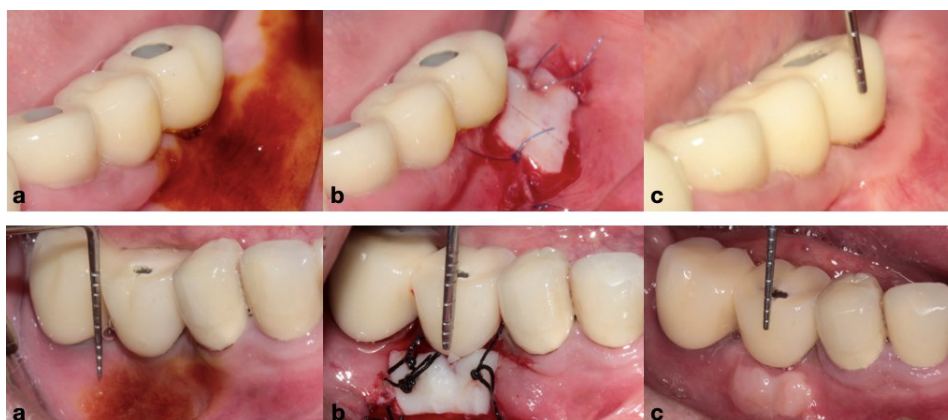
bed. Soft tissue and muscle attachments at the apical part of the recipient bed were removed with a half-thick blade. After the preparation of the recipient bed, the dimensions of the recipient bed were measured in the mesiodistal and apicocoronal directions to determine the amount of graft needed. A graft with a thickness of approximately 1-1.5 mm was obtained using a blade from the palate area between the canine and the first molar. The muscle/soft tissue attachments that caused its movement were eliminated by adapting the prepared graft to the recipient area. Then, the graft was adapted to the area with sutures so that it remained immobile. 6.0 polypropylene sutures (Ethicon, Johnson and Johnson Intl, St. Stevens, Woluwe, Belgium) were used in the periodontal microsurgery group, while 4.0 silk sutures (Ethicon, Johnson and Johnson Intl, St. Stevens, Woluwe, Belgium) were used in the conventional periodontal surgery group. After the procedure, periodontal paste was not placed on the recipient area. Compress was applied to the donor area with a tampon moistened with physiological saline for 10 minutes.

#### *Postoperative care*

The patients were given postoperative mouthwash (containing 0.12% chlorhexidine gluconate and 0.15% benzidamine hydrochloride, twice a day for 21 days). They were told that they could take pain relievers containing 500 mg of paracetamol if they felt pain, and they were told to indicate the drug they took in the feedback form and they were advised to consume a soft diet. The sutures were removed on the 10<sup>th</sup> day after the operation.

#### *Clinical follow-up measurements*

PD, CAL, mPI, mGI, mSBI and KMW were measured at baseline and at the 3<sup>rd</sup> month (Figure 1). GB was calculated at the 3<sup>rd</sup> month after the operation. MT was evaluated at baseline and at the 3<sup>rd</sup> month. WH was evaluated on the 10<sup>th</sup> day and 3<sup>rd</sup> month after the operation. Visual analog scale (VAS) was used for postoperative pain. Participants were asked to mark their pain level on a 10 cm horizontal scale with "never" on the left and "highest" on the right at the same time each evening for 10 days.



**Figure 1.** Presentation of a patient. (a) Immediately preoperative. Periodontal microsurgery (test) site (up) and conventional periodontal surgery (control) site (down). (b) Suturing the free gingival graft to the recipient area. (c) Peri-implant area at the 3<sup>rd</sup> postoperative month.

### *Determination of the graft shrinkage ratio*

The shrinkage rate of the grafts was determined with standard photographs. Measurements were repeated 3 times and the average was taken. A calibrated William's periodontal probe (Hu-Friedy, Chicago, IL.) was used for standardization. To assess GS, images were imported into a Java-based analysis program (ImageJ, National Institutes of Health, Bethesda, MD) using the same magnification size. The length of the probe in the photograph was calibrated to its actual length, and the number of pixels in the marked area was converted to square millimeters by the program to calculate the graft area (Figure 2). GS rate was calculated as the difference between the graft area at baseline and at the 3<sup>rd</sup> month post-operatively. The following formula was used for the GS rate:

$$\text{Graft shrinkage} = \frac{\text{Preoperative area} - \text{Postoperative area}}{\text{Preoperative area}} \times 100$$



**Figure 2.** Measurement of graft shrinkage

### *Statistical analysis*

Statistical evaluation of the obtained data, it was made in SPSS for Windows 11.5 package program. For intergroup comparisons, T-test and Mann-Whitney U test were used for independent samples for normally and non-normally distributed variables, respectively. In comparison of time-dependent changes in groups; Paired t-test and Wilcoxon test for normally and non-normally distributed variables were used, respectively. Intergroup comparisons of time-dependent variances were performed using repeated measures of two-way analysis of variance. Spearman-rank correlation analysis was used for relationships between variables. All tests were performed at the  $\alpha = 0.05$  significance level.

## **RESULTS**

PD, CAL, mPI, mGI, mSBI values were not significantly different between the groups at baseline and at the 3<sup>rd</sup> month after the operation ( $p > .05$ ). A statistically significant decrease was observed in PD, CAL, mPI, mGI and mSBI values in both groups at the 3<sup>rd</sup> month after the operation compared to the baseline values ( $p < .05$ ). Time-dependent changes did not differ significantly between the two groups ( $p > .05$ ) (Table 1). There was no significant difference between the groups at the beginning and at the 3<sup>rd</sup> month after the operation for

KMW values ( $p > .05$ ). A statistically significant increase was observed in KMW values in both groups at the 3<sup>rd</sup> month after the operation compared to the baseline ( $p < .05$ ). Time-dependent changes did not differ significantly between the two groups ( $p > .05$ ; Table 1).

**Table 1.** Clinical parameters at baseline and postoperative 3<sup>rd</sup> month in groups

Clinical parameters (Mean±SD)	Group	Baseline	3 <sup>rd</sup> month	P**	P***
PD (mm)	Test	1.89±0.51	1.63±0.37	0.011	0.686
	Control	1.80±0.60	1.50±0.47	0.001	
	P*	0.719	0.491		
CAL (mm)	Test	1.17±0.61	0.94±0.47	0.008	0.366
	Control	1.17±0.46	0.86±0.36	0.001	
	P*	0.994	0.646		
mPI	Test	1.50±0.49	0.63±0.41	0.001	1.000
	Control	1.48±0.48	0.60±0.39	0.001	
	P*	0.909	0.891		
mGI	Test	1.65±0.29	0.18±0.39	0.001	0.364
	Control	1.63±0.43	0.40±0.54	0.001	
	P*	0.881	0.302		
mSBI	Test	1.63±0.38	0.08±0.24	0.001	0.306
	Control	1.33±0.67	0.03±0.08	0.001	
	P*	0.232	0.535		
KMW (mm)	Test	0.44±0.50	5.54±1.64	0.001	0.175
	Control	0.61±0.47	4.80±1.84	0.001	
	P*	0.434	0.359		

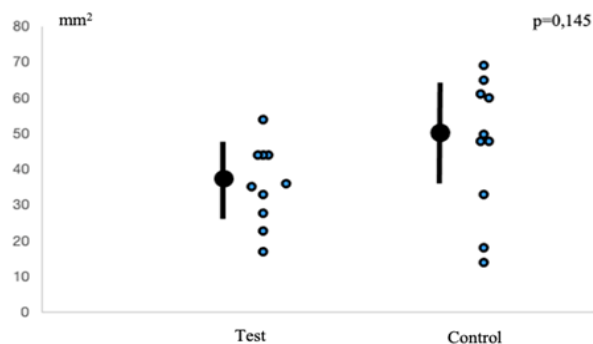
\*: Intergroup comparison results, \*\*: Intragroup comparison results, \*\*\*: Comparison results regarding the time-dependent changes of the groups. PD= probing depth; CAL= clinical attachment level; mPI= modified plaque index; mGI= modified gingival index; mSBI= modified sulcus bleeding index.

GS percentage was similar in both groups at the 3<sup>rd</sup> month postoperatively ( $p > .05$ ). GS in the test group was less than in the control group, but this difference was not statistically significant ( $p > .05$ ) (Table 2, Figure 3).

**Table 2.** Graft shrinkage values in the 3<sup>rd</sup> month after the operation in the groups

	Test Group	Control Group	p
GS (% mm <sup>2</sup> ) (Mean±SD)	35.80±11.15	46.60±19.17	0.145

GS= graft shrinkage



**Figure 3.** Graft shrinkage values of the groups at the 3<sup>rd</sup> month after the operation

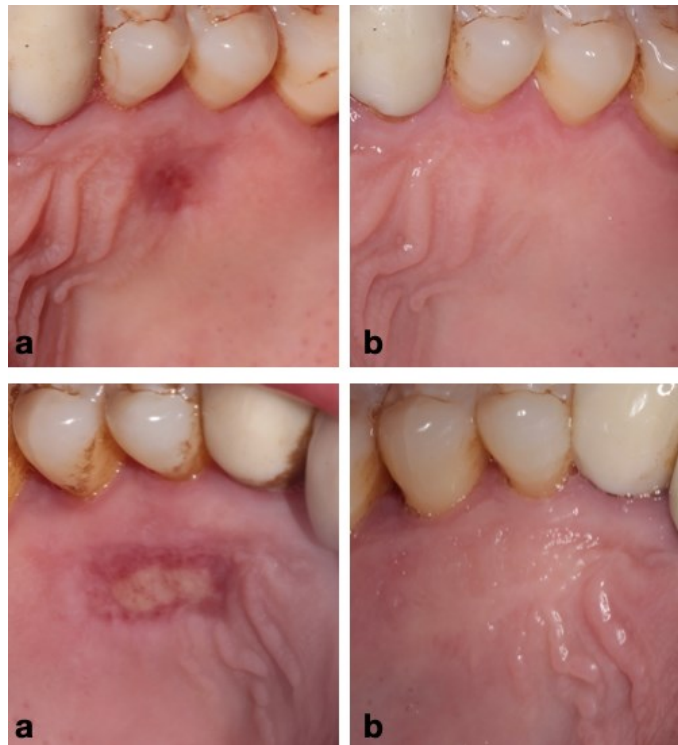
There was no significant difference between the groups in terms of MT at the beginning and at the 3<sup>rd</sup> month after the operation ( $p > .05$ ). In the test group and control group, the 3<sup>rd</sup> month postoperative MT was statistically significantly less than the initial MT ( $p = .02$ ,  $p = .001$ ). However, the decrease was greater in the control group at the 3<sup>rd</sup> month after the operation compared to the baseline ( $p = .001$ ). The time-dependent variation of MT values in the groups was statistically significant ( $p = .035$ ; Table 3).

**Table 3.** Donor area mucosal thickness values at the beginning and at the 3<sup>rd</sup> month after the operation and the wound healing values at the 10<sup>th</sup> day and 3<sup>rd</sup> month after the operation in the groups

	Group	Baseline	3 <sup>rd</sup> month	P**	P***
<b>MT (mm)</b> <b>(Mean±SD)</b>	Test	4.85±0.78	4.67±0.64	0.020	0.035
	Control	4.79±0.72	4.38±0.71	0.001	
	P*	0.858	0.348		
	Group	10 <sup>th</sup> day	3 <sup>rd</sup> month	P**	P***
<b>Wound Healing</b> <b>(Mean±SD)</b>	Test	3.30±0.67	5.00±0.00	0.001	0.140
	Control	2.50±1.08	4.90±0.32	0.001	
	P*	0.062	0.331		

\*: Intergroup comparison results, \*\*: Intragroup comparison results, \*\*\*: Comparison results regarding the time-dependent changes of the groups, MT= mucosal thickness

In terms of WH, there was no significant difference between the groups at the 10<sup>th</sup> day and 3<sup>rd</sup> month after the operation ( $p > .05$ ). In both groups, the scores at the 3<sup>rd</sup> month after the operation were statistically significantly higher than the scores at the 10<sup>th</sup> day ( $p = .001$ ). Time-dependent changes did not differ significantly between the groups ( $p > .05$ ; Table 3, Figure 4).



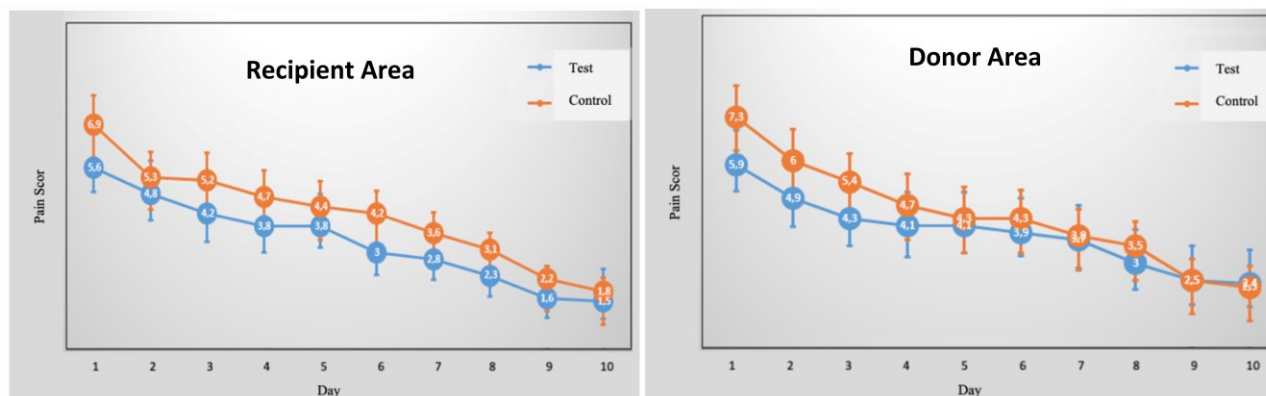
**Figure 4.** Wound healing in periodontal microsurgery (test) group (up) and conventional periodontal surgery (control) group (down). (a) 10<sup>th</sup> day. (b) 3<sup>rd</sup> month.

Pain levels and the amount of analgesics taken in the recipient and donor regions by days did not differ significantly between the groups ( $p > .05$ ). As a result of the comparison of the 1<sup>st</sup> day and the other days in the recipient and donor regions, there was a significant decrease in all days in both groups ( $p < .05$ ). When the transitions on consecutive days were examined, the reductions in pain levels were earlier in the test group in the donor and recipient regions ( $p < .05$ ; Figure 5).

## DISCUSSION

This study evaluated the effect of periodontal microsurgery and conventional periodontal surgical procedures on augmentation of the peri-implant keratinized mucosa. This is the first study to compare the effect of these surgical techniques on peri-implant FG and KMW augmentation over 3 months, attributed both clinical and patient-based parameters.





**Figure 5.** Pain levels related to days in groups for donor and recipient site

The soft tissues around the implant are less resistant to inflammatory conditions compared to the gingiva and the barrier they form to prevent the spread of inflammation to the deep tissues is weaker. In the presence of an ideally wide band of keratinized mucosa around the implant, peri-implant soft tissues form a functional barrier that is more resistant to external mechanical and chemical effects (10). It has been argued in many studies that adequate width of keratinized mucosa is necessary (11,12).

FGG is the gold standard treatment method to increase the width of keratinized mucosa around both teeth and dental implants, however, one of the biggest disadvantages of this procedure is GS. In order to reduce GS, ideal graft thickness, use of atraumatic surgical technique, rapid stabilization of the graft and protection of graft vessels from damage and dehydration are important (3). Factors affecting the stabilization of the graft affect avascular plasmatic circulation and GS (13).

In our study, all clinical parameters were similar in both groups at baseline and at the postoperative 3<sup>rd</sup> month. PD, CAL, mPI, mGI, mSBI parameters decreased in both groups at the 3<sup>rd</sup> month after the operation compared to baseline and the results are consistent with the literature (14,15). In our study, the mean KMW was measured as  $5.54 \pm 1.64$  mm in the periodontal microsurgery group at the 3<sup>rd</sup> month after FGG operation; in the conventional periodontal surgery group, mean KMW was  $4.80 \pm 1.84$  mm. The amount of KMW obtained in the 3<sup>rd</sup> month in both groups was statistically significant. Considering the time-dependent changes in the groups; in the periodontal microsurgery group, the KM gain obtained at the 3<sup>rd</sup> month after the operation was  $5.10 \pm 1.37$  mm, and in the conventional group at the 3<sup>rd</sup> month after the operation, the KMW gain was  $4.19 \pm 1.50$  mm. Mingdeng et al. (2018) applied FGG to 20 implant sites with insufficient KMW using microsurgical technique (6). After a 1-year follow-up period, the mean width of the keratinized mucosa was  $3.05 \pm 0.44$  mm, and the GS rate was  $41.22 \pm 5.04\%$  mm<sup>2</sup>. The results showed that microsurgery can increase the success rate of keratinized mucosa augmentation with peri-implant FGG.

In our study, periodontal microsurgery techniques were compared with conventional periodontal surgical techniques, considering that it is an atraumatic technique in the augmentation of the keratinized mucosa around the implant and the wound surface created is smaller, considering that it will improve clinical outcomes. In the literature, different clinical studies have presented a wide range of shrinkage percentages between 12% and 48% (16,17). Considering the differences in peri-implant and periodontal tissues, it can be

thought that the percentages of GS will also be different. Golmayo et al. (2021) compared the keratinized tissue width gain and GS after FGG operation in areas where the width of the keratinized mucosa around the implant and tooth is <2 mm (18). Significantly less gain in width of keratinized tissue and greater GS were observed at implant sites.

Graft shrinkage occurs during wound healing in the first month after surgery (16,17). In previous studies, horizontal and vertical measurements were made with the periodontal probe to indicate the shrinkage of the FGG and the area was calculated by multiplying these two values (19). Since the post-operative shrinkage is irregular, a java-based computer program was used for area measurement in our study. In studies conducted in the literature, it has been reported that the shrinkage in FGG is mostly in the 3-month period (19,20). Parvini et al. (2021) aimed to evaluate the soft tissue augmentation performed with FGG in the implant sites during the 3-month follow-up period (21). The mean surface area of FGG applied to 12 implants was measured immediately after surgery, at the 30<sup>th</sup> and the 90<sup>th</sup> days. The shrinkage rate of the graft was 16.3% between the baseline and the 30<sup>th</sup> day, and 33% between the baseline and the 90<sup>th</sup> day. It was observed that the shrinkage was more on the 90<sup>th</sup> day. In the light of this information, in our study, the GS rate was calculated as the difference between the graft area at the beginning and the 3<sup>rd</sup> month. 70% of the implants included in our study were in the posterior region. In our study, the graft was stabilized on the recipient bed with sutures in order to prevent the FGG from moving with the effect of the cheek muscles and traumas that may occur to the area during chewing. The number of sutures was kept to a minimum in both groups, based on the fact that factors effective during graft stabilization would affect avascular plasmatic circulation and GS. In our study, while GS was  $35.80 \pm 11.15\%$  mm<sup>2</sup> in the periodontal microsurgery group, it was  $46.60 \pm 19.17\%$  mm<sup>2</sup> in the conventional periodontal surgery group. GS was found to be less in the periodontal microsurgery group than in the conventional periodontal surgery group. In parallel with the data of our study, in the studies of Gümüş and Buduneli (2014), stabilization of the FGG around the implant; provided with conventional technique, microsurgical technique and cyanoacrylate adhesive (13). As a result of their studies, while GS was significantly less in the cyanoacrylate group than in the other groups, those in the conventional and microsurgery groups showed similar results.

The palate, edentulous ridges and tuber area are the most commonly used donor sites in connective tissue graft and FGG operations. In our study, the palate region was used to obtain FGG. In our study, the parameters we evaluated in the donor area were MT and wound healing. Invasive methods such as periodontal probe, endodontic reamer, injection needles and histological section measurements and non-invasive methods such as ultrasonic instruments and computed tomography can be used to measure gingival and palatal mucosa thickness (22). In this study, palatal MT was measured using an endodontic reamer. In both groups, MT did not reach their initial levels at the 3<sup>rd</sup> month after the operation; however, in the periodontal microsurgery group compared to the conventional periodontal surgery group, the MT at the 3<sup>rd</sup> month was closer to the initial value. Keskiner et al. (2016) in their study in which they examined the change in donor site thickness over time in spontaneous palatal wound healing after FGG removal; however, they stated that the thickness increased over time (23). The fact that the periodontal microsurgery technique causes a faster increase in palatal MT can be explained by the fact that it is an atraumatic technique and may provide a significant advantage.

In our study, wound healing in the donor area was evaluated according to "Landry's wound healing index" (9). Tissue color, presence of bleeding on palpation, presence of granulation tissue, presence of epithelialization at the incision margin and presence of suppuration were evaluated and data were collected. On the 10<sup>th</sup> day, wound healing indices in the test group ( $3.30 \pm 0.67$ ) were found to be higher than in the control group ( $2.50 \pm 1.08$ ). As expected, wound healing indices at the 3<sup>rd</sup> month were higher than at the 10<sup>th</sup> day. Wound healing indices at the 3<sup>rd</sup> month were similar in both groups.

It has been stated that microsurgical methods in periodontal treatments offer a faster and more comfortable wound healing process (24). One of the important goals of our study was to evaluate the pain levels in the donor and donor regions. In both groups, the first day was the day when the patients felt the most severe pain in the donor and donor areas. Significant reduction was observed in all other days compared to the first day in both groups, and the reduction in pain level was faster in the test group than in the control group.

Although it is thought that the fact that the patients themselves record the pain occurring after the operation and the amount of pain relief tablets they use may affect the results, when the total amount of pain relief tablets taken by the patients in the postoperative period is examined, it was seen that the patients in the periodontal microsurgery group used 3.5 (0-8) tablets and conventional periodontal surgery group used 6.00 (1-9) tablets. As a result, patients took less pain reliever tablets after the operation performed with the periodontal microsurgery technique and their stated pain scores were lower.

## **CONCLUSION**

This study shows that both periodontal microsurgery and conventional periodontal surgical techniques in the augmentation of the keratinized mucosa around the implant with FGG reduce plaque accumulation and inflammation findings and provide effective oral hygiene. GS and postoperative pain appears to be lower using periodontal microsurgery.

### **Acknowledgments**

The authors report no conflicts of interest.

### **Authorship contributions**

Surgical and Medical Practices: G.S., Concept: G.S., M.K.H., E.O., Design: G.S., M.K.H., E.O., Data Collection or Processing: G.S., M.K.H., Analysis or Interpretation: G.S., M.K.H., E.O., Literature Search: G.S., M.K.H., Writing: G.S., M.K.H., E.O.

### **Data availability statement**

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

### **Declaration of competing interest**

The authors declare that they have no conflict of interest.

## **Ethics**

The study was conducted in accordance with the ethical rules of the Declaration of Helsinki and it was approved by Kirikkale University Clinical Research Ethics Committee (No: 16/08 Date: 01.08.2019).

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