J Clin Periodontol 2016; 43: 976–984 doi: 10.1111/jcpe.12592

Journal of Clinical Periodontology

Quantitative changes in palatal donor site thickness after free gingival graft harvesting: a pilot study

Ilker Keskiner[1], Ahmet Aydogdu[2], Umut Balli[3] and Ayca E. Kaleli[1]

[1]Department of Periodontology, Faculty of Dentistry, Ondokuz Mayis University, Samsun, Turkey; [2]Department of Periodontology, Istanbul Application and Research Center, Faculty of Dentistry, Baskent University, Istanbul, Turkey; [3]Department of Periodontology, Faculty of Dentistry, Bulent Ecevit University, Zonguldak, Turkey

Keskiner I, Aydogdu A, Balli U, Kaleli AE. Quantitative changes in palatal donor site thickness after free gingival graft harvesting: a pilot study. J Clin Periodontol 2016; 43: 976–984. doi: 10.1111/jcpe.12592.

Abstract

Aim: The aim of this study was to investigate how donor sites thickness quantitatively change over time and at different points of donor site in spontaneous palatal wound healing after free gingival graft (FGG) harvesting.

Materials and Methods: Forty individuals were enrolled and divided into the following two groups based on the residual tissue thickness (RTT) after harvesting: Group 1, <2 mm; and Group 2, ≥2 mm. FGGs were standardized according to their dimensions and thickness and then harvested. Tissue filling was measured at three points of the defect area (mesial, central and distal) at various time points (baseline, after harvesting, and at 1, 3 and 6 months).

Results: The thickness of newly formed tissue from the baseline to 1 month after harvesting was greater in Group 2 than in Group 1, whereas the thickness from 1 to 3 months and from 3 to 6 months after harvesting was greater in Group 1 than in Group 2 ($p < 0.005$). RTT was positively correlated with tissue filling in all the groups at all time points ($p < 0.05$).

Conclusions: The palatal mucosal thickness after FGG harvesting might affect the filling of the defect. Within the study period, the periphery of the palatal wounds filled earlier and to a greater extent compared with the centre of the wounds.

Key words: palate; plastic surgery; wound healing

Accepted for publication 18 June 2016

Mucogingival defects create aesthetic and functional problems for patients (Camargo et al. 2001). To resolve these issues, autogenous soft tissue grafts such as subepithelial connective tissue grafts and free gingival grafts (FGG) are frequently used (Zucchelli & Mounssif 2015). Because the palatal mucosa is commonly used as a graft donor site, the thickness of the palatal mucosa is a major consideration for mucogingival surgery (Sanz & Simion 2014).

Gingival grafts harvested with the epithelium can be directly used as FGG and employed as subepithelial connective tissue grafts after de-epithelialization inside or outside the oral cavity (Zucchelli et al. 2010, Ozcelik et al. 2016). The donor site in this approach heals by secondary intention and the procedure is relatively easy to perform and can obtain large quantities of connective tissue (Del Pizzo et al. 2002). Graft necrosis or over-shrinkage may be observed at the recipient site when

ClinicalTrials.gov ID NCT02659904.

Conflict of interest and source of funding statement

The authors declare that they have no financial relationships related to any products involved in this study. The study was self-funded by the authors.

the graft thickness is insufficient (Mörmann et al. 1981, Miller 1985, Borghetti & Gardella 1990). In addition, necrosis and delayed wound healing can occur after gingival graft harvesting at the donor site if there is insufficient thickness of soft tissue on the palatal bone after a gingival graft of adequate thickness is harvested from a thin palatal mucosa (Zucchelli et al. 2010). Insufficient thickness at both the recipient and donor areas can increase the probability of complications related to mucogingival defect treatments. Moreover, re-harvesting from the same donor site might be necessary (Yen et al. 2007). The wound depth at the graft area is positively correlated with pain levels (Burkhardt et al. 2015), and Zucchelli et al. (2010) suggested that tissue with a thickness of 2 mm or more should be retained for better post-operative healing and pain reduction. Furthermore, tissue healing is known to occur earlier at wound edges, and this healing pattern can result in a difference between the centre and periphery of the wound area (Velnar et al. 2009). Based on this information, residual tissue thickness (RTT) and gingival graft thickness could be important clinical parameters in the planning stages of surgery (Mörmann et al. 1981, Zucchelli et al. 2010). However, the remaining questions pertain to the amount of tissue that must remain at the donor site after harvesting to avoid adverse effects that retard the healing process and to the effects RTT has on the filling and on the regain of the previous thickness of the tissue.

The thickness of palatal mucosa and autogenous soft tissue grafts have been widely studied (Mörmann et al. 1981, Studer et al. 1997, Muller et al. 1999, Zucchelli et al. 2003, 2014, Song et al. 2008, Barriviera et al. 2009, Yaman et al. 2014). However, to our knowledge, studies have not previously performed quantitative evaluations of the changes in spontaneous palatal wound healing after FGG harvesting at different locations and over time. Therefore, our primary objective in this clinical (case–control intervention) study was to explore the effects of RTT on palatal mucosa healing from the baseline measurement to 1, 3 and 6 months after FGG harvesting and to determine the time point at which to re-harvest autogenous soft tissue grafts from the same site. As a secondary objective, we examined whether the healing process differed in terms of the palatal thickness between the centre and periphery of the harvesting site. We also tested the correlations between the filling percentage and RTT after harvesting at the donor site. The primary efficacy variables were changes in the thickness of the palatal mucosa from the baseline measurement to 1, 3 and 6 months after graft harvesting.

Material and Methods

Study population

Forty individuals (21 men and 19 women; age range from 22 to 31 years old) who had been admitted to the Periodontology Department of Ondokuz Mayis University, Faculty of Dentistry (from February 2015 until December 2015), were enrolled in the study. All the individuals provided their written informed consent, and the study protocol was approved by the Ethics Committee of Ondokuz Mayis University in accordance with Helsinki Declaration revised in 2008 (2015/103).

Inclusion and exclusion criteria

All the individuals received oral hygiene instructions, and initial periodontal therapy was performed to establish optimal plaque control and gingival health conditions. The subjects were instructed to perform a non-traumatic brushing technique (roll) using an ultra-soft toothbrush and then re-evaluated at 8 weeks after the initial therapy, and only those with full mouth plaque score and full mouth bleeding score <15% were enrolled in the surgical procedure (Dogan et al. 2015). Furthermore, the following selection criteria were applied: (i) mucogingival defects (gingival recession with lack of keratinized tissue in the mandibular anterior area) that required soft tissue graft applications; (ii) for Group 1, a palatal thickness at each measurement point before graft harvesting between 2.5 and 3.4 mm and (iii) for Group 2, a palatal thickness at each measurement point before graft harvesting between 3.5 and 4.4 mm. After the study population was enrolled, a standardized FGG (thickness: 1.5 mm; width: 9 mm; length: 14 mm) was harvested in all individuals. Based on the RTT after the surgery, Group 1, RTT at the donor site after harvesting of 1.00–1.9 mm (between 2.5 and 3.4 mm before the thickness of 1.5 mm graft harvesting) ($n = 20$; nine men and 11 women; age: 26.95 ± 4.78 years old); and Group 2, RTT at the donor site after harvesting of 2.00–2.9 mm (between 3.5 and 4.4 mm before the thickness of 1.5 mm graft harvesting) ($n = 20$; 12 men and eight women; age: 25.90 ± 3.52 years old). The thickness of the palatal mucosa was measured before surgery and at 1, 3 and 6 months after surgery.

The exclusion criteria were periapical or palatal pathologies, absence of teeth from the canine to first molar, excessive forces (e.g. mechanical forces from orthodontics and traumatic occlusions), systemic diseases that would contraindicate periodontal surgery or interfere with tissue healing, chronic high-dose steroid therapy, radiation or immunosuppressive therapy, pregnancy, lactation, smoking or allergy or sensitivity to any drug. No individual has smoking history. The study participants did not present histories of drug therapies that are known to interfere with healing or cause gingival enlargement. Individuals who had prolonged bleeding, and delayed healing, were excluded to eliminate possible effects of these conditions on healing process and standardize the procedure.

Clinical measurements and intra-examiner reproducibility

Prior to the actual measurements, 10 individuals and 20 sites (two different sites for each individual) were selected. The thickness of the palatal mucosa was used to calibrate the investigator and correlate the reamer (No. 20 endodontic reamer; Bahadır Dis Malz, Istanbul, Turkey) with a computer-assisted automated periodontal probe (CPP) with stent tip (Florida Probe Corp., Gainesville, FL, USA). An endodontic reamer with a silicone stopper was placed in the palatal mucosa after greater

palatine and incisive nerves blockage with local anaesthesia. After carefully removing the reamer, the thickness of the palatal mucosa was measured with digital callipers with a 0.05 resolution (Stainless Steel Digital Caliper 75 mm; Shan, Guangxi, China). The tip of the CPP was replaced with an injection needle and calibrated, and then the thickness of the palatal mucosa was directly measured.

The investigator evaluated the sites on two separate occasions at 10 min. apart using both the reamer and the CPP (Studer et al. 1997). The investigator's measurements were considered sufficiently reproducible if the values obtained at baseline and at 10 min. differed by no more than 10% at the 0.5 mm level (Aroca et al. 2009). In addition, Pearson's correlation coefficient was obtained between the two measurement methods, and it revealed a strong correlation (0.94, $p < 0.05$). All the measurements were performed 30 min. following anaesthetic injection (Studer et al. 1997).

Three plastic stents were prepared for each subject. The first stent was used to standardize the location and size of the graft site; the second was used to standardize the measurement points; and the third was used to fabricate a healing stent for use following graft harvesting. The FGG was obtained from the distal part of the canine to the mesial part of the first molar and 2 mm apical to the gingival margin. The length and width of the graft site were 14 and 9 mm respectively.

After the length and width of the graft site were determined, the three measurement points (mesial, central, distal) on the central line of donor site in the mesial-distal direction were obtained from study models. The mesial point was marked perpendicularly to the centre of the first premolar located 6.5 mm from the gingival margin (this ensures 4.5 mm from the coronal and apical borders of the incision lines because of the coronal incision line is 2 mm apical to the gingival margin and the graft width is 9 mm) and 2 mm distal to the mesial margin of the graft site on the study model. The central and distal points 5 mm equidistant from one another were also marked (Rocha et al. 2012). A fissure diamond burr was used to create notches at the marked measurement points on the stent at 90° to the surface of the stent, and this prepared stent provided a consistent location for the assessment of mucosal thickness (Rocha et al. 2012). The three measurement points were marked with disclosing solution (Mira-2-Ton, Hager & Werken GmbH & Co. KG, Duisburg, Germany) by means of the notches of the second stent in the mouth.

After the correlation and calibration were approved, the thickness of the palatal mucosa before harvesting and the FGG thickness after harvesting were measured from the same marked points using the CPP with stent tip (Fig. 1). The arithmetic mean of three measurements at each point was calculated.

All the clinical examinations and group allocations were performed by the same investigator (A.E.K.), who was blinded to the study design. The RTT was calculated by subtracting the thickness of the FGG (1.5 mm was verified by measuring from the same points on the graft) from the thickness of the palatal mucosa at baseline. At the other time points, the palatal mucosa thickness was measured directly from the points determined by the stent. The percentage of newly formed tissue was calculated according to the following formula:

$$\text{newly formed tissue thickness} = \frac{(\text{tissue thickness at time point}) - (\text{RTT after harvesting})}{1.5} \times 100.$$

Surgical procedure

All the periodontal surgical procedures were performed with local anaesthesia on an outpatient basis under aseptic conditions by one experienced periodontal clinician (I.K.). The greater palatine and incisive nerves were blocked with a 2% lidocaine and 1:100,000 epinephrine injection. After preparing the recipient bed, a FGG was harvested at the palatal donor site using an instrument (ACE Surgical Supply Co. Inc., Brockton, MA, USA) and plastic stent guidance (Fig. 1d). This instrument consists of two parts: (i) a handle and (ii) a replacement shoe which is a standardized knife (1.5 mm in depth and 9 mm in width). This knife allows for the harvesting of a standard and uniform gingival graft from the palatal donor site, as shown in Fig. 1e. After harvesting, the graft thickness was measured with the CPP, and the dimensions (length and width) were measured by digital callipers to verify the graft size. Then, the gingival graft was sutured at the recipient bed, and the bleeding at the donor site was stopped by applying gentle external pressure with a gauze sponge for 5 min. The palatal donor site was covered with the third stent for 2 days, and it was not in contact with the harvesting area and no applications or procedures were performed at the donor site during this time. All the clinical measurements were recorded again at 1, 3 and 6 months after surgery (Fig. 2). At each recall visit, a plastic stent was emplaced, and the measurements were repeated from the same point. At first month, epithelialization was evaluated clinically and by means of a colour slide as described by Del Pizzo et al. (2002).

Post-operative care

The individuals were prescribed ibuprofen (600 mg/day for 4 days) and a 0.2% chlorhexidine gluconate mouth rinse (twice per day for 1 week) to reduce pain and inflammation. Post-operative instructions were provided to each individual.

Statistical analysis

The primary outcome variable (changes in the newly formed tissue thickness at the donor site) was used to calculate the sample size and determine the power of the study. However, sample size calculations could not be performed because precise information on newly formed tissue thicknesses after FGG harvesting was not available. Therefore, we based our estimates on a pilot study, which included 10 patients in each group. We estimated that a sample

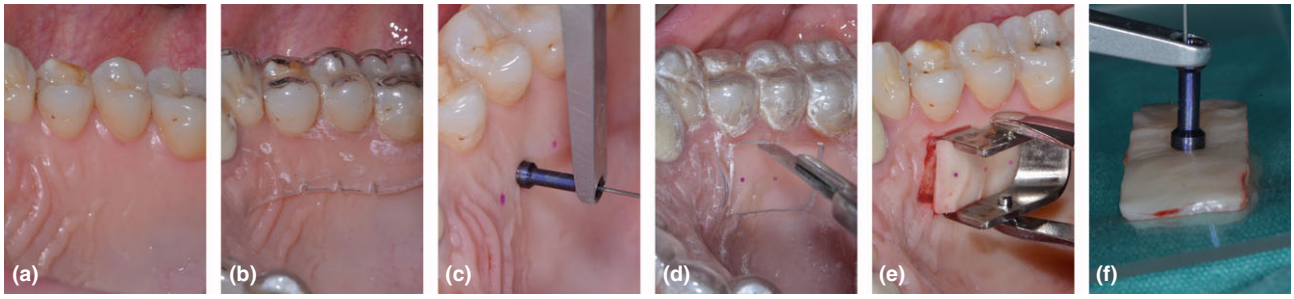

Fig. 1. (a) Baseline view. (b) Stent placed to standardize and mark the measurement points. (c) Measurement of palatal tissue thickness from the marked points with computer-assisted automated periodontal probe. (d) Stent placed to standardize the location and size of the graft. (e) Standard graft thickness obtained using handle with knife. (f) Measurement of graft thickness.

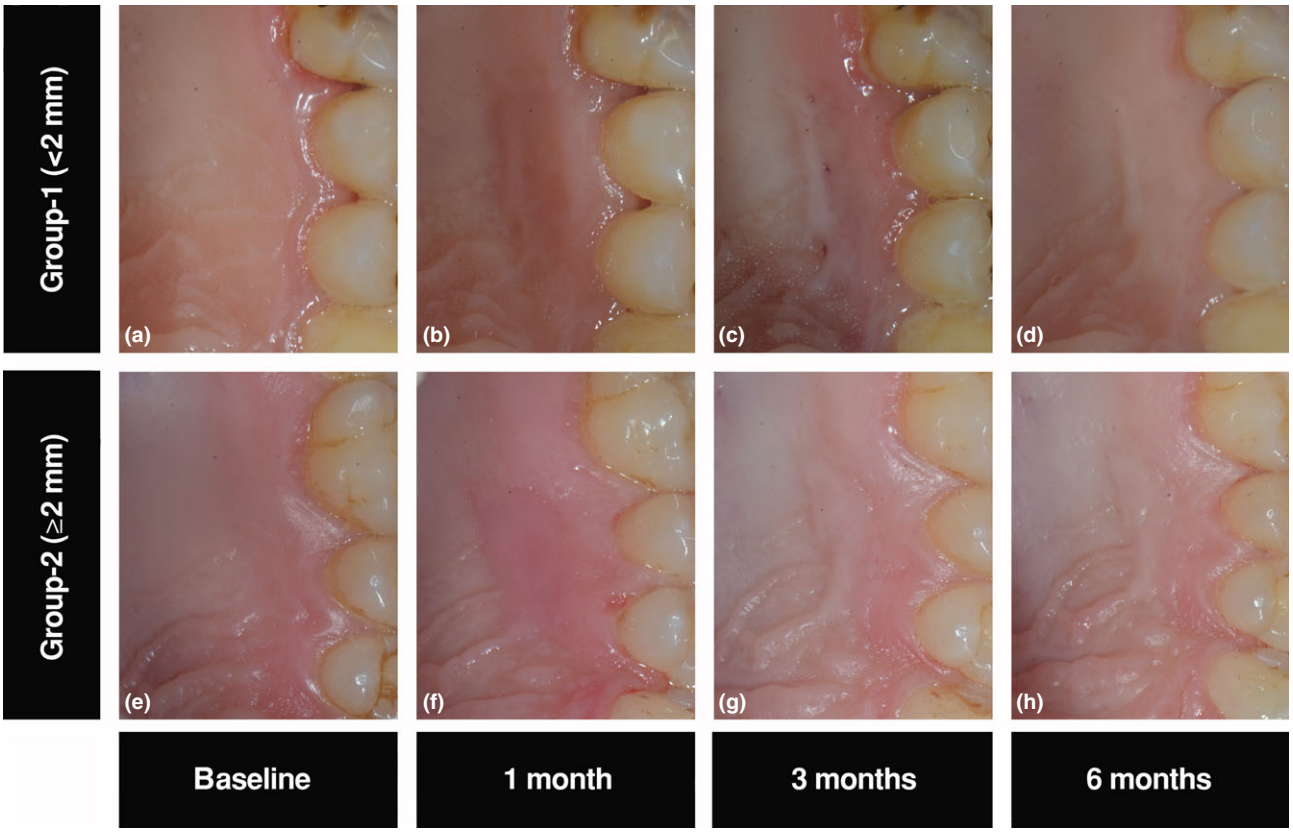

Fig. 2. Baseline, 1, 3 and 6 months views of palate. (a–d) Group 1, <2 mm residual tissue thickness (RTT). (e–h) Group 2, ≥2 mm RTT.

size of 18 patients in each group would allow for a type II error level of $\beta = 0.10$ (90% power) and a type I error level of $\alpha = 0.05$ (5% probability). To account for possible drop-outs, we included 20 patients in each group. Because sample size calculations could not be performed a priori, the retrospective power was calculated. The a posteriori power calculation yielded a power of 94% to detect differences in the outcomes before and after treatment.

The Shapiro–Wilk test was used to determine whether the data were normally distributed, and the Mann–Whitney *U*-test (unpaired observations) was used to compare the inter-group values if a normal distribution was not observed. Comparisons of the intra-group values (changes in the time- and location-dependent variables) were analysed using the Friedman test and then by post hoc group comparisons with the Bonferroni-adjusted Wilcoxon's signed-rank test (paired observations). Spearman's rank correlation test was used to detect the relationships between newly formed tissue thicknesses and RTT. All the tests were performed using statistical software (version 21.0; SPSS Inc., Chicago, IL, USA). The medians with 25th–75th percentile values were calculated for each parameter using the patients as the statistical units. A p-value < 0.05 was considered statistically significant.

Results

Since different measurements in the same patient at different follow-ups were performed, patients were questioned about the patient-related factors such as smoking, medication use and systemic condition for the management of statistical analysis. Patient-related factors were not changed along the study period.

Significant differences were not observed in the distribution of age (Group 1: 26.95 ± 4.78; Group 2: 25.90 ± 3.52) and sex (Group 1: nine male, 11 female; Group 2: 12 male, eight female) between Group 1 (<2 mm of RTT) and Group 2 (≥2 mm of RTT) ($p > 0.05$). Five individuals (11%: 3 in Group 1 and 2 in Group 2) were excluded from the study because of prolonged bleeding, and one individual from Group 1 (2%) was excluded because of delayed healing (not completed epithelialization) at the 1 month appointment.

Our results showed that there were significant differences in both Group 1 and Group 2 among all the measurement time points ($p < 0.005$).

The measurement and percentage of tissue thickness are provided in Tables 1 and 2. The measurement and percentage of newly formed tissue are given in Tables 3 and 4. The post-operative tissue thickness increased in a time-dependent manner.

At 1 month, the average of the three measurements points in Group 2 [3.06 mm (2.96–3.36 mm)] was significantly higher than that in Group 1 [2.22 mm (1.97–2.39 mm)] ($p < 0.005$). In addition, the tissue thickness percentiles at the baseline measurements were significantly higher in Group 2 [84.51% (83.71–86.28%)] than in Group 1 [71.90% (69.93–75.07%)] ($p < 0.005$), the tissue thickness difference between the baseline measurement and 1 month measurement was significantly higher in Group 2 [0.94 mm (0.89–0.98 mm)] than in Group 1 [0.66 mm (0.62–0.70 mm)] ($p < 0.005$). Similarly, the tissue thickness percentiles were significantly higher in Group 2 [62.11% (59.15–63.90%)] than in Group 1 [43.58% (41.10–46.45%)] ($p < 0.005$). At 3 months, the inter-group comparisons showed that Group 2 [3.37 mm (3.27–3.65 mm)] presented a significantly greater tissue thickness compared with Group 1 [2.63 mm (2.36–2.85 mm)], which is similar to the 1 month statistical analysis ($p < 0.005$). The tissue thickness percentiles at the baseline measurement were significantly higher in Group 2 [92.56% (92.10–93.36%)] than in Group 1 [85.67% (83.92–87.90%)] ($p < 0.005$). The newly formed tissue thickness from 1 to 3 months was significantly greater in Group 1 [0.41 mm (0.38–0.45 mm)] than in Group 2 [0.29 mm (0.27–0.30 mm)] ($p < 0.005$). The newly formed tissue thickness percentile from 1 to 3 months was significantly higher in Group 1 [27.07% (25.58–29.76%)] than in Group 2 [19.34% (17.72–19.96%)] ($p < 0.005$). A statistical evaluation of the 6 month intergroup comparisons demonstrated that the tissue thickness in Group 2 [3.59 mm (3.47–3.91 mm)] was significantly greater than that in Group 1 [2.91 mm (2.60–3.09 mm)] ($p <$ 0.005). The palatal tissue thickness percentiles at the baseline measurement were significantly higher in Group 2 [97.14% (98.33–99.28%)] than in Group 1 [93.74% (92.79–95.21%)]. The newly formed tissue thickness from 3 to 6 months was significantly greater in Group 1 [0.26 mm (0.23–0.28 mm)] than in Group 2 [0.22 mm (0.17–0.26 mm)] ($p < 0.005$). The newly formed tissue thickness percentile from 3 to 6 months was significantly higher in Group 1 [17.18% (15.04–18.38%)] than in Group 2 [14.30% (11.09–17.01%)] ($p < 0.005$).

The inter-group comparisons between Group 1 and Group 2 indicated significant differences at all the time points and for all measurement points ($p < 0.005$).

Although palatal tissue thickness at 6 months was statistically higher compared to RTT after harvesting in all individuals, only 11 of 40 returned to the baseline palatal tissue thickness at the mesial and distal measurement points, with two in Group 1 and nine in Group 2, but none at central point. The maximum percentage of central palatal tissue thickness was 98.45% and 99.70% in Group 1 and 2 respectively.

A statistical analysis (pair-wise tests) of the comparisons between measurement points (mesial, central, distal) demonstrated that significant differences occurred among these points ($p < 0.016$).

The correlation coefficients are presented in Table 5. Statistically significant, moderate and positive correlations were found between the tissue thickness and the newly formed tissue thickness at the measurements points for all the time intervals.

Discussion

To the best of our knowledge, this is the first clinical study that has explored the effects of RTT on palatal mucosa filling from the baseline measurement to 1, 3 and 6 months after FGG harvesting and evaluated whether the newly formed tissue thickness differed between the centre and periphery of the harvesting site.

There are various methods for measuring the thickness of the gingiva or palatal mucosa, including invasive methods, such as periodontal probes, endodontic reamers, injection needles and histological section measurements and non-invasive methods, such as ultrasonic devices and computed tomography (Olsson et al. 1993, Eger et al. 1996, Studer et al. 1997, Muller et al. 1999, Song et al. 2008, Lehmann et al. 2012, Yu et al. 2013, Yaman et al. 2014). However, the distance between the reference points should be measured with an additional device, such as the callipers described in the Material and Methods section or a silicone stopper if more precise and accurate quantitative measurements are desired. Measurements can be affected by tissue displacement during probing, including probing against the palate during transmucosal probing. In addition, displacement of the silicone stopper can influence the measurements (Ronay et al. 2011). Histological sections are a reliable method of measuring cadaver jaws, although they are not applicable in vivo (Yan et al. 2014). Furthermore, ultrasonic tissue thickness measurements are atraumatic, rapid and easily applicable; however, they have low reproducibility and present limitations when the mucosal thickness is >6 mm (Muller et al. 1999). Computed tomography can provide high-resolution images of the masticatory mucosa; however, the high radiation dose might not be applicable for the mandibular lingual

Table 1. Distribution in measurements of palatal tissue thickness (mm) in study groups

	Measurement points	Baseline*,†	After harvesting*,†	1 month*,†	3 months*,†	6 months*,†
Group 1	Mesial‡	3.20 (2.90–3.40)	1.70 (1.40–1.90)	2.40 (2.20–3.10)	2.80 (2.53–3.00)	3.10 (2.73–3.20)
(<2 mm of RTT)	Central‡	3.10 (2.83–3.30)	1.60 (1.33–1.80)	2.15 (1.90–2.38)	2.50 (2.30–2.78)	2.90 (2.63–3.08)
n = 20	Distal‡	2.95 (2.73–3.10)	1.45 (1.23–1.60)	2.15 (1.90–2.30)	2.50 (2.30–2.80)	2.75 (2.60–3.00)
Group 2	Mesial‡	3.75 (3.60–4.05)	2.25 (2.10–2.55)	3.20 (3.10–3.50)	3.50 (3.32–3.78)	3.70 (3.60–4.05)
(≥2 mm of RTT)	Central‡	3.65 (3.50–3.95)	2.15 (2.00–2.45)	3.05 (2.80–3.38)	3.35 (3.20–3.65)	3.55 (3.40–3.88)
n = 20	Distal‡	3.55 (3.50–3.75)	2.05 (2.00–2.25)	3.00 (3.00–3.18)	3.30 (3.20–3.48)	3.50 (3.40–3.75)

RTT, residual tissue thickness.
Values given as median (25–75 percentiles).
*Statistically significant differences among the measurements points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/3 = 0.016.
†Statistically significant differences between groups in each measurements point (Mann–Whitney *U*-test).
‡Statistically significant differences among the time points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/10 = 0.005.

Table 2. Distribution in percentage of palatal tissue thickness (%) in study groups

	Measurement points	Baseline	After harvesting*,†	1 month*,†	3 months*,†	6 months*,†
Group 1	Mesial‡	100	53.13 (48.28–55.88)	75.17 (73.56–76.78)	88.64 (87.03–89.25)	95.18 (93.88–96.89)
(<2 mm of RTT)	Central‡	100	51.61 (46.89–54.55)	68.71 (65.82–75.00)	82.40 (79.19–85.21)	92.30 (92.10–92.67)
n = 20	Distal‡	100	49.14 (44.94–51.61)	72.17 (68.24–74.24)	88.32 (83.40–90.01)	93.93 (92.38–95.42)
Group 2	Mesial‡	100	59.99 (58.33–62.94)	86.83 (85.27–87.48)	94.05 (92.78–94.90)	98.83 (97.89–100.00)
(≥2 mm of RTT)	Central‡	100	58.90 (57.14–62.01)	81.62 (79.76–84.11)	91.42 (89.62–92.47)	97.13 (96.24–97.99)
n = 20	Distal‡	100	57.74 (57.14–59.98)	85.08 (84.04–86.68)	92.39 (91.85–93.21)	98.17 (97.24–100.00)

RTT, residual tissue thickness.
Values given as median (25–75 percentiles).
*Statistically significant differences among the measurements points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/3 = 0.016.
†Statistically significant differences between groups in each measurements point (Mann–Whitney *U*-test).
‡Statistically significant differences among the time points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/6 = 0.008.
palatal tissue thickness (%) = (palatal tissue thickness at the measurement time points/baseline palatal tissue thickness) × 100.

Table 3. Measurements of newly formed tissue thickness (mm) in study groups

	Measurements point	After harvesting-1 month*,†	1–3 months*,†	3–6 months*,†
Group 1 (<2 mm of RTT)	Mesial‡	0.70 (0.63–0.81)	0.40 (0.36–0.47)	0.23 (0.17–0.28)
n = 20	Central‡	0.56 (0.51–0.65)	0.37 (0.34–0.40)	0.33 (0.26–0.37)
	Distal‡	0.65 (0.62–0.70)	0.43 (0.38–0.51)	0.29 (0.21–0.34)
Group 2 (≥2 mm of RTT)	Mesial‡	0.99 (0.91–1.05)	0.28 (0.26–0.29)	0.18 (0.14–0.22)
n = 20	Central‡	0.83 (0.78–0.88)	0.35 (0.29–0.36)	0.19 (0.17–0.22)
	Distal‡	0.96 (0.91–0.99)	0.27 (0.25–0.28)	0.21 (0.17–0.25)

RTT, residual tissue thickness.
Values given as median (25–75 percentiles).
*Statistically significant differences among the measurements points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/3 = 0.016.
†Statistically significant differences between groups in each measurements point (Mann–Whitney *U*-test).
‡Statistically significant differences among the time points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/3 = 0.016.

gingiva under the tongue (Yan et al. 2014). In this study, the tip of the CPP was replaced with an injection needle and calibrated at 0, 3 and 10 mm. After the measurements were performed, differences in the tissue thickness as measured by the reamer with callipers or the CPP were not observed (data not shown). Tissue thickness measurements can be directly and easily performed to one decimal place with the CPP method. Thus, the CPP could be used to determine tissue thicknesses, especially when measurements at several points are required.

An important step in periodontal plastic surgery is planning the surgical procedure prior to beginning surgery. Insufficient palatal thickness can complicate the treatment of

Table 4. Percentages of newly formed tissue (%) in study groups

	Measurements point	After harvesting-1 month*,†	1–3 months*,†	3–6 months*,†
Group 1 (<2 mm of RTT)	Mesial‡	46.92 (42.03–54.20)	26.34 (23.85–31.63)	15.08 (11.09–18.72)
n = 20	Central‡	37.30 (33.80–43.33)	24.87 (22.59–26.67)	21.70 (17.55–24.82)
	Distal‡	43.30 (41.47–46.70)	28.60 (25.33–33.96)	14.09 (11.57–19.14)
Group 2 (≥2 mm of RTT)	Mesial‡	66.33 (60.96–70.26)	18.65 (17.51–19.43)	11.86 (9.53–14.79)
n = 20	Central‡	55.35 (52.00–58.35)	23.10 (19.41–23.94)	12.44 (11.12–14.48)
	Distal‡	64.15 (60.60–65.64)	18.27 (16.64–18.77)	13.84 (11.40–16.35)

RTT, residual tissue thickness.
Values given as median (25–75 percentiles).
*Statistically significant differences among the measurements points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/3 = 0.016.
†Statistically significant differences between groups in each measurements point (Mann–Whitney *U*-test).
‡Statistically significant differences among the time points in each group (Friedman test with Bonferroni-adjusted *Wilcoxon* signed-rank test) Bonferroni adjustment = 0.05/3 = 0.016.
newly formed tissue thickness = ((tissue thickness at time point) − (RTT after harvesting))/1.5 × 100.

mucogingival defects at both the recipient and donor sites. Therefore, evaluating the pre-operative palatal soft tissue thickness is imperative for each individual case. There is evidence that complete epithelialization of the palatal donor site within 4 weeks after surgery is considered normal healing for FGG harvesting areas (Farnoush 1978, Del Pizzo et al. 2002, Silva et al. 2010, Keceli et al. 2015), and all the patients enrolled in this study were evaluated to determine their epithelialization status. As we know, bleeding is the normal component of the surgical procedures. Haemostasis is the first phase of the normal wound healing process (Guo & Dipietro 2010). So, prolonged bleeding can affect the normal stage of healing. Therefore, control the bleeding time becomes important factor to evaluate the healing process. In the routine clinical procedure, bleeding can be stopped with conventional gauze compress method at the time of surgery. However, extra applications such as electrocautery instrument, laser, haemostatic sponge, haemostatic agents, periodontal dressing may be needed to control prolonged bleeding. However, these applications can compromise the palatal wound healing. To eliminate possible effects of the extra applications on healing process and standardize the healing procedure, a total of six individuals were excluded from the analysis (five individuals because of prolonged bleeding and one because of delayed healing). In this study, prolonged bleeding was observed in an average of 11% of the patients, whereas Del Pizzo et al. (2002) and Silva et al. (2010) reported immediate bleeding in 25% and 75% of the non-smoking individuals respectively. Differences in pressure application time (2 *versus* 5 min.) might explain this discrepancy between the studies. We preferred a longer pressure time (standardized to 5 min.) to reduce the risk of prolonged bleeding, which was achieved in this study.

Several clinical studies have explored palatal wound healing after harvesting with/without various applications; however, we examined the changes in palatal donor site thickness after FGG harvesting without additional applications. Only two clinical studies have explored the changes in tissue thickness after graft harvesting from the palatal mucosa (Yen et al. 2007, Shanmugam et al. 2010). A clinical and histological study by Shanmugam et al. (2010) compared the efficacy of applying two dressing materials for palatal wound healing after FGG harvesting, and they observed a 0.7% reduction in donor site thickness in the non-eugenol-based dressing application group 42 days after FGG harvesting from the palatal

Table 5. The Spearman's rank correlation (rho) among groups with respect remaining tissue thickness and newly formed tissue thickness at measurements points

	1 month				3 months				6 months			
	Mesial	Central	Distal	All points	Mesial	Central	Distal	All points	Mesial	Central	Distal	All points
Group 1 (<2 mm of RTT)												
r	0.538	0.641	0.670	0.581	0.767	0.462	0.740	0.505	0.446	0.627	0.519	0.544
p	0.014	0.002	0.001	0.007	0.000	0.040	0.000	0.023	0.049	0.003	0.019	0.013
Group 2 (≥2 mm of RTT)												
r	0.941	0.949	0.850	0.946	0.626	0.608	0.597	0.617	0.524	0.453	0.497	0.447
p	0.000	0.000	0.000	0.000	0.003	0.002	0.005	0.002	0.018	0.045	0.026	0.048
All groups												
r	0.538	0.641	0.685	0.603	0.717	0.895	0.862	0.854	0.735	0.754	0.692	0.731
p	0.014	0.002	0.001	0.003	0.000	0.000	0000	0.000	0.000	0.000	0.000	0.000

RTT, residual tissue thickness.
Statistically significant ($p < 0.05$).

mucosa and a 10.4% reduction in the non-friable collagen sponge application group at the same time point. These results are inconsistent with our findings, palatal tissue thickness (the average of three measurement points) at 1 month is ~28% and ~16% less than the preoperative measurements in Group 1 and group 2 respectively. In the study by Shanmugam et al. (2010), localization of the donor area and the dimensions of the grafts were not reported, only one point (1 cm from the marginal gingiva between the premolars) was selected to measure the tissue thickness, and the measurements were rounded off to the nearest 0.5 mm. These differences in the study protocol as well as differences in the measurement times might explain the discrepancies among the tissue filling results. Yen et al. (2007) explored the effects of platelet concentration on palatal donor sites after connective tissue-graft harvesting by a modified single incision technique. In their study, the graft thickness was not standardized and the dimensions were not reported. However, the authors showed that a 0.4 mm reduction in tissue thickness occurred between the baseline measurement and 6 weeks after connective tissue harvesting (Yen et al. 2007). In a recent study, 6 mm punch biopsies were performed at palatal sites, and wound healing with or without a collagen matrix application was investigated (Thoma et al. 2012). The defect area (unkeratinized tissue) was smaller with the collagen matrix application than with spontaneous healing at 4 and 8 days after the biopsies. The defect areas were reduced by 24% and 52% at 4 and 8 days in the spontaneous healing group respectively. In this study, the tissue filling at the donor site was approximately 47% (0.70 mm), 37% (0.56 mm) and 43% (0.65 mm) in Group 1 and 66% (0.99 mm), 55% (0.83 mm), 64% (0.96 mm) in Group 2 within the first month at the mesial, central and distal sites, respectively, and the differences between the groups were significant at each time point. These findings showed that the tissue filling at donor sites was slower when the RTT was <2 mm. In addition, newly formed tissue at the palatal wound site was directly associated with RTT after harvesting. However, the quantitative changes at 3 or 6 months after FGG harvesting have not been previously reported; thus, a direct comparison with other studies is not possible. Patients with uncontrolled bleeding during surgery were eliminated from the study and this is a limitation of this pilot study.

This study showed that significant differences in tissue thickness over time occurred at all time points in groups. The inter-group comparisons showed that the tissue filling was greater in RTT of ≥2 mm compared with RTT of <2 mm. The majority of tissue filling at the donor site was observed within the first month, and then a significant reduction in tissue filling was observed. The recovery process also varied according to the measurement point location, with increases observed from the mesial, distal and central regions (in that order), and it also varied over time in both groups. In addition, palatal wound filling after FGG harvesting was directly affected by the RTT at the donor site. Complete recovery was only observed in two individuals with a thickness of <2 mm, whereas complete recovery was observed in nine individuals with a thickness of ≥2 mm at the mesial and distal measurement points at 6 months after FGG harvesting.

The results suggested that defect filling after FGG harvesting is more rapid with a RTT at ≥2 mm relative to thickness at <2 mm, and complete regain of the tissue thickness at the defect requires more than 6 months if RTT is <2 mm, especially in the centre of the donor site. If the thickness of the masticatory mucosa as well as the thickness of the required graft render it possible, it is recommended to leave at least 2 mm of tissue at the palatal donor site after the first graft harvesting. Additional long-term (more than 6 months) interventional studies with greater participation should be undertaken to confirm these results and elucidate the volumetric changes at the donor site after graft harvesting.

References

Aroca, S., Keglevich, T., Barbieri, B., Gera, I. & Etienne, D. (2009) Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. *Journal of Periodontology* **80**, 244–252.

Barriviera, M., Duarte, W. R., Januario, A. L., Faber, J. & Bezerra, A. C. (2009) A new method to assess and measure palatal masticatory mucosa by cone-beam computerized tomography. *Journal of Clinical Periodontology* **36**, 564–568.

Borghetti, A. & Gardella, J. P. (1990) Thick gingival autograft for the coverage of gingival recession: a clinical evaluation. *The International Journal of Periodontics & Restorative Dentistry* **10**, 216–229.

Burkhardt, R., Hammerle, C. H. & Lang, N. P. (2015) Self-reported pain perception of patients after mucosal graft harvesting in the palatal area. *Journal of Clinical Periodontology* **42**, 281–287.

Camargo, P. M., Melnick, P. R. & Kenney, E. B. (2001) The use of free gingival grafts for aesthetic purposes. *Periodontology 2000* **27**, 72–96.

Del Pizzo, M., Modica, F., Bethaz, N., Priotto, P. & Romagnoli, R. (2002) The connective tissue graft: a comparative clinical evaluation of wound healing at the palatal donor site. A preliminary study. *Journal of Clinical Periodontology* **29**, 848–854.

Dogan, S. B., Dede, F. O., Balli, U., Atalay, E. N. & Durmuslar, M. C. (2015) Concentrated growth factor in the treatment of adjacent multiple gingival recessions: a split-mouth randomized clinical trial. *Journal of Clinical Periodontology* **42**, 868–875.

Eger, T., Muller, H. P. & Heinecke, A. (1996) Ultrasonic determination of gingival thickness. Subject variation and influence of tooth type and clinical features. *Journal of Clinical Periodontology* **23**, 839–845.

Farnoush, A. (1978) Techniques for the protection and coverage of the donor sites in free soft tissue grafts. *Journal of Periodontology* **49**, 403–405.

Guo, S. & Dipietro, L. A. (2010) Factors affecting wound healing. *Journal of Dental Research* **89**, 219–229.

Keceli, H. G., Aylikci, B. U., Koseoglu, S. & Dolgun, A. (2015) Evaluation of palatal donor site haemostasis and wound healing after free gingival graft surgery. *Journal of Clinical Periodontology* **42**, 582–589.

Lehmann, K. M., Kasaj, A., Ross, A., Kammerer, P. W., Wagner, W. & Scheller, H. (2012) A new method for volumetric evaluation of gingival recessions: a feasibility study. *Journal of Periodontology* **83**, 50–54.

Miller, P. D. Jr (1985) Root coverage using the free soft tissue autograft following citric acid application. III. A successful and predictable procedure in areas of deep-wide recession. *The International Journal of Periodontics & Restorative Dentistry* **5**, 14–37.

Mörmann, W., Schaer, F. & Firestone, A. R. (1981) The relationship between success of free gingival grafts and transplant thickness. Revascularization and shrinkage–a one year clinical study. *Journal of Periodontology* **52**, 74–80.

Muller, H. P., Schaller, N. & Eger, T. (1999) Ultrasonic determination of thickness of masticatory mucosa: a methodologic study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **88**, 248–253.

Olsson, M., Lindhe, J. & Marinello, C. P. (1993) On the relationship between crown form and clinical features of the gingiva in adolescents. *Journal of Clinical Periodontology* **20**, 570–577.

Ozcelik, O., Seydaoglu, G. & Haytac, C. M. (2016) Diode laser for harvesting de-

epithelialized palatal graft in the treatment of gingival recession defects: a randomized clinical trial. *Journal of Clinical Periodontology* **43**, 63–71.

Rocha, A. L., Shirasu, B. K., Hayacibara, R. M., Magro-Filho, O., Zanoni, J. N. & Araujo, M. G. (2012) Clinical and histological evaluation of subepithelial connective tissue after collagen sponge implantation in the human palate. *Journal of Periodontal Research* **47**, 758–765.

Ronay, V., Sahrmann, P., Bindl, A., Attin, T. & Schmidlin, P. R. (2011) Current status and perspectives of mucogingival soft tissue measurement methods. *Journal of Esthetic and Restorative Dentistry* **23**, 146–156.

Sanz, M. & Simion, M. (2014) Surgical techniques on periodontal plastic surgery and soft tissue regeneration: consensus report of Group 3 of the 10th European Workshop on Periodontology. *Journal of Clinical Periodontology* **41** (Suppl. 15), S92–S97.

Shanmugam, M., Kumar, T. S., Arun, K. V., Arun, R. & Karthik, S. J. (2010) Clinical and histological evaluation of two dressing materials in the healing of palatal wounds. *Journal of Indian Society of Periodontology* **14**, 241–244.

Silva, C. O., Ribeiro Edel, P., Sallum, A. W. & Tatakis, D. N. (2010) Free gingival grafts: graft shrinkage and donor-site healing in smokers and non-smokers. *Journal of Periodontology* **81**, 692–701.

Song, J. E., Um, Y. J., Kim, C. S., Choi, S. H., Cho, K. S., Kim, C. K., Chai, J. K. & Jung, U. W. (2008) Thickness of posterior palatal masticatory mucosa: the use of computerized tomography. *Journal of Periodontology* **79**, 406–412.

Studer, S. P., Allen, E. P., Rees, T. C. & Kouba, A. (1997) The thickness of masticatory mucosa in the human hard palate and tuberosity as potential donor sites for ridge augmentation procedures. *Journal of Periodontology* **68**, 145–151.

Thoma, D. S., Sancho-Puchades, M., Ettlin, D. A., Hammerle, C. H. & Jung, R. E. (2012) Impact of a collagen matrix on early healing, aesthetics and patient morbidity in oral mucosal wounds – a randomized study in humans. *Journal of Clinical Periodontology* **39**, 157–165.

Velnar, T., Bailey, T. & Smrkolj, V. (2009) The wound healing process: an overview of the cellular and molecular mechanisms. *Journal of International Medical Research* **37**, 1528–1542.

Yaman, D., Aksu, S., Disci, R. & Demirel, K. (2014) Thickness of palatal masticatory mucosa and its relationship with different parameters in Turkish subjects. *Internation Journal of Medical Sciences* **11**, 1009–1014.

Yan, S., Shi, S. G., Niu, Z. Y., Pei, Z. H., Shi, S. M. & Mu, C. (2014) Soft tissue image reconstruction using cone-beam computed tomography combined with laser scanning: a novel method to evaluate the masticatory mucosa. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* **118**, 725–731.

Yen, C. A., Griffin, T. J., Cheung, W. S. & Chen, J. (2007) Effects of platelet concentrate on palatal wound healing after connective tissue graft harvesting. *Journal of Periodontology* **78**, 601–610.

Yu, S. K., Lee, B. H., Lee, M. H., Cho, K. H., Kim do, K. & Kim, H. J. (2013) Histomorphometric analysis of the palatal mucosa associated with periodontal plastic surgery on cadavers. *Surgical and Radiologic Anatomy* **35**, 463–469.

Zucchelli, G., Amore, C., Sforza, N. M., Montebugnoli, L. & De Sanctis, M. (2003) Bilaminar techniques for the treatment of recession-type defects. A comparative clinical study. *Journal of Clinical Periodontology* **30**, 862–870.

Zucchelli, G., Mele, M., Stefanini, M., Mazzotti, C., Marzadori, M., Montebugnoli, L. & de Sanctis, M. (2010) Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: a comparative randomized-controlled clinical trial. *Journal of Clinical Periodontology* **37**, 728–738.

Zucchelli, G. & Mounssif, I. (2015) Periodontal plastic surgery. *Periodontology 2000* **68**, 333–368.

Zucchelli, G., Mounssif, I., Mazzotti, C., Montebugnoli, L., Sangiorgi, M., Mele, M. & Stefanini, M. (2014) Does the dimension of the graft influence patient morbidity and root coverage outcomes? A randomized controlled clinical trial. *Journal of Clinical Periodontology* **41**, 708–716.

Address:
Ilker Keskiner
Department of Periodontology
Faculty of Dentistry
Ondokuz Mayıs University
Samsun 55139
Turkey
E-mail: ilkerkeskiner@yahoo.com

Clinical Relevance

Scientific rationale for the study: Data on changes in the thickness of donor sites over time and according to the location after free gingival graft harvesting are currently not available.

Principal findings: Defect filling primarily occurred within the first month. The newly formed tissue at the donor sites was ≥2 mm of the residual tissue thickness at all time points. The tissue filling in the centres of the defects was less than that in the mesial and distal parts.

Practical implications: Residual tissue thickness of ≥2 mm in spontaneous palatal wound healing is an important consideration when planning subsequent surgery.