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De-epithelialized gingival graft versus subepithelial connective tissue graft in the treatment of multiple adjacent gingival recessions using the tunnel technique: 1-year results of a randomized clinical trial

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Abstract

Aim: To compare the clinical efficacy and postoperative morbidity of de-epithelialized gingival graft (DGG) with subepithelial connective tissue graft (SCTG) on treatment of multiple adjacent gingival recessions (MAGRs) with tunnel technique (TUN) and to evaluate histological characteristics of the palatal grafts.

Materials and methods: Twenty-seven patients with MAGRs affecting at least 2 adjacent teeth were treated with either DGG + TUN or SCTG + TUN. Recession depth(RD) and width(RW), probing depth(PD), clinical attachment level(CAL), keratinized tissue height(KTH), gingival thickness(GT), and complete and mean root coverage(CRC, MRC) were evaluated at 6 and 12 months postoperatively. Multilevel analysis was performed to identify patient- and tooth/site-related predictors for the 12-month MRC outcomes. Postoperative patient morbidity and histological characteristics of palatal graft samples obtained during harvesting were investigated.

Results: At the 12-month follow-up, MRC was 91.72% ± 16.59% and 84.72% ± 19.72% in DGG + TUN and SCTG + TUN groups (p = .001). Multilevel regression analysis identified RD, KTH and GT as variables associated with MRC. No significant difference between the groups was observed regarding postoperative patient morbidity parameters. Cellularity was found significantly higher in the SCTG samples compared to the DGG samples (p < .05).

Conclusions: Although DGG + TUN presented higher MRC and CRC compared to SCTG + TUN in the treatment of MAGRs, treatment method was not a significant predictive factor for the amount of MRC outcomes while RD, KTH and GT were significant predictive factors.

KEYWORDS
connective tissue graft, gingival recession, histology, patient morbidity, root coverage

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Clinical Relevance

Scientific rationale for study: Limited information is available about the effects of connective tissue grafts (CTGs) obtained by different harvesting techniques on root coverage outcomes as well as patient's postoperative morbidity and aesthetic evaluation in the treatment of MAGRs using the tunnel technique (TUN). On the other hand, patient- and tooth/site-related prognostic indicators may have a critical role in obtaining the treatment outcomes.

Principal findings: DGG combined with TUN showed higher mean root coverage (MRC) and complete root coverage (CRC) compared to SCTG in the treatment of MAGRs. Nevertheless, tooth/site-related prognostic factors could impact root coverage outcomes regardless of CTG harvesting technique. Both procedures showed similar outcomes in terms of patient's postoperative morbidity and aesthetic evaluation at the 1-year follow-up.

Practical implications: DGG could be a predictable and safe option as an autologous palatal graft when combined with TUN for the treatment of MAGRs. Baseline defect and gingival anatomical characteristics should be considered when appraising the therapeutic prognosis of MAGRs.

1 | INTRODUCTION

The predictable treatment of multiple adjacent gingival recessions (MAGRs) represents a major challenge in periodontal plastic surgeries due to MAGRs' complicated predisposing anatomic features, such as thin gingival phenotype or limited keratinized tissue, variations in the depth and width of the adjacent recession defects, shallow vestibules and high frenum attachments (Aroca et al., 2018). Furthermore, in these cases, wound healing may be compromised due to factors such as larger avascular surface and poor blood supply (Sculean et al., 2016).

The coronally advanced flap (CAF) or the tunnel technique (TUN) with graft-based subepithelial connective tissue procedures has been reported to be the most predictable methods for the treatment of MAGRs (Tatakis et al., 2015; Sculean et al., 2016; Tavelli et al., 2018). On account of the advantages of TUN, including superior blood supply and advanced wound healing owing to its conservative characteristics associated with the flap elevation without papillae dissection or vertical releasing incisions, this approach has recently achieved popularity compared to other methods (Aroca et al., 2013; Tavelli et al., 2018).

Although the use of connective tissue grafts (CTGs), together with various surgical techniques, has been accepted as "the gold standard" for the treatment of GRs (Buti et al., 2013; Chambrone & Tatakis, 2015), some disadvantages of CTG are underlined. Limited donor tissue in the cases where inadequate amount of palatal tissue thickness and/or a bigger dimension of CTG is needed, as in the treatment of MAGRs, is claimed to be among those disadvantages (Zucchelli et al., 2010). Moreover, subepithelial CTG (SCTG), either harvested by "trap-door" (TD) (Bruno, 1994; Harris, 1997) or "single-incision" (SI) approaches (Hurzeler & Weng, 1999; Lorenzana & Allen, 2000), has been frequently associated with postoperative pain and discomfort, as well as palatal flap necrosis/dehiscence at the donor site (Del Pizzo et al., 2002; Griffin et al., 2006; Wessel & Tatakis, 2008; Zucchelli et al., 2010; Gobbato et al., 2016; Isler et al., 2018).

To overcome these limitations and obtain a firmer and uniform CTG, de-epithelializing of the free gingival grafts (FGG) has been proposed, especially when palatal fibromucosal tissue thickness is inadequate (≤2.5 mm) and a large graft dimension in apico-coronal or mesio-distal directions is required (Harris, 2003; Bosco & Bosco, 2007; Zucchelli et al., 2010; de Sanctis et al., 2011). De-epithelialized gingival graft (DGG) has also been suggested to be less prone to postoperative shrinkage, because of obtaining larger amount of collagen-rich connective tissue from lamina propria (Harris, 2003; Zuhr et al., 2014; Azar, Rojas, Patricia, et al., 2019), minimal amount of fatty/glandular tissue (Zucchelli et al., 2010; Bertl et al., 2015) and less number of medium to large vessels (Tavelli et al., 2020). Previously, it was reported that DGG applied with the TUN presented better manipulation in recipient area and reduced postoperative morbidity compared to a conventional CTG, and resulted in a successful root coverage outcome with increased both width and thickness of the keratinized tissue in the treatment of MAGRs (McLeod et al., 2009). In a randomized clinical trial (RCT), Zucchelli et al. (2010) found no significant differences regarding post-operative pain and root coverage outcomes when compared SCTG and DGG in combination with CAF in the treatment of single or multiple GRs. However, a significant difference was observed in terms of keratinized tissue thickness (KTT) in favour of DGG, and this finding was attributed to the its characteristics of better stability, low shrinkage rates and to allow the incorporation of the portion of connective tissue closest to the epithelium. Despite all biologically advantages of DGG, there is scarce information in the literature on its clinical significance in combination with TUN compared to conventional CTG procedures in the treatment of MAGRs. Therefore, the present study aimed to investigate the clinical efficacy and postoperative morbidity of DGG compared to SCTG on treatment of MAGRs with TUN and to evaluate the characteristics of the grafts harvested by these two different techniques histopathologically.

2 | MATERIALS AND METHODS

This study was a single-centre, single-blinded and parallel-group RCT and was structured according to the CONSORT statement (http://www.consort-statement.org/). The study design was approved by The Clinical Research Ethics Committee of Ankara University, Faculty of Dentistry (36290600/110) and also registered at U.S. National Institutes of Health Clinical Trials Registry (NCT04291963). The procedures, which were in full accordance with the principles of the Declaration of Helsinki of 1975, as revised in 2013, were explained to all the subjects and informed consents were provided before their participation. A total of 38 patients, who have been referred to the Gazi University Faculty of Dentistry Department of Periodontology, between December 2017 and June 2018 were recruited based on the following inclusion criteria:

- Age between 18 and 60 years,
- Non-smoker and systemically healthy,
- No active periodontal disease,
- Presence of at least two single-rooted teeth with Miller class I and/or II (RT1) (Miller, 1985; Cairo, Nieri, Cincinelli, Mervelt, & Pagliaro,2011) buccal gingival recession defects ≥2 mm in depth,
- Full-mouth plaque and bleeding score of <15% and no probing depths >3 mm,
- Absence of non-carious cervical lesions (NCCLs) and non-detectable cemento-enamel junction (CEJ) at the defect sites,
- No history of previous periodontal plastic surgery at the affected sites.

Exclusion criteria were as follows:

- Presence of caries lesions or restorations in the cervical area,
- Intake of medications which impede periodontal tissue health and healing,
- Medical contraindications for periodontal surgical procedures,
- Pregnancy and lactation.

The patients were randomly assigned to receive TUN in combination with either DGG or SCTG. Randomization was performed by using a permuted block design with a computer random-number generator (allocation ratio of 1:1) to choose DGG or SCTG. Allocation concealment was obtained by sequentially numbered, opaque, sealed envelopes containing the treatment to the specific subject. Treatment assignment was registered by the clinician who assisted in the operations. The envelopes were opened immediately after the preparations of the recipient sites, and the treatment procedures were communicated to the operator.

2.1 | Sample size

The present study was powered to detect a true mean difference of at least 0.50 mm with the standard deviation (SD) of the differences of 0.45 mm between both treatment groups regarding recession depth (RD) after the 12-month follow-up according to previous studies (Zuhr et al., 2014). The minimum sample size thus required to be approximately 12 patients in per treatment group using $\alpha = .05$ and a power = %80 within a 95% confidence.

2.2 | Initial therapy and clinical examinations

Each study participant underwent a comprehensive periodontal examination and received full-mouth supragingival scaling, polishing and oral hygiene instructions (non-traumatic brushing technique). Surgical treatment of the recession defects was scheduled in case of full-mouth plaque score and full-mouth bleeding score <15%.

A single calibrated examiner (S.C.I.), who was masked to the treatment allocation, performed all clinical measurements. A calibration exercise was carried out to assess the intra-examiner reproducibility. RD measurements at a set of 20 recession defects were evaluated twice within 48 hours for each patient. Calibration was accepted if measurements at baseline and the 48- hour appointments were similar to the millimetre at ≥90% level.

Clinical measurements were recorded at baseline and at 3, 6 and 12 months after surgeries. The measurements were performed at the mid-buccal point of the treated tooth by a Williams periodontal probe (Nordent Manufacturing Inc.), using an individual resin stent to standardize the positioning of periodontal probe throughout the duration of the study. All measurements were rounded to the closest 0.5 mm. The following parameters were taken at each gingival recession defect:

- Plaque index (PI) (O'Leary et al., 1972) (+/−, %);
- Gingival index (GI) (Löe & Silness, 1963) (0–3);
- Bleeding on probing (BOP) (Ainamo & Bay, 1975) (+/−, %);
- Probing depth (PD);
- Clinical attachment level (CAL);
- RD was measured from gingival margin to CEJ.
- Recession width (RW) was defined as horizontal measurement of gingival recession at the CEJ level.
- Keratinized tissue height (KTH) was recorded as the distance from gingival margin to mucogingival junction (MGJ), with the MGJ location determined using a functional method (Guglielmoni et al., 2001).
- Gingival thickness (GT) was measured at mid-buccal aspect of the tooth, 1 mm apically from the base of the gingival sulcus by using K-file 15. The reamer was penetrated perpendicularly to the mucosal surface until a hard tissue was felt. The endodontic instrument was fixed with silicone stopper resin block-out material (Ultradent, USA) and the measurement was completed with an electronic caliper with 0.05 resolution (Alpha Tools Digital Caliper) outside the mouth.
- Mean root coverage (MRC) was calculated as [(Baseline RD) – (12 months RD)/Baseline RD] × 100%.
- Complete root coverage (CRC) was calculated as the percentage

of the teeth with recession defects having complete coverage achieved [(Teeth with CRC)/(All treated teeth)] × 100%.

An independent examiner (B.O.) evaluated the 12-month follow-up aesthetic outcome according to the root coverage aesthetic score (RES) (Cairo et al., 2009).

2.3 | Study outcomes

The primary endpoint of the present study was to investigate the difference of MRC values between study groups at 12 months postoperatively.

The secondary outcomes were as follows: (1) to evaluate the mean RD, RW, PD, CAL, KTH, GT and CRC values after 6 and 12 months between study groups; (2) to assess postoperative patient morbidity parameters (postoperative pain, patients' discomfort, sensitivity and bleeding) and the characteristics of the grafts harvested by these two different techniques histologically.

2.4 | Surgical procedure

All surgical procedures were implemented by the same periodontist (H.B.) in a microsurgical manner. Immediately before the surgery, the root surfaces were cleaned with plastic brushes and the contact points of the affected teeth were temporarily splinted to prevent the collapse of suspended sutures with a flowable, light-curing resin material (Dynamic flow, President Dental). Following local anaesthesia (Maxicaine FORT, Vem), preparation of the exposed root surfaces was done with Gracey curettes (Nordent). The modified TUN was performed at all recipient sites (Zuhr et al. 2007; Aroca et al., 2010). Initial sulcular incisions were performed with a microsurgical blade (Swann-Morton). A partial thickness tunnel was created by means of tunnelling knives (Helmunt Zepf) and extended beyond the MGJ. The adjacent papillary complex was carefully separated by full-thickness preparation and elevated in the buccal direction to achieve complete coronal tension-free advancement of the whole soft tissue complex.

Before harvesting the palatal grafts, the thickness of the palatal mucosa was measured using a 15 endodontic reamer at the point of 6 mm apical of the gingival margin in the area of the first or second premolar. In the DGG group, CTG was obtained from the area between distal line angle of the maxillary canine to the distal line angle of the maxillary first molar by means of intra-oral de-epithelialization of the FGG using a 2.3 mm diameter diamond burr (801G/023, EMS, Aldrich Co.) with NaCL 0.9% saline irrigation. After de-epithelialization, the graft was trimmed by using a N°15C blade (Swann-Morton) to achieve an optimal thickness of 1–1.5 mm (Figure 1). In SCTG group, graft was harvested through the SI approach (Hurzeler & Weng, 1999; Lorenzana & Allen, 2000). A horizontal single

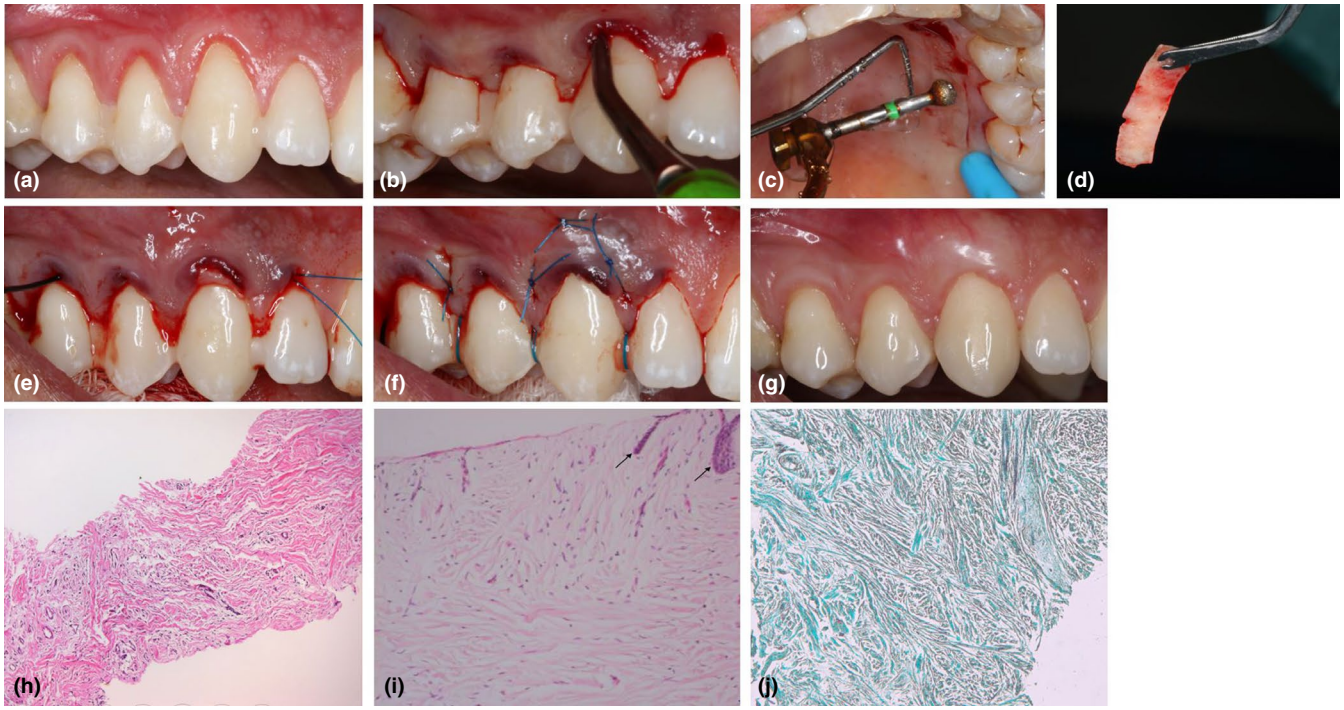


FIGURE 1 Treatment of multiple adjacent gingival recessions (MAGRs) with de-epithelialized gingival graft (DGG) plus tunnel technique (TUN). (a) Baseline; (b) Tunnelling flap preparation using tunnelling knives, coronal displacement of the flap; (c) Trimming of DGG; (d) Clinical view of DGG; (e) Placement of the graft into the tunnel; (f) Suturing; (g) 1 year after surgery; (h) A histological section of DGG biopsy sample, haematoxylin–eosin staining, original magnification ×10); (i) Epithelial remnants (arrow) in DGG sample, haematoxylin–eosin staining, original magnification ×20); (j) Masson's trichrome staining, original magnification ×10)

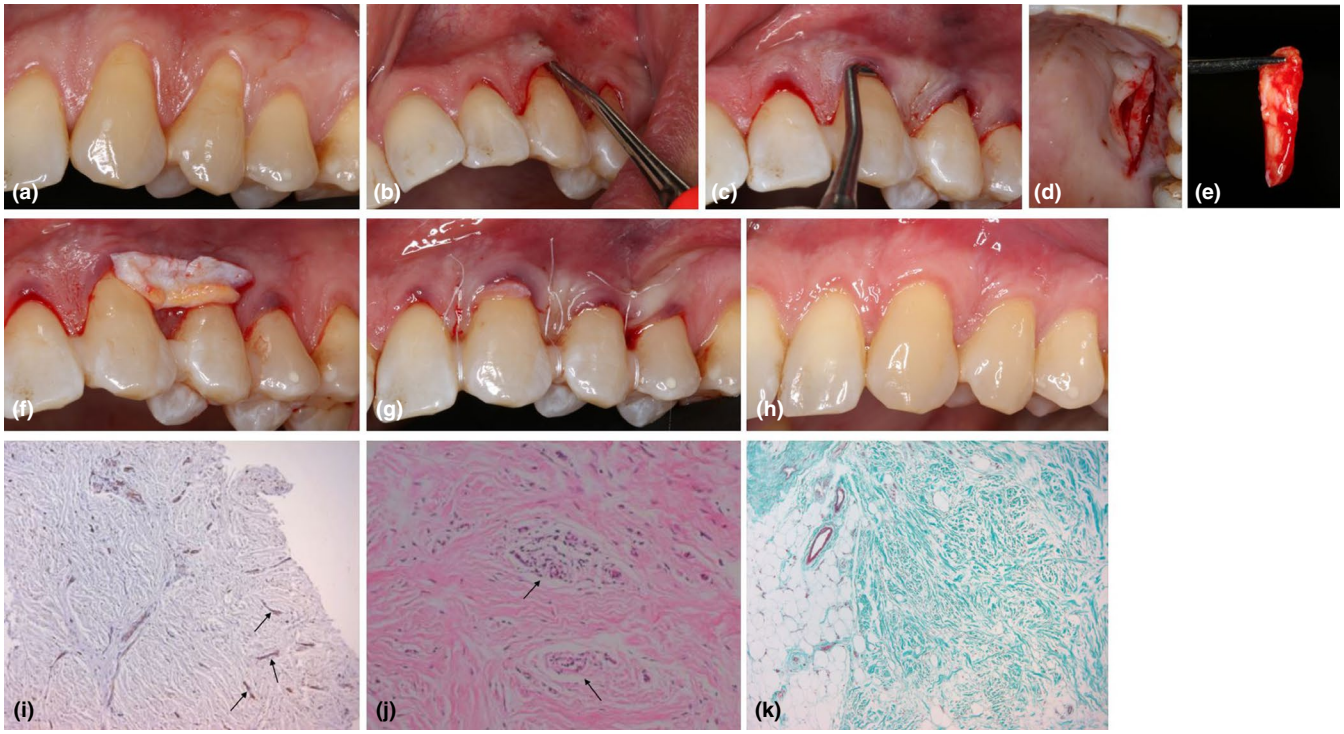


FIGURE 2 Treatment of multiple adjacent gingival recessions (MAGRs) with subepithelial connective tissue graft (SCTG) plus tunnel technique (TUN). (a) Baseline; (b) Tunnelling flap preparation using tunnelling knives; (c) Coronal displacement of the flap; (d) Harvesting of SCTG using single-incision technique; (e) Clinical view of SCTG; (f) Placement of the graft into the tunnel; (g) Suturing; (h) 1 year after surgery; (i) Vascular structures (arrow) in SCTG sample, (smooth muscle actin (SMA) original magnification ×10); (j) Perivascular lymphocytic cell infiltration (arrow) in SCTG sample (haematoxylin–eosin, original magnification ×20); (k) Wide adipose tissue adjacent to green stained collagen fibres, (Masson's trichrome, original magnification ×10)

incision was made parallel to palatal midline 2 mm apical to the gingival margin of the maxillary teeth, then a split-thickness dissection was performed in order to maintain a 1–1.5 mm uniform thickness (Figure 2). In both groups, palatal donor sites were closed using perpendicular cross sutures (5-0 silk sutures, Dogsan Sutures, Trabzon, Turkey) without performing any other intervention. Immediately after harvesting the grafts, the thickness, the width (mesial–distal dimension) and the height (apical coronal dimension) of the grafts were measured with a digital caliper (Alpha Tools Digital Caliper) with 0.05 resolution. The histological samples of the both groups were obtained by removing a 2 mm thick slice from the most distal portion of the grafts including the most coronal and apical parts of the grafts according to a recent study reported by Azar, Rojas, Mandalunis, e al. (2019).

Subsequently, the grafts were placed in the tunnel using mattress sutures at the mesial and distal aspects and adapted to the recipient site. The grafts were placed in the tunnel by positioning with the connective tissue section coronally and the adipose tissue section apically. Vertical double-crossed sutures (5-0 monofilament suture, Dogsan Sutures, Trabzon, Turkey) were used to stabilize the buccal soft tissue complex 1–2 mm coronally to the CEJ (Zuhr et al., 2009), and no additional suturing was performed for the stabilization of the grafts individually to the teeth. Duration of the each operation was recorded in minutes.

2.5 | Post-surgical protocol

All patients were advised to avoid mechanical trauma, tooth brushing and flossing at the surgical sites for postoperative 2 weeks until suture removal. To achieve plaque control, a 0.12% clorhexidine-gluconate mouthrinse (Klorhex Oral Rinse; Drogsan Pharmaceuticals) was instructed to use 2 times/day for 1 min. for the first 2 weeks. The patients were prescribed 600 mg ibuprofen (Brufen, Abbott) to take one at the end of the surgical intervention and one after 6 h after the surgery for anti-inflammatory therapy. They were also instructed to take additional analgesics in case of pain and to keep record the quantity of analgesics used (number and dosage) on a daily basis during the second weeks postoperatively. Following suture removal 2 weeks after surgery, plaque control was maintained using a soft tooth brush and a roll technique. At the 2-month postsurgery, patients were reinstructed to return to the regular oral hygiene habits. All patients received one session of prophylaxis, and oral hygiene reinforcement once a week in the first month and quarterly until the end of the study.

2.6 | Patient-reported evaluations

Immediately after surgery, a questionnaire was given to each patient evaluating postoperative pain, patients' discomfort,

sensitivity and bleeding considering their palatal sites at 1, 2, 3, 7, 14 and 28 days after surgery. All patients were asked to record their postoperative morbidity using a visual analog scale (VAS) from 0 to 10 and also to assess dichotomously (yes or no) for postoperative bleeding.

2.7 | Histological evaluation

Histological evaluation was performed by a senior oral pathologist (B.Y.), who was unaware of which CTG harvesting procedure the samples belong to, in serial sections (5 section for each sample). Soft tissue biopsies (2 × 2 × 1.5 mm) were obtained from harvested palatal grafts and fixed in 10% neutral buffered formalin solution and embedded in paraffin. The specimens were prepared at 5-μm-thick histological sections and stained with haematoxylin–eosin (H&E) and Masson's trichrome stain. Evaluation of epithelial remnants, inflammatory infiltrate and connective tissue cellularity was conducted on H&E sections. Structure of collagen fibres was investigated with Masson's trichrome stain. SMA antibody, which stains smooth muscle cells of capillaries so as to detect vascular structures, was used for immunostaining procedures. The immunohistochemical analysis was made using UltraVision Large Volume Detection System Anti-Polyvalent, HRP kit (Lab Vision Corp/Neomarkers). The sections were incubated in non-immune blocking serum at 5–10 min. Subsequently, they were incubated in ready-to-use SMA (anti α smooth muscle actin antibody, NeoMarkers) primary antibody for 3 h. The sections were then washed with phosphate-buffered saline (PBS) and allowed to stand for 5 min. They were incubated in secondary antibody 1 (HRP Universal kit) for 15 min. and washed again with PBS and left for 5 min. The same procedure was applied for secondary antibody 2 (HRP Universal kit). Finally, they were stained with AEC (aminoethylcarbazole) chromogen, passed through distilled water for 3 min. and stained with Mayers haematoxylin. The sections were kept in PBS for 3 min. and closed using water-based medium.

Descriptive morphologic analysis was performed evaluating the presence of inflammatory cells, connective tissue cellularity, vascularization, adipose tissue and collagen structure with a digital virtual microscopy system (Olympus) in 5 high power field (HPF) and a resolution of 0.321 mm/pixel (Bertl e al., 2015). Meanwhile, Masson's trichrome stained sections were photographed with a Leica DM4000 B (Leica Microsystems GmbH, Wetzlar, Germany) and the size and shape of collagen fibres and adipose tissue ratio were assessed at these images. The ratio of connective tissue cellularity was assessed into three categories as hypocellular (under 30%), normocellular (30%–70%) and cellular (over 70%). Evaluation of vascularization was scored (based on the number of vessels in 5 HPF) as following: 1 (1–10 capillaries), 2 (11–20 capillaries), 3 (21–30 capillaries) and 4 (31–40 capillaries). The entire evaluations were performed by the same oral pathologist using a random of 20 sample sections two times in different sessions, 1 week apart, to assess reproducibility.

2.8 | Statistical analysis

SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0) Statistics package program was used in data analysis. The recession defect was considered as the unit of statistical analysis. Mixed design ANOVA was used to compare the mean differences between groups that include between-subjects and within-subjects factors. The homogeneity of the variances, which is one of the prerequisites of parametric tests, was checked through Levene's test. Repeated measures ANOVA was analysed by Mauchy's sphericity test and Box's Test of Equality of Covariance Matrices. If parametric tests (factorial design for repeated measures analysis) do not provide the preconditions, Greenhouse–Geisser or Huynh–Feldt correction was used for corrections to the Degrees of Freedom. The Corrected Bonferroni test was used for multiple comparisons. To compare the RES value between the groups, Independent Student's t test was performed. Univariate parametric and nonparametric regression methods considering both patient and tooth/site levels in addition to the treatment modalities were performed in order to investigate factors (age, gender, tooth location, graft and palatal thickness, GT, KTH, RD, RW, connective tissue cellularity and vascularization) influencing the outcomes variables (MRC). $p < .05$ was accepted for the significance level of the tests.

3 | RESULTS

Twenty-seven patients (12 females, 15 males; mean age: 41.26 ± 11.43 years) contributed a total of 61 recession defects completed the 12-month follow-up study without any uneventful healing effects. Thirty of the recessions were treated in the DGG + TUN group, whereas 31 of them were treated in the SCTG + TUN group. Figure 3 shows the CONSORT flowchart diagram reporting the numbers of participants, who were randomly assigned, receiving intended treatments and analysing for the primary outcome of the present study. The details of patients' demographics and the characteristics of affected tooth, palatal tissues and grafts and surgical chair time for the study groups were presented in Table 1.

3.1 | Clinical outcomes

There were no statistically significant differences between the study groups in all the clinical parameters evaluated at baseline examination except for RW and also graft width. Plaque was not observed in the operated area in both groups at any study follow-up periods. The mean GI values decreased at 12 months compared to baseline for both groups; however, this reduction was statistically significant for only DGG + TUN group ($p = .007$). Similarly, significant differences were also seen at 12 months compared to baseline in DGG + TUN and SCTG + TUN groups for RD ($p = .001$, $p = .001$), RW ($p = .001$, $p = .001$), PD ($p = .014$, $p = .012$), CAL ($p = .001$, $p = .001$), KTH ($p = .001$, $p = .001$) and GT ($p = .033$, $p = .04$), respectively.

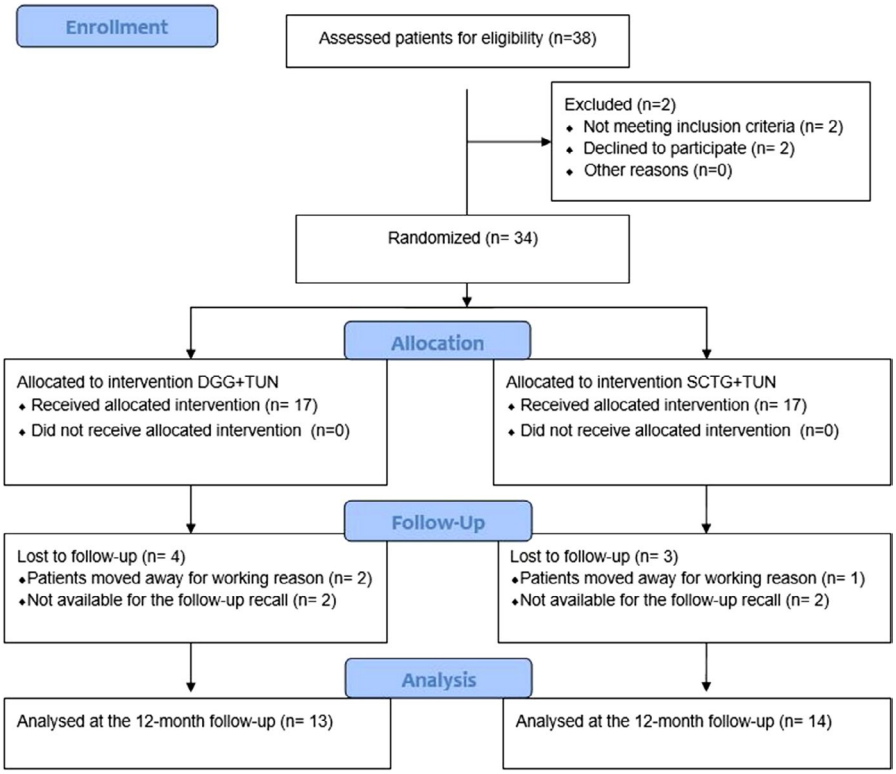


FIGURE 3 The CONSORT flow chart of the study

	DGG + TUN group (*n* = 30 teeth)	SCTG + TUN group (*n* = 31 teeth)
Patient	13 (5 males and 8 females)	14 (10 males and 4 females)
Age (range)	43.5 ± 9.26 years (26–60)	40.57 ± 12.17 years (22–60)
Tooth location		
Maxilla	15	19
Mandible	15	12
Type of teeth		
Upper Incisors	6	6
Lower Incisors	5	6
Upper Canines	4	4
Lower Canines	3	4
Upper Premolars	6	6
Lower Premolars	6	5
Palatal thickness (mm)	3.08 ± 0.42	3.0 ± 0.36
Graft thickness (mm)	1.40 ± 0.11	1.37 ± 0.11
Graft width (mm)	20.42 ± 3.53	17.57 ± 2.38[a]
Graft height (mm)	4.67 ± 0.49	4.64 ± 0.50
Surgical chair time (min)	70.42 ± 12.87	66.07 ± 10.59

TABLE 1 Demographic data on patients and the characteristics of affected tooth, palatal tissues and grafts and surgical chair time

Abbreviations: DGG, de-epithelialized gingival graft; SCTG, subepithelial connective tissue graft; TUN: Tunnel technique.

[a]Between groups, statistically significant difference ($p < .05$), Mann–Whitney *U* test.

At the12-month follow-up, the mean RD was 0.27 ± 0.99 mm in DGG + TUN group and 0.68 ± 0.99 mm in SCTG + TUN group and the difference was found to be significant in favour of DGG + TUN group. However, no significant difference was observed for the changes in RD value at 12 months compared to baseline between the groups. Whereas, KTH significantly increased at all study time-points during 12-month period in both groups without demonstrating statistically significant inter-group differences ($p > .05$). MRC

TABLE 2 Clinical parameters at baseline and 6 and 12 months postoperatively

	DGG + TUN group (n = 30 teeth)	SCTG + TUN group (n = 31 teeth)	p Value
PI (%)			
Baseline	0 ± 0	0 ± 0	
6 months	0 ± 0	0 ± 0	
12 months	0 ± 0	0 ± 0	
p Value†			
GI (0–3)			
Baseline	0.46 ± 0.50[a]	0.27 ± 0.42[a]	.061*
6 months	0.23 ± 0.43[b]	0.12 ± 0.34[b]	.297*
12 months	0.13 ± 0.34[b]	0.10 ± 0.30[b]	.661*
p Value†	.007	.437	
RD (mm)			
Baseline	2.63 ± 0.89[a]	3.0 ± 1.19[a]	.181*
6 months	0.52 ± 0.73[b]	0.76 ± 0.91[b]	.261*
12 months	0.27 ± 0.54[b]	0.68 ± 0.99[b]	.049*
p Value†	.001	.001	
RW (mm)			
Baseline	2.95 ± 0.87[a]	3.45 ± 0.68[a]	.015*
6 months	0.80 ± 0.99[b]	1.19 ± 1.42[b]	.217*
12 months	0.77 ± 1.16[b]	1.06 ± 1.41[b]	.374*
p Value†	.001	.001	
PD (mm)			
Baseline	1.03 ± 0.18[a]	1.16 ± 0.37[a]	.960*
6 months	1.13 ± 0.34[a]	1.35 ± 0.49[b]	.045*
12 months	1.40±056[b]	1.42 ± 0.56[b]	.894*
p Value†	.014	.012	
CAL (mm)			
Baseline	3.67 ± 0.93[a]	4.16 ± 1.30[a]	.094*
6 months	1.65 ± 0.72[b]	2.08 ± 1.13[b]	.083*
12 months	1.66 ± 0.67[b]	2.09 ± 1.11[b]	.074*
p Value†	.001	.001	
KTH (mm)			
Baseline	2.70 ± 1.29[a]	2.42 ± 1.18[a]	.378*
6 months	3.97 ± 1.03[b]	3.48 ± 0.93[b]	.059*
12 months	4.20 ± 1.03[c]	4.13 ± 0.96[c]	.781*
p Value†	.001	.001	
GT (mm)			
Baseline	0.73 ± 0.19[a]	0.74 ± 0.20[a]	.840*
6 months	1.54 ± 0.44[b]	1.51 ± 0.38[b]	.460*
12 months	1.58 ± 0.54[b]	1.57 ± 0.29[b]	.845*
p Value†	.033	.04	
MRC (%)			
6 months	84.72 ± 19.72	79.62 ± 22.51	.001‡
12 months	91.72 ± 16.59	83.16 ± 23.32	.001‡
CRC (%)			
6 months	70.0 (21/30)	48.4 (15/31)	.001‡
12 months	76.7 (23/30)	61.3 (19/31)	.001‡

Abbreviations: CAL, Clinical attachment level; CRC, Complete root coverage; DGG, De-epithelialized gingival graft; GI, Gingival index; GT, Gingival thickness; KTH, Keratinized tissue height; MRC Mean root coverage; PD, Probing depth; PI, Plaque index; RD, Recession depth; RW, Recession width; SCTG, Subepithelial connective tissue graft; TUN, Tunnel technique.

*The Corrected Bonferroni test (after the mixed design ANOVA DGG + TUN group- SCTG + TUN group), $p < .05$ considered statistically significant.

†Repeated measures of analysis of variance, $p < .05$ considered statistically significant. Different letters in the same column represent statistically significant difference between values ($p < .05$).

‡Mann–Whitney U test (DGG + TUN group- SCTG + TUN group).

was 91.72% ± 16.59% in DGG + TUN group, while 84.72% ± 19.72% in SCTG + TUN group at the 12-month examination with showing significant differences between the two groups (p = .001). CRC was achieved in 23 of the 30 recession defects in DGG + TUN group (76.7%), while in 19 of 31 recessions in SCTG + TUN group (61.3%) and the difference was statistically significant in favour of DGG + TUN group, as was MRC (p = .001). In both groups, MRC and CRC were found to be slightly increased at the 12-month follow-up, as compared to the 6-month follow-up. Regarding the aesthetic assessment, the final average RES was 8.76 ± 1.52 and 8.58 ± 1.85 in DGG + TUN and SCTG + TUN groups, respectively, without any statistically significant inter-group differences (p = .67). Table 2 shows the clinical outcomes of the study groups at 6 and 12 months postoperatively.

3.2 | Postoperative patient morbidity outcomes

No significant differences were observed between DGG + TUN and SCTG + TUN groups for each time point in the follow-up period in respect to postoperative pain, patients' discomfort, sensitivity and the amount of systemic analgesic consumption (Table 3). Postoperative bleeding was seen only at the first day after the operations for both group. It was observed in 4 (33.3%) and 2 (15.3%) cases for DGG + TUN and SCTG + TUN groups, respectively (p = .544).

3.3 | Histological assessments

DGG samples showed partly epithelial remnants in superficial portions. The collagen fibres were mostly observed as normal and coarse, and demonstrated normal organization in the cells poor hypocellular connective tissue. Only three of the samples showed

TABLE 3 Postoperative patient morbidity parameters for the study follow-up periods

	DGG + TUN (n = 13)	SCTG + TUN (n = 14)	p Value
Postoperative pain			
1 day	3.83 ± 1.9[a]	3.5 ± 2.44[a]	.70*
2 days	1.67 ± 0.89[b]	1.79 ± 2.04[b]	.85*
3 days	1.25 ± 1.76[b]	1.36 ± 1.69[b]	.88*
7 days	0.58 ± 1.0[c]	0.79 ± 1.72[c]	.72*
14 days	0.08 ± 0.29[c]	0.07 ± 0.27[c]	.91*
28 days	0 ± 0	0 ± 0	
p Value	.001‡	.001‡	
Patients' discomfort			
1 day	3.17 ± 2.08[a]	3.21 ± 2.99[a]	.96*
2 days	1.42 ± 1.08[b]	1.0 ± 1.47[b]	.43*
3 days	1.33 ± 2.06[b]	0.71 ± 0.99[c]	.36*
7 days	0.42 ± 0.9[c]	0.32 ± 0.82[c]	.78*
14 days	0 ± 0	0.64 ± 2.13[c]	.28*
28 days	0 ± 0	0 ± 0	
p Value	.001‡	.001‡	
Sensitivity			
1 day	2.08 ± 2.64[a]	2.57 ± 2.34[a]	.62*
2 days	0.92 ± 1.16[b]	1.71 ± 1.86[b]	.20*
3 days	0.92 ± 1.38[b]	1.21 ± 1.85[b]	.65*
7 days	0.5 ± 0.9[c]	0.64 ± 1.34[c]	.76*
14 days	0.33 ± 0.89[c]	0.29 ± 0.83[c]	.89*
28 days	0 ± 0	0.08 ± 0.28[d]	.34*
p Value	.001‡	.001‡	
The amount of systemic analgesic§ consumption (mg)	2968.4 ± 2167.7	1989.4 ± 1918.8	.149†

Abbreviations: DGG, de-epithelialized gingival graft; SCTG, subepithelial connective tissue graft; TUN: Tunnel technique.

*General linear mixed model (multiple comparisons by group) Bonferroni–Dunn test.

†General linear mixed model (one of the factors in factorial order is repeated analysis of variance) with interaction, $p < .05$ considered statistically significant. Different letters in the same column represent statistically significant difference between values ($p < .05$)

‡General linear mixed model (multiple comparisons on time basis) Bonferroni–Dunn test.

§Ibuprofen.

minimal mono-nuclear inflammatory cell infiltration while adipose tissue was found in five samples. The mean number of vessels seen in the DGG samples was determined as 16.7. In SCTG samples, the collagen fibres were observed as in DGG samples; however, the connective tissue presented more cellularity compared to the SCTG samples. Four of the SCTG samples showed mono-nuclear inflammatory cell infiltration. Adipose tissue was present in all the samples (100%). The mean number of vessels seen in the samples was 18.

TABLE 4 Evaluation of the graft samples in terms of presence of inflammatory cell, connective tissue cellularity and vascularization

Parameters % (n)	DGG + TUN (n = 13)	SCTG + TUN (n = 14)	p Value
Inflammatory infiltrate			
Absent	76.9% (10)	78.6% (11)	.918*
Present	23.1% (3)	21.4% (3)	
Connective tissue cellularity (fibroblasts)			
Hypocellular	61.5% (8)[bA]	21.4% (3)[aA]	.024†
Normocellular	30.8% (4)[aB]	42.9% (6)[aB]	
Cellular	7.7% (1)[bC]	35.7% (5)[aA]	
Vascularization			
0–10	7.7% (1)	7.1% (1)	.749*
11–20	53.8% (7)	64.3% (9)	
21–30	30.8% (4)	14.3% (2)	
31–40	7.7% (1)	14.3% (2)	

Abbreviations: DGG, de-epithelialized gingival graft; SCTG, subepitelial connective tissue graft; TUN, Tunnel technique.

*Chi-squared test, $p < .05$ considered statistically significant.

†Repeated measures of analysis of variance, $p < .05$ considered statistically significant. Differences between groups are expressed in lower case letters and intra-group changes are expressed in capital letters. Different letters in the same column represent statistically significant differences between values ($p < .05$).

Percentage of inflammatory infiltrate was similar in both groups. When the groups were examined in terms of cellularity, SCTG group was more cellular than the DGG group ($p < .05$). Although normocellularity rate was higher in SCTG group, there was no significant difference between the groups. No significant difference was observed between the groups regarding vascularization (Table 4).

3.4 | Regression analyses

A multilevel linear regression analysis was performed to investigate the impact of the treatment modalities, patient characteristics (age, gender and tooth location), baseline clinical tooth- and site-related parameters (graft and palatal thickness, GT, KTH, RD, RW), and histomorphometric features of the grafts (connective tissue cellularity and vascularization) on the outcome variable MRC at 12 months of follow-up surgery. Regression analysis demonstrated that the mean GT and KTH values at baseline were correlated with statistically significant positive effects on MRC ($p = .004$, and $p = .013$, respectively) while mean RD values at baseline were a significant negative prognostic factor in the amount of MRC ($p = .02$). However, treatment method was not a predictive factor for MRC outcome ($p = .911$). The multilevel regression model is presented in Table 5.

4 | DISCUSSION

The present study aimed to compare clinical efficacy and postoperative morbidity of DGG + TUN and SCTG + TUN in the treatment of

TABLE 5 Multilevel linear regression analysis of variables affecting the primary outcome

	Unstandardized coefficients		Standardized coefficients			95% Confidence Interval for *B*		Collinearity statistics	
Model	*B*	*SE*	Beta	*t*	*p*	Lower bound	Upper bound	Tolerance	VIF
(Constant)	111.442	68.488		1.627	.111	−26.772	249.656		
Age	−0.049	0.259	−0.023	−0.189	.851	−0.572	0.474	0.772	1.296
Gender	−5.375	5.941	−0.130	−0.905	.371	−17.365	6.615	0.538	1.859
Tooth location	1.416	7.349	0.034	0.193	.848	−13.416	16.247	0.351	2.845
GT	50.425	16.311	0.457	3.092	.004*	17.508	83.341	0.509	1.965
Palatal thickness	2.460	7.224	0.046	0.341	.735	−12.118	17.039	0.603	1.660
Graft thickness	−9.600	24.010	−0.051	−0.400	.691	−58.020	38.821	0.675	1.482
Cellularity	−7.041	3.784	−0.247	−1.861	.070	−14.677	0.596	0.631	1.585
Vascularization	−1.451	3.746	−0.055	−0.387	.700	−9.011	6.108	0.553	1.808
KTH	5.146	1.994	0.293	2.581	.013*	9.169	1.122	0.864	1.157
RW	−0.872	4.217	−0.031	−0.207	.837	−9.382	7.638	0.507	1.971
RD	−6.009	2.475	−0.319	−2.428	.020*	−11.003	−1.015	0.645	1.549
Treatment methods	−0.709	6.308	−0.017	−0.112	.911	−13.438	12.021	0.496	2.018

Abbreviations: GT, gingival thickness; KTH, keratinized tissue height; RD, recession depth; RW, recession width; VIF, variance inflation factor.
*R^2 = 74.6%, $p < .05$.

RT1 multiple-recession type defects which, to the best of our knowledge, have not been previously assessed. The results demonstrated that DGG + TUN showed higher MRC and CRC outcomes as well as lower RD at 12 months compared to SCTG + TUN. Nevertheless, CTG harvesting approach (DGG or SCTG) was not a predictive factor of MRC outcome. Both groups revealed comparable postoperative patient-centred outcomes during 28 days after the surgery.

The localization of palatal harvesting and the composition of CTG have been suggested to influence clinical outcomes of RC procedures as well as postoperative patient morbidity (Zucchelli et al., 2010; Tavelli et al., 2019). DGG approaches obtained with both extra-oral (Harris, 2003; McLeod et al., 2009; Zucchelli et al., 2010; de Sanctis et al, 2011; Vieira et al., 2017) and intra-oral (Ozcelik et al., 2016) de-epithelialization of the FGGs- in combination with CAF or TUN procedures have been shown promising results in periodontal plastic surgeries (McLeod et al., 2009; Zucchelli et al., 2010, 2014; Ozcelik et al., 2016). Because of the fact that DGG has been demonstrated to have higher amount of fibrous connective tissue and less amount of fatty and glandular tissue compared to conventional SCTG substitutes (Bertl et al., 2015; Pall et al., 2017), DGG has been found to associate with decreased shrinkage of the grafts and increased GT (Zuhr et al., 2014). Tavelli et al. (2019) highlighted in their systematic review that these features of DGG could contribute to enhanced GR reduction and MRC. It was also indicated slightly superior outcomes regarding GR reduction, MRC, KT gain, PD reduction and CAL gain in favour of DGG + CAF, compared to SCTG + CAF. The present findings are particularly in line with those previous ones that DGG + TUN, in comparison with SCTG + TUN, presented significantly higher MRC (91.72% ± 16.59% and 84.72% ± 23.32%, respectively) and CRC (76.7% and 61.3%, respectively) while, both groups presented similar KTH and GT at the 12-month postoperatively. However, Zucchelli et al. (2010) reported comparable MRC and CRC for DGG + CAF versus SCTG + CAF (96.2% ± 8.93% and 92.28% ± 13.06%; 84% and 72%, respectively) at 12-month follow-up. They explained that the lack of significance between the groups regarding RC outcomes could be due to the great within-group variability, which would have required a larger sample size. However, they found a significant difference in respect of GT gain favouring DGG + CAF, compared to SCTG + CAF (0.80 ± 0.17 mm and 0.61 ± 0.16 mm, respectively). In the present study, GT gain was observed as 0.86 ± 0.52 mm for DGG + TUN and 0.83 ± 037 mm for SCTG + TUN at the 12-month follow-up, demonstrating no significant difference between the groups. On the other hand, a slightly increasing trend of the clinical results regarding MRC, CRC, KTH and GT obtained for both study groups was noted between 6 and 12 months follow-ups, although these differences resulted statistically not significant except for KTH measurements in this study. These increases may be explained, at least in part, by the creeping attachment occurred during the 12-month study period.

The limited data in the available literature regarding the evaluation of DGG and conventional SCTG efficacy in combination with TUN on RC outcomes made the exact comparison of the current findings with previous ones difficult. In a recent RCT, Pietruska et al. (2019) compared DGG + TUN and collagen matrix (CM) + TUN in the treatment of MAGRs and reported significant differences regarding MRC, CRC, RD and RW favouring DGG + TUN at 12 months after the surgery. MRC and CRC were 83.10% and 67%, respectively, whereas, RD reduction was 1.54 ± 0.58 mm in their study, which was

slightly lower compared to the present data of DGG + TUN group. In another previous report, Zuhr et al. (2014) performed the treatment of GR defects with SCTG harvesting by SI technique in combination with TUN. It was demonstrated that MRC was 98.4 ± 3.6% and CRC was 78.6%, which were higher according to the present findings of SCTG + TUN group. The difference could be attributed with that both single and multiple GR defects were included to that study by Zuhr et al. (2014). It was speculated that recession defects features, either being single or multiple adjacent, might interfere with passive displacement of the flap in apico-coronal position because of the flap extension at a larger/smaller area (Zuhr et al., 2014; Tavelli et al., 2018). Our results are also in accordance with a previous research reported by Aroca et al. (2013), which evaluated the efficacy of SCTG + TUN procedures in the treatment of Miller class I and II MAGRs and showed similar RC outcomes (MRC: 90 ± 18%; CRC: 59%) at the 12-month follow-up.

Several prognostic factors including patient-, defect- and procedure-related factors have been reported as affecting the RC outcomes and should be taken into consideration in the clinical setting (Chambrone et al., 2012; Pini-Prato et al., 2015). The predictive impact of baseline defect and gingival anatomical characteristics on the results of RC procedures have been investigated in many studies (Pini Prato et al., 2018; Rasperini et al., 2018; Rasperini et al., 2020). According to those studies, the present multilevel model analysis showed that the initial GR and KTH were significant positive prognostic factors for MRC. On the other hand, initial RD was found to be negatively associated with MRC. Although mean RW at baseline was significantly higher in SCTG + TUN group compared to DGG + TUN, the present model revealed no significant effect of initial RW on MRC. It is also noteworthy that treatment was not a prognostic factor for MRC, despite significant differences in MRC between the two treatments.

Although DGG approaches give rise to palatal wound healing by secondary intention, which was previously associated with increased postoperative patient morbidity (Del Pizzo et al., 2002; Wessel & Tatakis, 2008). Zucchelli et al. (2010) demonstrated comparable postoperative patient-centred outcomes (pain, discomfort and bleeding) for DGG and SCTG harvesting procedures. Similarly, the present results did not reveal any significant differences between the groups in terms of postoperative patient-related parameters. Meanwhile, the amount of systemic analgesic consumption during postoperative 2 weeks was reported as 2016 ± 1896.4 mg and 1656 ± 1532.2 mg for DGG + CAF and SCTG + CAF, respectively, presenting no significant difference between the groups, in the same study performed by Zucchelli et al. (2010). In line with these findings, the amount of systemic analgesic consumption of the present study was 2968.4 ± 2167.7 mg and 1989.4 ± 1918.8 mg in DGG + TUN and SCTG + TUN, with no statistically significance between the groups. Our results were higher from that study, most probably because of the fact that the present study was performed for the treatment only in MAGRs, which made the recipient sites considerably wider compared to single GRs. Moreover, it has been reported that TUN, which is a more technique-sensitive approach, is required longer surgical chair time and associated with higher patient morbidity than CAF procedures (Gobbato et al., 2016).

The present study also demonstrated that after the de-epithelialization procedures, epithelial remnants were partly observed in superficial portions of DGG as stated in the previous studies (Pall et al., 2017; Romano et al., 2017; Azar, Rojas, Mandalunis, et al., 2019). The effect of epithelial remnants on long-term RC outcomes of DGG procedures remains unknown (Romano et al., 2017). In the present study, DGG showed superior MRC and CRC results at the 1-year follow-up than SCTG, even if all DGG samples exhibited epithelial remnants. On the other hand, the present study revealed that the ratio of connective tissue cellularity in DGG samples was found to be significantly lower than SCTG samples. This finding supports a previous histologic study in fresh human cadavers in which de-epithelialization harvested CTGs appeared to have larger amounts of fibrous connective tissue and lower cellular structures (Bertl et al., 2015). Our study also revealed no difference between DGG and SCTG samples in terms of the number of vessels and graft vascularity. The current findings were particularly in line with a recent cadaver study, which presented no significant difference regarding the mean total number of vessels and small vessels between DGG and SCTG obtained with TD technique (Tavelli et al., 2020). Nevertheless, SCTG samples exhibited a significantly higher mean number of medium and large vessels compared to DGG samples in that study. This finding was attributed to that SCTG harvested by TD technique has been obtained from a deeper layer of palatinal mucosa including larger blood vessels and nerves. Similarly, a recent histological and immunohistochemical study was also demonstrated larger blood vessels in deeper connective tissue compared to the connective tissue closest to the epithelial layer (Perotto et al., 2017). An interesting finding of the present study was that some samples showed minimal mono-nuclear inflammatory cell infiltration in both groups. Indeed, the lamina propria of oral mucosa contains a collection of immune cells (macrophages, langerhans cells, B cells, and T cells) that either initiate a response to pathogenic micro-organisms, or induce a state of immune non-responsiveness (Novak et al., 2008; Merry et al., 2012). The lymphocyte and plasma cell can be histologically observed in small numbers scattered throughout the lamina propria; however, other inflammatory cells such as monocytes and macrophages are found in significant numbers only in connective tissue following an injury or as apart of a disease process (Nanci, 2017).

It is reasonable to assume that the cellularity component can vary and be dependent upon the area of the graft which was analysed and does not necessarily dictate the entire section. However, it is possible to make an accurate comparison of tissue composition between the grafts obtained by different techniques by standardizing a particular sample area, which can be a limitation of the present study. In this study, the histological samples were obtained by removing a 2 mm thick slice from the most distal portion of the grafts in both groups. The present study included MAGRs in both upper and lower jaws which could be considered as another limitation of the present study. Taking into consideration that tooth location could play a critical role in determining the amount of achievable

RC, the distribution of maxillary and mandibulary recessions in the groups was revealed to present no significant difference. It should be emphasized that treatment outcomes may be influenced by patient-, defect-, and procedure-related factors. RD, KTH and GT, which were significantly correlated with MRC in the present study, exhibited favourable baseline values in the DGG + TUN group, although baseline inter-group differences for these parameters were not significant. These discrepancies might have affected mean RC outcomes (MRC and CRC), which showed a statistically significant difference in favour of DGG + TUN group and might have contributed to the finding that treatment was not a prognostic factor for MRC outcome. Another limitation of the present study could be the lack of histological analysis of the recipient sites after the surgery. However, it seemed to not possible to take samples from each recipient site because of all treated sites could not demonstrate CRC or excessive gingival tissue allowing gingivoplasty procedures at the end of the treatment. Therefore, this study was aimed to compare the histopathological characteristics of CTG harvesting by two different techniques and to correlate these features with clinical outcomes.

5 | CONCLUSION

Within its limitations, the present investigation showed that DGG combined with TUN resulted in higher MRC and CRC compared to the SCTG + TUN combination when treating MAGRs, although treatment method was not a prognostic factor for MRC outcome. This apparent discrepancy is possibly explained by the fact that treatment outcomes can be influenced by certain identified prognostic parameters such as baseline defect (RD) and gingival anatomical (GT, KTH) characteristics were presented higher MRC and CRC compared to SCTG in the treatment of MAGRs. Both procedures showed similar outcomes in terms of patient's postoperative morbidity and aesthetic evaluation at the 1-year follow-up.

CONFLICT OF INTEREST

The authors report no conflict of interests related to the study

AUTHOR'S CONTRIBUTION

Burcu Ozdemir and Sila Cagri Isler involved in study design and drafting manuscript: Hikmat Bakhishov conceived the ideas and data analysis; Hikmat Bakhishov and Bejna Bozyel involved in data collection; Benay Yıldırım and Hikmat Bakhishov conducted histological evaluation of the samples; Mustafa Agah Tekindal performing statistical analysis; Burcu Ozdemir and Sila Cagri Isler involved in concept and critical revision of the manuscript.

DATA AVAILABILITY STATEMENT

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

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