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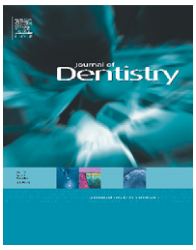

Root coverage stability of the subepithelial connective tissue graft and guided tissue regeneration: A 30-month follow-up clinical trial

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ABSTRACT

Objectives: The aim of this study was to compare the long-term clinical effects produced by subepithelial connective tissue graft (SCTG) and guided tissue regeneration combined with demineralized freeze-dried bone allograft (GTR-DFDBA) in the treatment of gingival recessions in a 30-month follow-up clinical trial.

Methods: Twenty-four defects were treated in 12 patients who presented canine or premolar Miller class I and/or II bilateral gingival recessions. GTR-DFDBA and SCTG treatments were performed in a randomized selection in a split-mouth design. The clinical measurements included root coverage (RC), gingival recession (GR), probing depth (PD), clinical attachment level (CAL) and keratinized tissue width (KTW). These clinical parameters were evaluated at baseline and after 6, 18 and 30 months post-surgery.

Results: The changes in RC, GR, PD and CAL did not show significant differences between groups ($p > 0.05$). Both procedures promoted similar RC (GTR-DFDBA: 87% and SCTG: 95.5%) and similar reduction in GR (GTR-DFDBA: 3.25 mm and SCTG: 3.9 mm), PD (GTR-DFDBA: 1.6 mm and SCTG: 1.2 mm) and CAL (GTR-DFDBA: 4.9 mm and SCTG: 5.0 mm). The increase in KTW was significantly higher ($p = 0.02$) in the SCTG group (3.5 mm) than in the GTR-DFDBA group (2.4 mm).

Conclusions: Both techniques for treatment of gingival recession (SCTG and GTR-DFDBA) lead to favourable and long-term stable results, but SCTG promoted a more favourable increase in keratinized tissue.

1. Introduction

Different treatment modalities have been described to obtain root coverage in gingival recessions. Among them, subepithelial connective tissue graft (SCTG) is the surgical procedure that promotes the most predictable and satisfactory results for root coverage and aesthetics. [1–17] However, the SCTG technique requires a second surgical site for a donor area of connective tissue, which may cause more discomfort to the patient. [3,18] Hence, the guided tissue regeneration (GTR) technique with the use of barriers was proposed by Tinti and Vincenzi [19] in an attempt to promote stable root coverage and the formation of new cementum (the periodontal ligament and alveolar bone covering the exposed root surface). There are nonresorbable and resorbable membranes for GTR

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procedures[19,20]; however, the use of nonresorbable barriers requires a second intervention for membrane removal, whereas the resorbable barrier does not need a second surgical intervention for that purpose. Thus, the resorbable membranes seem to be more comfortable for patients during the healing phase.[20]

Several comparative studies have been performed between SCTG and GTR techniques[2,4–11,21]; however, few studies have compared the procedures of GTR and SCTG to each other over a longer period of time. Some authors have demonstrated that the amount of root coverage presents no significantly difference between GTR and SCTG procedures, [2,4,5,7,8,10,11,21] whereas other studies have shown that SCTG yields significantly better results than GTR.[6,9] A recent systematic review showed that SCTG, coronally advanced flaps alone or combined with biomaterials and also GTR may be used as root-coverage procedures for the treatment of localized recession-type defects, but the authors highlighted that the use of SCTG seemed to be more adequate where root coverage and gain in keratinized tissue were expected.[22] The aim of this study was to clinically evaluate the long-term (30-month) results of 2 therapeutic modalities for the treatment of gingival recessions: GTR with a bioabsorbable barrier membrane and SCTG.

2. Materials and methods

2.1. Subject population

Twelve patients (3 men and 9 women; mean age = 39 years) were selected for the study among those undergoing periodontal treatment at Araraquara Dental School of São Paulo State University (UNESP) from October 1996 to 2000. To be enrolled in the study, the patients had to fulfil the following inclusion criteria: age between 25 and 60 years, probing depth $\leq$4 mm in all teeth, and a minimum of 3 mm of gingival recession (Miller Class I and II) at mid-buccal surfaces of maxillary canines or pre-molars bilaterally. Patients were excluded from the study if they met any of the following exclusion criteria: diabetes mellitus status, pregnancy or lactation, physical or mental handicap that could interfere with adequate oral hygiene control, current smoker or former smoker (of five years or greater standing), use of systemic and/or topical steroidal and non-steroidal anti-inflammatory drugs during the last 3 months, antibiotic consumption during the last 6 months before the surgical procedures, a condition requiring prophylactic antibiotic coverage before invasive dental procedures or occlusal interferences. Gingival tissues with thinner phenotypes were excluded from the study, which showed features of translucency and prominent dental roots following the bone contour in the vestibular region.

Before entering surgery, patients were given oral and written information on the study design and purpose in order to obtain informed consent. The study design was approved by the local ethics committee of UNESP, and it conformed to the requirements of the World Medical Association Declaration of Helsinki. This study is a follow-up of a previously published paper with an 18-month follow-up period.[10]

2.2. Sample size and power

The sample size was based on earlier studies.[6,23,24] A posteriori power statistical test was performed based on our results for dependent means, considering root coverage data on the 30-month post-surgery for the GTR-DFDBA (87 $\pm$ 17.2) and SCTG (95.5 $\pm$ 10.1) groups. The power analysis was performed at the end of the study to ensure that the sample used was sufficient to uncover clinically and statistically significant differences. The power was calculated by a post hoc *t* test (G Power® 3.0.10., Faul F et al., Bonn, Germany), and it was estimated as 0.99 with error $\alpha = 0.01$.

2.3. Examiner calibration

A single examiner evaluated all periodontal clinical parameters (RACM). For calibration, clinical attachment level was randomly evaluated in two sextants per patient in four subjects on 2 different occasions, one week apart. The data were submitted to the Student's *t*-test, and calibration was approved, shown by the fact that there were no statistically significant differences in the evaluation between the two occasions ($p > 0.05$). Pearson's correlation between the two measurements revealed a very high correlation ($r = 0.92$). The calibration was repeated before each re-evaluation.

2.4. Experimental design

Six to eight weeks before entering the surgical procedures, the patients were enrolled in the study according to the inclusion criteria. Then, scaling and root planning by quadrants were performed and oral hygiene instruction was provided. After completion of the non-surgical periodontal treatment, the following periodontal parameters were assessed: gingival recession (GR), probing depth (PD), clinical attachment level (CAL) and keratinized tissue width (KTW).

The percentage of root coverage 30 months post-surgery was also calculated as described by Rosetti et al. [10] All measurements were obtained at the mid-buccal portion of each tooth by a single trained calibrated examiner (RACM) at baseline (0 week), 6, 18 and 30 months after the surgical procedures. The data of previous findings at baseline, 6 and 18-month follow-up were compared with the data obtained in the 30-month follow-up.

2.5. Surgical procedure and postoperative care

The surgical treatments were randomized by aleatory selection in a split-mouth design by an experienced clinician (EPR). A simple randomization was performed by flipping a coin, for example, heads as control and tails as treatment determined the assignment of each treatment group. The Guided Tissue Regeneration combined with demineralized freeze-dried bone allograft (DFDBA, Dembone, Pacific Coast Tissue Bank, Los Angeles, CA) was considered as the test group (GTR-DFDBA), and the subepithelial connective tissue graft (SCTG) procedure group was the control group (''gold standard''). The same surgical preparation was performed for SCTG and GTR-DFDBA at the recipient sites with an intrasulcular incision and 2 vertical incisions made in a trapezoidal formation with the

removal of epithelial tissue at the mesial and distal papillae. The donor connective tissue was obtained from the palate using the technique described by Bruno[25] for the SCTG procedure, while a bioabsorbable collagen membrane[26] (University of São Paulo, São Carlos, SP, Brazil) was placed over the root surface, which overextended at least 3 mm over the margin of the bone defect. This membrane is not commercially available, but previous study performed by our research group demonstrated that the membrane provided effective blockade of epitelial tissue and promoted regeneration of lost periodontal tissues.[26]

DFDBA was placed under this membrane and the flaps of SCTG and GTR-DFDBA were coronally positioned and sutured. The postoperative care and medications for pain control were prescribed according to Rosetti et al.[10] Silk sutures were removed eight days after surgery, and chlorhexidine 0.12% (Periogard, Colgate–Palmolive, Osasco, SP, Brazil) was applied topically on the surgical sites with a cotton swab twice a day for 2 months without mechanical cleaning of the surgical sites.

2.6. *Statistical analysis*

Data analyses were performed using BioStat 5.0 software (BioEstat®, Belém, PA, Brazil). All data were analyzed for distribution using the Kolmogorov–Smirnov test. Data from the periods (baseline, 6, 18 and 30 months) that proved to be parametric were compared by repeated measures ANOVA, whereas the non-parametric data were compared by Friedman's test. The statistical analysis for comparisons between treatment groups (GTR-DFDBA versus SCTG) was performed using Paired-*t* test for parametric variables, and Wilcoxon for non-parametric data. Differences were considered significant when $p \leq 0.05$.

3. Results

All patients were followed for a period up to 30 months, and the differences among periods between the GTR-DFDBA and SCTG groups are shown in Table 1. Comparisons between 18 and 30 months revealed no statistically significant differences for parameters RC, GR, PD and CAL in the GTR-DFDBA and SCTG groups ($p > 0.05$), except for KTW, which showed significant changes in the GTR-DFDBA group ($p < 0.01$, Table 1). Table 2 shows the differences between treatments at the 30-month follow-up. A statistically significant difference between GTR-DFDBA and SCTG groups was only verified in the increase in KTW; the SCTG group presented higher values than the GTR-DFDBA group (3.5 mm × 2.4 mm). Overall, GTR-DFDBA and SCTG treatments showed similar changes in RC (87.0 × 95.5%), GR (3.25 mm × 3.9 mm), PD (1.6 mm × 1.2 mm) and CAL (4.9 mm × 5.0 mm). The aesthetic analysis demonstrated improvement using both treatments without significant differences between them at 18 months.[10] There are no differences between the results of aesthetic evaluation conducted after 18 and 30 months.

4. Discussion

Several studies have evaluated the treatment outcomes for GTR alone[23,27–32,33] and SCTG alone.[1,3,12–16,28,34–36] However, few studies have compared these two surgical procedures over a long period of time. Our findings showed that the GTR-DFDBA and SCTG treatments for gingival recessions promoted stable root coverage (RC) in a long-term follow-up. The high percentage of RC was verified at 18 months for GTR-DFDBA (84.25%) and SCTG groups (95.7%) and was maintained up to 30 months (GTR-DFDBA: 87% and SCTG: 95.9%). Some differences were verified among the results from the present study and the previous by Rosetti et al.[10] The previous study showed that GTR was superior to SCTG when PD was evaluated at 18 months, while the present data at 30-month follow-up revealed that PD values were similar between GTR and SCTG groups, and only KTW showed statistically significant difference. The effectiveness of both treatments in obtaining stable RC was also demonstrated in other comparative studies using these same techniques.[5,7,11,21]

Table 1 – Comparisons (mean ± standard deviation) among periods of the periodontal parameters.

	Baseline	6 months	18 months	30 months
GTR-DFDBA				
Root coverage (%)§	N/A	76.4 ± 19.0^{a}	84.2 ± 17.9^{a}	87.0 ± 17.2^{a}
Gingival recession (mm)§	3.75 ± 0.9^{a}	$1.42 \pm 0.97^{b*}$	$1.1 \pm 0.9^{c*}$	$0.5 \pm 0.6^{c*}$
Probing depth (mm)†	2.4 ± 0.5^{a}	$1.0 \pm 0.7^{b**}$	$1.0 \pm 0.6^{b*}$	$0.8 \pm 0.4^{b*}$
Clinical Attachment level (mm)§	6.2 ± 1.0^{a}	$2.4 \pm 1.4^{b*}$	$2.1 \pm 1.0^{b*}$	$1.3 \pm 0.8^{b*}$
Keratinized tissue width (mm)§	1.0 ± 0.5^{a}	$2.1 \pm 1.0^{b*}$	$2.5 \pm 0.6^{b*}$	$3.4 \pm 0.6^{c*,‡}$
SCTG				
Root coverage (%)†	N/A	95.0 ± 8.0^{a}	95.7 ± 8.4^{a}	95.5 ± 10.1^{a}
Gingival recession (mm)†	4.2 ± 0.8^{a}	$0.4 \pm 0.6^{b*}$	$0.2 \pm 0.3^{b*}$	$0.3 \pm 0.65^{b*}$
Probing depth (mm)†	2.5 ± 0.7^{a}	1.7 ± 0.5^{a}	1.7 ± 0.65^{a}	$1.3 \pm 0.5^{b*}$
Clinical attachment level (mm)†	6.7 ± 0.8^{a}	$2.0 \pm 0.8^{b*}$	$1.9 \pm 0.6^{b*}$	$1.7 \pm 0.65^{b*}$
Keratinized tissue width (mm)†	1.0 ± 0.6^{a}	$4.7 \pm 1.1^{b*}$	$4.6 \pm 1.0^{b*}$	$4.5 \pm 1.2^{b*}$

[a,b,c] Different superscript letters in the rows indicate statistically significant differences among periods ($p > 0.05$).
§ Repeated measures ANOVA for parametric data.
† Friedman's test for non-parametric data, $p < 0.05$ indicate statistically significantly differences among periods. N/A: not applicable.
* $p < 0.001$ (compared to baseline data).
** $p < 0.05$ (compared to baseline data).
‡ $p < 0.01$ (compared to 18 months).

Table 2 – Mean differences between guided tissue regeneration (GTR-DFDBA) and subepithelial connective tissue graft (SCTG).

Variable/treatment	Δ 18 months -baseline (mean)	*p*-Value	Δ 30 months -baseline (mean)	*p*-Value
Root coverage (%)[b]				
GTR-DFDBA	84.2	0.073	87.0	0.14
SCTG	95.6		95.5	
Gingival recession (mm)[a]				
GTR-DFDBA	2.63	0.02	3.25	0.16
SCTG	3.96		3.9	
Probing depth (mm)[a]				
GTR-DFDBA	1.41	0.01	1.6	0.17
SCTG	0.84		1.2	
Clinical attachment Level (mm)[a]				
GTR-DFDBA	4.5	0.71	4.9	0.84
SCTG	4.71		5.0	
Keratinized tissue width (mm)[a]				
GTR-DFDBA	1.50		2.4	0.02*
SCTG	3.54	<0.0001	3.5	

[a] Wilcoxon test for non-parametric data.
[b] Paired *t*-test for parametric data.
* Statistically significant difference ($p < 0.05$).

In a recent study, Zucchelli et al.[36] compared the root coverage outcomes of a coronally advanced flap (CAF) with connective tissue (CTG) or de-epithelialized gingival (DGG) grafts. The results demonstrated that both types of grafts were effective in root coverage and clinical attachment gain for the treatment of gingival recession after 12 months. Other studies have evaluated the alteration in mucogingival line location following the use of a subepithelial connective tissue graft one year following the surgery.[1,36,37] The results demonstrated that MGL tends to revert back to its primary position, and this reversion is accompanied by an increase in the width of keratinized gingival tissue.

The predictability of SCTG in obtaining stable RC for a long time was especially verified in several studies using different periods of evaluation, such as 12 months,[14,16,34] 24 months,[16] and 30 months,[38] 49 months [13] and 60 months.[3] Satisfactory results in RC stability were also found for GTR treatment after 9 months,[29] 24 months [32] and 48 months.[28] Conversely, some studies have demonstrated poor RC for the GTR technique,[27,33] which could be explained by membrane exposure during the healing phase that leads to contamination.[6,9] Furthermore, bacterial colonization of membranes has been negatively correlated with clinical attachment gain and positively correlated with gingival recessions.[30]

Another factor that could interfere with the amount of RC is the initial recession depth. Deep and shallow recessions could respond differently.[7,28] A study showed that recessions ≥5 mm deep had greater RC after GTR treatment, whereas the amount of RC after mucogingival surgery was usually constant, irrespective of the initial recession depth and without significant differences in RC between GTR and SCTG after 48 months (73.07 × 72.3%).[28] Although the present study showed initial recession depths ≤5 mm for GTR (range from 2.85 to 4.65) and SCTG (range from 3.4 to 5.0), both procedures were predictable in obtaining a high percentage of RC at 18 months (84.25 × 95.7%), which remained stable up to 30 months (87.0 × 95.5%). Moreover, our findings for RC are similar to those obtained by Zucchelli et al.[7] 12 months post-surgery for GTR (85.7%) and SCTG (93.5%), which treated sites with initial recession depths ≥5 mm. Thus, the muco-gingival technique has been shown to be as effective as GTR procedures in the treatment of gingival recessions ≥4 mm. It has also been revealed that the recession depth is not the parameter that influences the selection of the surgical procedure.[7]

Our data showed a significant amount of keratinized tissue width (KTW) at 18 and 30 months. However, the amount of KTW in the SCTG group showed a more significant increase than GTR-DFDBA (3.5 × 2.4, $p = 0.02$) at 30 months. The mean increase of KTW when using SCTG at 18 months (3.54 mm)[10] remained stable up to the 30-month evaluation (3.5 mm), whereas the GTR-DFDBA group showed a significant increase in KTW between the periods of 18 and 30 months (2.5 mm × 3.4 mm, $p < 0.01$). The KTW gain in the SCTG group in earlier phases could be explained by the fact that the quality of the connective tissue ultimately determines the character of the surface epithelium.[39] Moreover, the mucogingival line that had been moved in coronal direction during the surgical approach tended to revert back to its original position following the use of SCTG, and this reversion has been shown to be accompanied with an increase in KTW and in the attached gingiva width.[37]

In clinical and histological case reports, Roman et al. [40] reported the treatment of gingival recessions with a coronally advanced flap and subepithelial connective tissue graft. The clinical and histological results were observed after 12 months. Clinically, the grafted tissues seemed to be attached to the root surfaces, and the histological results indicated that the healing resulted in a long connective tissue attachment, which was shown to be stable over time. The significant increase in KTW verified in the present study in the GTR-DFDBA group at 30 months is in agreement with the results of Pini Prato et al.[28] who also observed a later increase in KTW after 48 months. These authors reported that the increase was not the result of creeping attachment because the level of the gingival margin remained stable

between the two periods of evaluation (18 and 48 months), and they attributed the increase of KTW to the apical migration of the mucogingival junction towards its original location.

A hypothesis for the later increase in KTW in the GTR-DFDBA group was that the use of barriers could delay the proliferation of the granulation tissue from the gingival connective tissue and periodontal ligament. The granulation tissue that proliferates from those tissues may possess the ability to induce the formation of keratinized gingival epithelium.[41] Another hypothesis was that some creeping attachment might have occurred because there was a mean reduction in the gingival recession from 18 to 30 months of evaluation (1.12–0.5 mm); creeping attachment can be characterized by a postoperative migration of the gingival margin in a coronal direction over a previously denuded root, and it continues for a long post-surgical period until a constant marginal level is reached.[42]

In our study, the GTR-DFDBA technique was combined with a bone grafting, which could respond differently from the use of GTR alone; however, according to Sculean et al.[43] a histologically superior healing following the combination of barriers membranes and grafting materials can be obtained when compared with each material alone and the bone formation was more extensive in the groups receiving the combined approach. The discussion of our data was based on studies using GTR alone because a recent systematic review and meta-analyses by Chambrone et al.[22] showed that there were few clinical trials that have associated GTR with bone substitutes comparing with SCTG treatment.

The use of the DFDBA in the present study can be justified based on results of our research group. A histometric study in dogs showed that the treatment of the gingival recessions with GTR associated with collagen membrane and DFDBA resulted in larger extension of neoformed cementum and bone, and even in small proportions of residual recessions.[44]

Our data demonstrated better results in obtaining a KTW increase with the SCTG procedure (4.5 mm), as our results were higher than those reported by some long-term studies, which showed a range of KTW increase of 1.14[38], 2.0 and 2.1[11], 2.5 mm[5], and 3.7 mm.[7] Such discrepancies among the results of the studies may be related to different methodologies and inclusion criteria, such as classification of gingival recession and differences in values of keratinized tissue width at baseline and the initial recession depth. A limitation of our study was that the thickness of the gingival keratinized tissue was not measured, but the gingival tissues with thinner phenotypes were excluded.

In general, our findings demonstrated that SCTG and a combined technique of GTR with bone grafting can be used for RC with favourable results. The present study showed that SCTG seems to be more adequate in obtaining RC and a gain in KTW, in agreement with the systematic review conducted by Chambrone et al.[22] Those authors evaluated the effectiveness of different root-coverage procedures in the treatment of recession-type defects and concluded that both treatments (SCTG and GTR-DFDBA) may be used as root-coverage procedures for the treatment of localized recession-type defects. However, in cases where both root coverage and gain in keratinized tissue are expected, the use of SCTG treatment was more favourable.

5. Conclusions

The techniques of SCTG and GTR-DFDBA for the treatment of gingival recession lead to favourable and stable long-term results for root coverage, but SCTG showed a more favourable increase in keratinized tissue width.

Conflict of interest

The authors declare that they have no past or present financial interest or other personal interest of any nature regarding the manuscript.

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