Clinical parameters, histological analysis, and laser Doppler flowmetry of different subepithelial connective tissue grafts

Ricardo Rabelo Eustachio, Rafael Ferreira, Nair Cristina Margarida Brondino,1 Carla Andreotti Damante, Maria Lucia Rubo De Rezende, Adriana Campos Passanezi Sant’ana, Sebastião Luiz Aguiar Greghi, Mariana Schutzer Ragghianti Zangrando

Abstract: Subepithelial connective tissue graft (SCTG) presents favorable outcomes. However, the harvesting technique can influence the anatomical and histological composition of the SCTG. Within the limitations of a case report, the behavior of SCTGs removed by two techniques was evaluated bilaterally in one patient using double blade scalpel (DBS) and de-epithelialized graft (DE). Clinical parameters, laser Doppler flowmetry (LDF) and histological analysis were assessed. Complete root coverage was observed bilaterally, as well as improvement in width and thickness of keratinized tissue 2 years postoperatively. The LDF analysis demonstrated better revascularization in the DBS recipient area compared to DE. The histological evaluation showed differences in tissue composition and organization of collagen fibers. Similar clinical outcomes were observed bilaterally, nevertheless greater morbidity and aesthetic was reported in the DE harvesting area.

Key words: Connective tissue, histology comparative, laser-Doppler flowmetry, tissue harvesting

INTRODUCTION

Periodontal plastic surgery can be defined as surgical procedures performed to prevent or correct anatomic, developmental, traumatic or disease-induced defects of gingival tissues, mucosa or alveolar bone.[1-3] The indications of surgical procedures for root coverage (RC) are: to avoid abrasion or root caries, improve the inconsistency or disharmony of the gingival margin, for esthetic reasons, alveolar ridge defects, reduction of dentin hypersensitivity, and keratinized tissue augmentation.[2,4,5]

The subepithelial connective tissue graft (SCTG) associated with coronally advanced flap is considered the gold standard treatment for RC in Miller Class I and II gingival recessions.[4,6,7] However, SCTG can present different anatomical and histological characteristics depending on the harvesting technique.[4] Different techniques are available to obtain SCTG from palatal donor site,[5,6,8] including the de-epithelialized graft (DE), which is the removal of a free gingival graft with subsequent graft de-epithelization,[9,10] and double blade scalpel (DBS)[8] technique, using a specific scalpel with two parallel blades, usually 1.5 mm apart. Regardless of the technique employed, the selected site for harvesting the SCTG must provide an adequate amount of tissue, avoiding risks to the patient with lower postoperative morbidity.[7,9]

Thus, the objective of this case report was to compare, by clinical parameters, laser Doppler flowmetry (LDF), and histological analysis, the behavior of SCTG removed by two techniques: DBS and DE.

CASE REPORT

Patient, female, 29 years old, Caucasian, complaining about bilateral tooth hypersensitivity

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Eustachio RR, Ferreira R, Brondino NC, De Rezende ML, Sant’ana AC, et al. Clinical parameters, histological analysis, and laser Doppler flowmetry of different subepithelial connective tissue grafts. J Indian Soc Periodontol 2018;22:348-52.
at the region of premolars and molars, associated with esthetic impairment. Before performing any procedure, the patient was instructed about the actions involving the dental treatment and signed a free and informed consent and authorization form for the publication of clinical data in this study.

The patient presented thin periodontal biotype and Miller Class I\(^1\) multiple gingival recessions in these areas, as well as traumatic brushing habits. The patient was instructed to perform tooth brushing by the modified Stillman technique. Surgical treatment of the recession defects was not scheduled until the patient reached bleeding on probing (BOP) and plaque index (PI) lower than 20%.

Surgeries were performed with an interval of 30 days, and both sides had the same surgical protocol except for the graft harvesting technique. Both surgeries were performed by a single trained surgeon. Figure 1a and b demonstrate the initial clinical aspects of surgical areas with multiple gingival recessions in premolars and first molars. The technique employed in recipient sites was described by Zucchelli and De Sanctis\(^{14}\). The split-full-split flap was raised [Figure 1e] allowing complete graft and RC without tension [Figure 1f]. Papillae de-epithelization was performed [Figure 1g] with Goldman-Fox scissor, and the graft was stabilized at the level of the cementoenamel junction [Figure 1h]. Suspensory sutures complemented by simple suture [Figure 1i and j] promoted coronally advanced flap with total coverage of the SCTG and positioned 1 mm coronally to the cementoenamel junction using a 5-0 nylon suture. The right side received a DBS graft [Figure 2a-d] and the left recipient site received DE [Figure 2e-h], drawn with a coin. In the DBS surgery, the DBS was positioned parallel to the long axis of the tooth, moving from distal to mesial, deepening all the extension of the active surface of the scalpel into the palatal tissue in regular movements [Figure 2a]. Internal lateral and apical incisions were made to remove the graft, leaving the most external portion of connective tissue and epithelium in the palate. The size of each graft was previously marked with the back of the blade on the palatal donor site [Figure 2e]. In both techniques, grafts were removed with approximately 1.5 mm of thickness. Figure 2b and f demonstrate the anatomical differences of DBS graft and DE, respectively. Both grafts were de-epithelized using 15C Swann-Morton\(^\circ\) blades [Figure 2c and g]. DBS palatal donor site presents an approximation of the incised edges [Figure 2d], while the DE harvesting technique leaves an extensive area of secondary intention healing [Figure 2h].

Clinical parameters

The clinical measurements were performed by a single blinded and calibrated examiner. Data were collected at baseline and after 1 and 2 years postoperatively [Table 1].

**Figure 1:** (a) Intraoral view of multiple gingival recessions on the right side; (b) and left side; (c and d) surgical procedure involving premolars and first molars with oblique incisions by Zucchelli and De Sanctis; (e and f) split-full-split flap without tension; (g) papillae de-epithelization; (h) graft stabilization at the cementoenamel junction; (i and j) 5-0 nylon sutures with coronally advanced flap

**Figure 2:** Graft harvesting techniques used in surgeries on the right side (a, c, e and g) and left side (b, d, f and h) (a) double blade scalpel; (b) donor site marking for later removal of de-epithelialized graft; (c) graft obtained by the double blade scalpel– DBS; and (d) de-epithelialized graft– de-epithelialized graft; (e and f) epithelium removal on the table and (g and h) suture of donor bed sites
The following periodontal parameters were assessed: Probing depth, BOP, PI, keratinized tissue width (KTW), soft tissue thickness (STT), and recession depth. STT was determined 1.5 mm apically to the gingival margin at the mid-buccal point with an anesthetic needle and round endodontic stop, using a digital caliper (MTX, Digital Caliper).

To determine the mean %RC, percentage of the gain of KTW (%GKTW) and Gain of STT (%GSTT) the formula proposed by Zucchelli et al. was used [Figure 3].

Compared to the initial aspects [Figure 4a and b], evaluation after 1 [Figure 4c and d] and 2 years [Figure 4e and f] postoperatively revealed the stability of RC and gain of KTW. The coverage rate was 100% in premolars while the molars showed only partial coverage, but with an increase in keratinized tissue and reduction in recession depths. Only premolars received SCTG since they were the targeted elements in the RC and solely the coronally advanced flat was performed in both first molars.

Evaluations with visual analog scales were made considering the aesthetics in recipient areas and morbidity in donor sites. After 2 years, the patient reported esthetic preference for the left side (DE), but this technique caused greater discomfort in the postoperative period (in analyses at 0, 7, 14, and 28 days).

An esthetic analysis was also carried out (considering color, texture, and regularity of the gingival margin in comparison to adjacent tissues) by a periodontist blinded to the surgical procedures, who also considered the left side had the most favorable esthetics.

**Laser Doppler flowmetry**

For the blood flow assessment, a LDF (VMS-LDF2 DUAL CHANNEL-Laser Doppler Blood Flow and Temperature Monitor, trademark Moor instruments) was employed at baseline, 2, 7, 14, and 28 days after the surgical procedures. The appliance is equipped with two probes that were stabilized with the use of individual silicone guides, providing a standardized probe positioning for the consecutive measurements. The guide had two perforations corresponding to the central part of the grafted connective tissue, being one drilled in a more mesial position and the other more distally, both aligned. The measurements with the LDF lasted 2 min in the two points simultaneously, and the collected data were analyzed by the statistical software Matlab and Excel Worksheet, then plotted to generate centroid graphs, and drawn as a result of this flowmetry analysis [Figure 5].

The Doppler flowmetry showed a difference between values obtained on each side of the mouth (compared to the baseline values), demonstrating that the mean flow measured from the DE graft tended to be lower when compared to the graft removed by DBS, at all moments. The DBS graft presented a pattern of flow that returns and overtakes the baseline levels at 28 days, while the DE presented a much lower and variable flow rate in all measured periods. In addition, the lowest area of blue points in DE centroid graphs shows a more heterogeneous flow pattern. In DBS, the largest area of the ellipse represents greater homogeneity of the flow and the revascularization process as well.

**Histological analysis**

Incisional biopsies (fragment measuring 1 mm wide with graft length and thickness) were performed before de-epithelization of the grafts to confirm and analyze the nature of each harvested tissue. Histological sections were stained by H and E for evaluation on a microscope (Carl Zeiss, Germany) [Figure 6].

The palatal fibromucosa is characterized by a dense connective tissue (lamina propria) covered with ortho-keratinized epithelium and a layer composed of the considerable amount of fatty and glandular tissue (submucosa) with variable thickness, as shown in Figure 6a-j. The images [Figure 6e and j] evidence the different types of collagenization for each graft. In the DE graft, more organized collagen fibers [Figure 6e] give the appearance of denser connective tissue. In the DBS [Figure 6j], it is possible to observe greater spacing between the fibers, leading to a looser aspect. These differences may be due to the predominance of each type of tissue present in the grafts. Normally there is a predominance of lamina propria in the DE grafts and submucosa in DBS, as shown on the ×4, in which the DBS [Figure 6j] presents the apical portion with more space between the tissue structures, characterizing a more disorganized tissue. However, individual variations should be considered in setting the tissue type of each graft.

**Formula**

\[(\text{Baseline } P_x) - (\text{Px after 1 or 2 years}) 	imes 100 \text{ (Baseline } P_x)\]

**Table 1: Clinical parameters of double blade scalpel and de-epithelialized graft recipient sites at baseline and after 1 and 2 years postoperatively**

|                  | Baseline | 1 year | 2 years |
|------------------|----------|--------|---------|
|                  | DBS      | DE     | DBS     | DE     |
| PD               | 3        | 2      | 2       | 2      | 3       | 3       | 3       | 3       |
| RD               | 2        | 2      | 2       | 2      | 0       | 0       | 0       | 0       |
| KTW              | 3        | 3      | 3       | 3      | 3       | 4       | 4       | 4       |
| STT              | 1.03     | 1.08   | 1.12    | 1.71   | 1.84    | 2.53    | 1.18    | 1.81    | 1.91    | 2.84    | 1.22    | 2.05    |
| % RC             | -        | -      | 100     | 100    | 100     | 100     | 100     | 100     |
| % KTW            | -        | -      | 16      | 33     | 16      | 33      | 16      | 33      |
| % GSTT           | -        | -      | 121     | 6      | 126     | 16      |

DBS – Double Blade Scalpel technique; DE – De-epithelialized technique; PD – Probing depth (mm); RD – Recession depth (mm); KTW – Keratinized tissue width (mm); STT – Soft-tissue thickness (mm); % RC – Percentage of root coverage; % KTW – Percentage of gain in keratinized tissue width; % GSTT – Percentage of gain in soft-tissue thickness
FIGURE 4: Multiple gingival recessions at right (double blade scalpel) and left sites (de-epithelialized graft) (a and b); root coverage with double blade scalpel (c) and de-epithelialized graft (d) after 1 year; root coverage with Double Blade Scalpel (e) and de-epithelialized graft (f) after 2 years.

FIGURE 5: Centroid graphs generated by plotting of the measured data regarding flowmetry of grafts removed by Double Blade Scalpel and de-epithelialized graft technique. The flowmetry can be assessed in these graphs by the area and quantity of blue dots and the increase of these points in relation to flows at moments t−1 and t. Double Blade Scalpel: subepithelial connective tissue graft removed by double blade scalpel; de-epithelialized graft: subepithelial connective tissue graft removed by de-epithelialized graft technique.

DISCUSSION

Regardless of the type of connective tissue harvested, complete RC was achieved bilaterally. Enlargement of the KTW (%GKTW) reached 16% for DBS and 33% for DE and thickness (%GSTT) showing 126% for DBS and 16% for DE, were observed after 2 years postoperatively. In the esthetic analysis, both patient and periodontist considered best results at the DE site; however, the patient reported increased postoperative morbidity in DE donor area. The LDF showed a tendency of better and constant revascularization at all periods of measurement for DBS graft about DE. Histological analysis revealed differences in graft composition, with greater quantity of fibrous connective tissue and less spacing between fibers in DE compared to DBS. However, further studies are required to confirm these differences with a statistically significant sample.

In the present case report, better results were observed for DBS in %GSTT 2 years postoperatively. STT measurements were determined 1.5 mm apically to the gingival margin and partial marginal necrosis of DE graft was observed after 7 days postoperatively. Therefore, these factors may have influenced an inferior %GSTT for the DE site. This finding is by Zuhr et al.,[7] who suggested that very dense and coarse connective tissue appears to undergo necrosis more easily. The LDF analysis suggested more heterogeneous results, with the lower mean flow in the DE grafted area compared to DBS. This result can be associated with the greater difficulty of revascularization of a denser SCTG regarding collagen.
fibers and less spacing between them, as observed in the histological evaluation. The operating principle of the LDF has some measurement-related limitations (small movements).[16]

Although there are different techniques for obtaining SCTG,[5,8-13] some factors must be considered. The decision-making process regarding the best technique for harvesting the SCTG should be based on scientific evidence, but also should consider the amount of available tissue in the eligible sites, the morbidity to the patient[9] and in particular, the preference of the clinical operator.[7] Within the limitations of a clinical case, this report showed no differences in the clinical results achieved by two distinct types of SCTG, despite the histological differentiation and a tendency of smaller initial revascularization in the DE graft, as verified by LDF.

CONCLUSION

Both techniques (DBS and DE) presented satisfactory clinical and esthetic outcomes in this case report. The different histological pattern of these types of grafts may be associated with variations in the revascularization process, verified by LDF. However, randomized clinical trials are required to better assess the real clinical differences as well as the results, using different types of SCTG harvesting techniques aiming at RC.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

This study was supported by FAPESP (São Paulo Research Foundation) grant #2012/13331-2for the purchase of laser Doppler flowmeter (VMS-LDF2 DUAL CHANNEL- Laser Doppler Blood Flow and Temperature Monitor, trademark Moor instruments). The foundation had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Miller PD Jr. Root coverage grafting for regeneration and aesthetics. Periodontol 2000 2000;1993:1,118-27.
2. Zucchelli G, Mounssif I. Periodontal plastic surgery. Periodontol 2000 2015;68:333-68.
3. Cairo F. Periodontal plastic surgery of gingival recessions at single and multiple teeth. Periodontol 2000 2017;75:296-316.
4. Bertl K, Pfiff M, Hirtler L, Nrend B, Nünberger S, Stavropoulos A, et al. Relative composition of fibrous connective and fatty/glandular tissue in connective tissue grafts depends on the harvesting technique but not the donor site of the hard palate. J Periodontol 2015;86:1331-9.
5. Harris RJ. Histologic evaluation of connective tissue grafts in humans. Int J Periodontics Restorative Dent 2003;23:575-91.
6. Chambrone L, Chambrone D, Pustiglioni FE, Chambrone LA, Lima LA. Can subepithelial connective tissue grafts be considered the gold standard procedure in the treatment of miller class I and II recession-type defects? J Periodontol 2015;86:1331-9.
7. Zucchelli G, Mele M, Stefanini M, Mazzotti C, Marzadori M, Montebugnoli L, et al. Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: A comparative randomized-controlled clinical trial. J Clin Periodontol 2014;41 Suppl 15:S123-42.
8. Harris RJ. The connective tissue and partial thickness double pedicle graft: A predictable method of obtaining root coverage. J Periodontol 1992;63:477-96.
9. Zucchelli G, Mele M, Stefanini M, Mazzotti C, Marzadori M, Montebugnoli L, et al. Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: A comparative randomized-controlled clinical trial. J Clin Periodontol 2010;37:728-38.
10. Bosco AF, Bosco JM. An alternative technique to the harvesting of a connective tissue graft from a thin palate: Enhanced wound healing. Int J Periodontics Restorative Dent 2007;27:133-9.
11. Edel A. Clinical evaluation of free connective tissue grafts used to increase the width of keratinised gingiva. J Clin Periodontol 1974;1:185-96.
12. Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. J Periodontol 1985;56:95-102.
13. Harris RJ. A comparison of two techniques for obtaining a connective tissue graft from the palate. Int J Periodontics Restorative Dent 1997;17:260-71.
14. Zucchelli G, De Sanctis M. Treatment of multiple recession-type defects in patients with esthetic demands. J Periodontol 2000;71:1506-14.
15. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229-35.
16. Baab DA, Oberg PA. The effect of cigarette smoking on gingival blood flow in humans. J Clin Periodontol 1987;14:418-24.