Somatosensory evaluation of donor and recipient sites of subepithelial connective tissue graft: case-control preliminary study

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Abstract

Aim: The study aimed to qualitatively evaluate the somatosensory profile in the recipient and palatal donor sites after root coverage by subepithelial connective tissue graft (SCTG).

Materials and Methods: This preliminary observational case-control study included patients submitted to SCTG, after 6 months postoperatively (case-G6, palate and recipient sites), homologous non operated areas were selected for directly comparison (HNO site), and a complementary group that was not submitted to SCTG (control-G0) was selected to validate the measurements protocols. The qualitative sensory test (QualST), Douleur Neuropathique 4 questionnaire (DN4), and Two-point test were performed. The participant discriminated the areas into hypersensitive, hyposensitive, or normal sensitive.

Results: In the G6 group, the QualST test based in patient self-report after stimuli in comparison to G6 versus HNO areas, showed significant differences in pressure hyposensitivity in recipient sites and thermal and tactile hyposensitivity in donor sites (p=0.04). There were no significant differences in sensory acuity by the Two-point test. Comparisons in control G0 group did not present changes, and normal sensitivity was reported. The DN4 questionnaire revealed normal sensitivity in grafted and palatal donor sites.

Conclusion: This preliminary study reinforces the positive results of periodontal plastic surgery using SCTG, not reporting significant somatosensory changes.

Keywords: Periodontics; Surgical flaps; Graft; Sensory function.

Introduction

Root coverage procedures are the most studied techniques of periodontal plastic surgeries, not only because of the high prevalence of gingival recession and consequent esthetic discomfort and tooth sensitivity, but also because they are complex techniques that still require greater knowledge regarding the patient-centered outcomes (Tonetti and Jepsen, 2014). Root coverage is common evaluated by clinical parameters (Tonetti and Jepsen, 2014; Cairo *et al*., 2016; Kumar *et al*., 2017; Chambrone *et al*., 2018; Mounssif *et al*., 2018), which can be accurately measured by examiners, but these studies rarely provide information on how the patients feel and assess these improvements. For example, if there was loss or increase in soft tissue sensitivity in the recipient or donor sites.

One of the most used surgical approaches for root coverage is the subepithelial connective tissue graft (SCTG) associated with coronally advanced flap (CAF) (Zuccheli and De Sanctis, 2000). The patient related outcomes measures (PROMs), after these procedures, were evaluated in the immediate postoperative period, including pain and edema, or in the later periods, by evaluations of tooth sensitivity and/or esthetic aspects (Kumar *et al*., 2017; Mounssif *et al*., 2018).

There is a limited number of publications on the soft tissue sensory function after SCTG in recipient and donor sites. A previous study evaluated the nerve

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recovery by pressure (30 g) with a periodontal probe in 15 areas after 12 months of SCTG healing (Aimet *et al*., 2010). The patient complained with positive or negative answers when the pressure was applied in the graft area with a periodontal probe. Patients who responded negatively were associated with hyposensitivity reaction. It was observed that 50% of participants presented changes in sensations as anesthesia or paresthesia, not describing the evaluated area. By biopsies and immunocytochemical evaluation of the SCTG, authors observed a greater number of Merkel cells from the grafted tissue after 2 months compared to the initial recipient site. Thus, they described two hypotheses: the increase in Merkel cells would be related to tissue injury, or there was abnormal cell proliferation in tissue regeneration (Aimet *et al*., 2010). In palatal area, the loss of sensitivity was evaluated by different outcomes (Del Pizzo *et al*., 2002). The evaluations were performed in the first and eighth week after surgery by pressure and friction movements in the area. The classification was performed by a scale of verbal descriptors (none, mild/moderate or severe), and all patients reported no loss of sensation in the eighth week. Also, in the palatal area, the sensitivity after graft removal on the 14th postoperative day was evaluated (Roman *et al*., 2012), and 93% of patients reported absent or minimal change in sensitivity using a verbal scale (none/minimum, moderate or very important). A case-control study aimed to compare trigeminal somatosensory sensitivity between patients with a clinical diagnosis of symptomatic irreversible pulpitis (n: 533) and healthy participants (n: 533) to evaluate the impact of somatosensory stratification of symptomatic irreversible pulpitis on pulp sensibility testing. A standardized battery of qualitative sensory assessment measured intra- and extraoral sensitivity to touch, cold, and pinprick stimuli, dental pain intensity (0–100, numeric rating scale), and duration (seconds) evoked by cold stimuli (refrigerant spray) were applied to, respectively, the nonaffected and affected tooth (cases) and the upper right and left premolars (controls). Pain duration was more pronounced after the pulp sensibility test, and dental pain during the pulpectomy procedure was more frequent in the subgroup of patients who also reported intraoral hypersensitivity. Therefore, somatosensory evaluation may improve the endodontic diagnostic process (Costa *et al*., 2020). Nevertheless, the present study measures somatosensory assessment in SCTG recipient and donor sites by these validated tests that are widely used in sensory studies (Del Pizzo *et al*., 2002; Aimet *et al*., 2010; Roman *et al*., 2012; Costa *et al*., 2020), with a control group for comparison. Also, the evaluation of the present study was performed 6 months after surgery, a period when there is greater stabilization of the tissue healing.

Therefore, it is fundamental to understand whether these graft recipient and donor sites present somatosensory changes in the long term. This information about possible complications and sequelae after performing these procedures will contribute with further information about the postoperative response of SCTG, enabling adequate patient guidance. The study hypothesis was that the sensory function of operated areas was within normal standards. Thus, this preliminary observational case-control study aimed a) to compare the somatosensory profile in SCTG recipient and donor sites to homologous areas of individuals at 6 months postoperatively, and b) to compare these individuals to a control group not submitted to root coverage procedures.

Materials and methods

The study was approved (CAAE: 18008919.5.0000.5417) by the Institutional Review Board of Bauru School of Dentistry – University of São Paulo (FOB-USP). This is a retrospective observational case-control study, with participants undergoing surgical procedures with SCTG selected from the clinics of the Discipline of Periodontology, Bauru School of Dentistry, University of São Paulo.

Sample selection

The study included participants aged between 18 and 60 years, with good general health, absence of active periodontal disease, and who had undergone surgery for root coverage by the SCTG technique associated with CAF, with postoperative period of 6 months (case group - G6). Individuals with the same profile and who had not undergone surgery with SCTG+CAF (control group -G0) were also selected.

Enrolled patients were evaluated in the SCTG recipient and donor sites (G6 group: recipient and palate areas at 6 months postoperatively) and compared with the homologous non operated (HNO) areas. The complementary group included individuals not submitted to root coverage procedures (control G0 group) to compare, in a parallel way, the somatosensorial parameters and evaluate their reproducibility.

Surgical procedures

After local anesthesia (40 mg of articaine hydrochloride 3% with 10 μg of epinephrine), root planing of the exposed root surface was performed with Gracey curettes. Subsequently, a modification of the CAF associated with SCTG technique was done as proposed previously (Zuccheli and De Sanctis, 2000). A SCTG was harvested from palatal area with thickness of 1.0mm, using double blade scalpel technique (ICE dental instruments, Brazil). The SCTG was sutured in

recipient site by simple suture technique. Finally, the flap was coronally advanced without tension to obtain a complete coverage of the graft. Post surgically anti-inflammatory (nimesulide 100 mg twice a day for 5 days) were administered for all patients. Patients were instructed to not brush treated areas, but to rinse chlorhexidine solution (0,12%) twice daily for 1 minute for 2 weeks. Sutures were removed after 14 days. Follow ups included professional supragingival tooth cleaning and individually oriented oral hygiene instructions and were scheduled at 1, 3 and 6 months postoperatively.

Calibration

The evaluations were performed by a single calibrated examiner (TSJ). A representative sample of ten random individuals were selected exclusively for study calibration, and the Two-point test (quantitative test) was applied and re-applied after 1 week. The intra-examiner agreement was evaluated by the statistical test of intraclass correlation coefficient (ICC). The ICC statistical test revealed a value of 0.79, demonstrating good examiner calibration.

Somatosensory evaluations

In G6 group (operated and HNO sites), the evaluation was performed 6 months after surgery, period when tissue healing is in considerable stabilization. The Qualitative Sensory Test (QualST) was used to assess the presence or absence of altered somatosensory function. This simple and rapid test provides information on gross hyposensitivity or hypersensitivity to touch, cold, and pin prick stimuli. Although other stimulation modalities exist, these modalities cover the function of Aβ, Aδ and C fibers 11 (Baad-Hansen *et al*., 2013).

The four different stimuli were applied in this sequence:

- Dynamic sensitivity to touch with friction of a cotton swab in a straight movement of an area of 1 to 2 cm near to the gingival margin – tactile graft (Tact G) / tactile palate (Tact P) (Figure 1)
- Standardized circular movements using the toothbrush bristles in the central region of the graft (for 1-2 seconds) – brush graft (Brush G) / brush palate (Brush P); (Figure 2)
- Sensitivity to needle prick stimulation was performed using a toothpick (applied for 1-2 seconds) with moderate force (no mucosal penetration, only until ischemia) – prick graft (Prick G) / prick palate (Prick P); (Figure 3)
- Sensitivity to cold stimulus was assessed using a cooled stainless-steel spatula (soaked in ice for at least 5 minutes before application) applied for 1-2 seconds – thermal graft (Ther G) / thermal palate (Ther P); (Figure 4)

The main outcome of study was acquired after applying the stimuli and the participant was asked if the sensations were similar (norm sensitive=0), less sensitive (hyposensitive= -) or more sensitive (hypersensitive= +), in the graft recipient and donor sites compared to the HNO areas, in the G6 group. In the G0 group (control group without surgical intervention), a region was chosen (usually the premolars, the most evaluated recipient and palatal areas in G6 group) and compared to the gingival region of the homologous tooth under the same conditions.

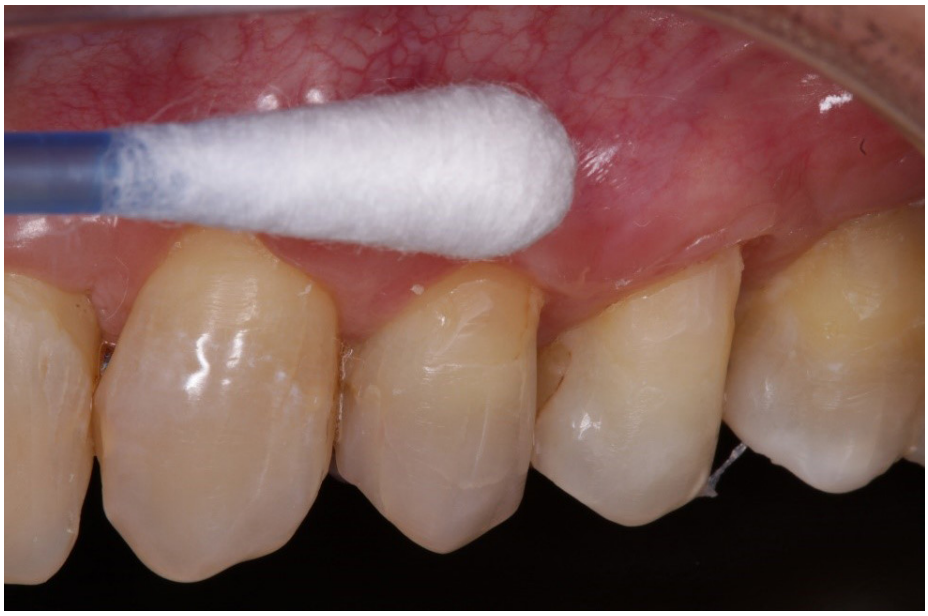

Figure 1. Friction test.

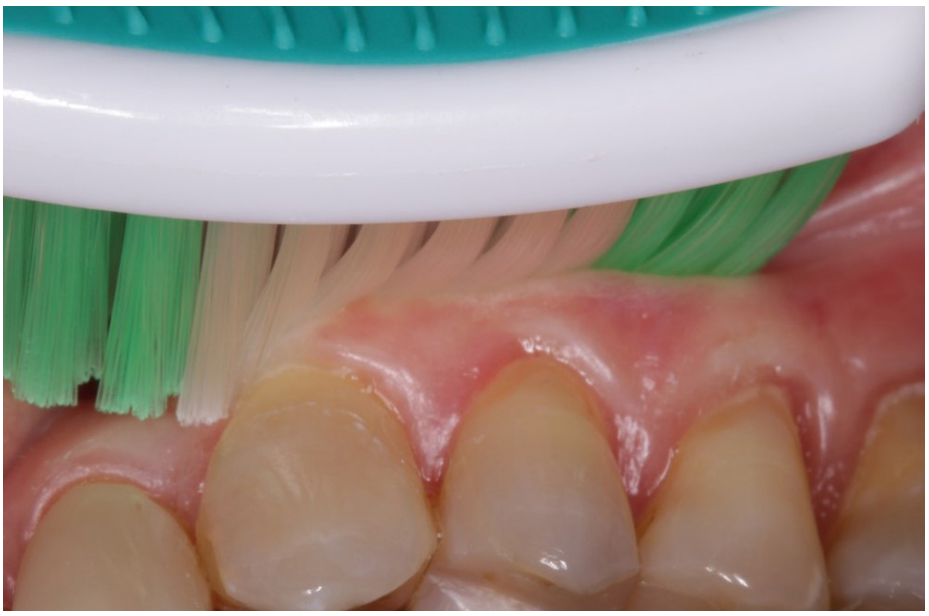

Figure 2. Toothbrush test.

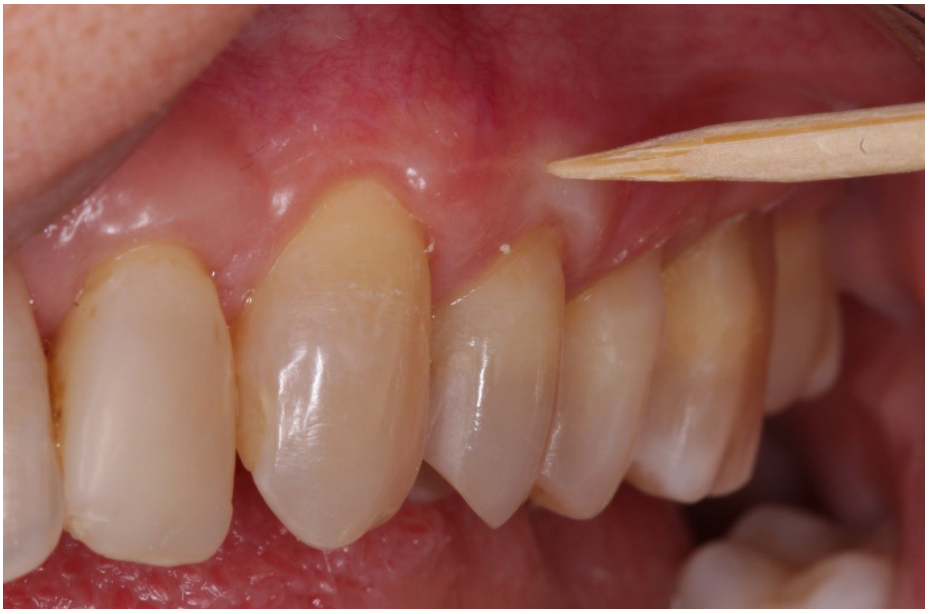

Figure 3. Needle prick test.

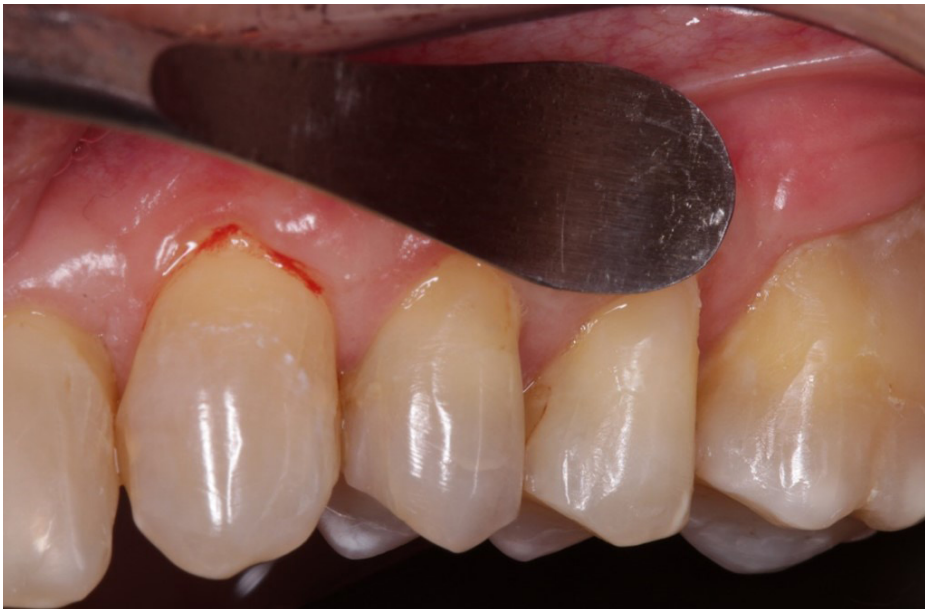

Figure 4. Cooled stainless steel spatula test.

The sensory acuity test (two-point discrimination/Two-point Test) used a dry-point compass (with rounded ends) (Figure 5) that were spaced (using a millimeter ruler) at standard intervals from 1 mm to 15 mm. When the participant could distinguish the 2-point touch sensation, the distance between the two points was recorded (Ziccardi *et al*., 2012). The evaluation was performed in horizontal and vertical directions on the G6 group (recipient and donor sites) and on the HNO areas. In G0 group the assessment was similar, but both sides were not operated.

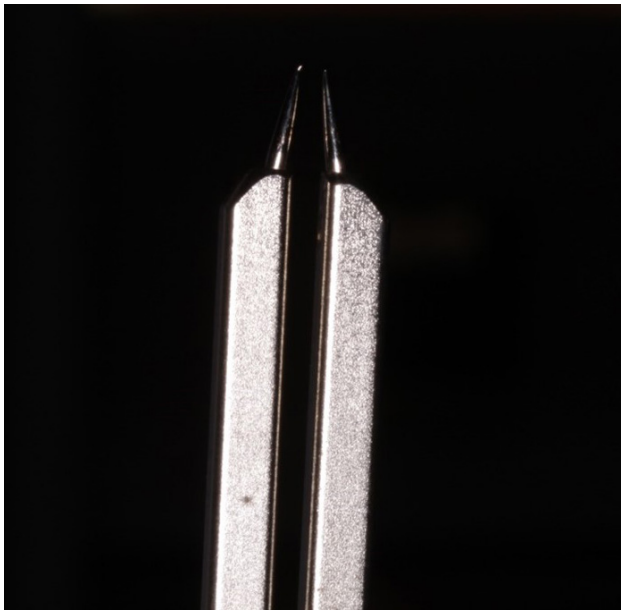

Figure 5. Dry-point compass.

All participants answered the Douleur Neuropathique 4 questionnaire (DN4) with McGill descriptors (Santos *et al*., 2010). This questionnaire is a widely used tool to assess sensory, affective, and evaluative characteristics of the painful experience and/or non-painful sensory alteration. This multidimensional pain assessment is mainly performed by an extensive list of descriptors (burning, electric shock, tingling, numbness, itching, painful cold sensation, pinprick or other) that are presented for participants to choose the terms that best represent their sensory and/or painful experience. The study applied the version translated previously (Santos *et al*., 2010). The Visual Analogue Scale (VAS) was added to this questionnaire, so that the participants could indicate the pain intensity, if present in the operated region in the 6-month postoperative period (considering "no pain: 0" to "worst possible pain: 100"). To evaluate the extension of sensation and/or pain changing and the incapacity or unable to perform oral functions as speaking, chewing, and brushing, the VAS was also used (considering "nothing: 0" to "impossible: 100" to execute dairy activities). The participants were also asked to report the frequency and duration of this pain and/or sensory change.

Statistical analysis

The results were tabulated on the Excel software and statistically analyzed, adopting a significance level of 0.05 for all tests. To assess the QualST test, the Chi-Square test was used, while the Two-point test was evaluated by analysis of variance (ANOVA). A descriptive analysis of sensory characteristics was used for the DN4 questionnaire.

Results

This preliminary study included 18 participants, being 9 in each group (G6 and G0). A total of 72 areas were evaluated, involving graft recipient, donor sites, and HNO areas. The regions evaluated included 1st and 2nd premolars, in maxilla (n = 14 patients) and mandible (n =4 patients). Fourteen patients were female (G6 n: 7, G0 n: 7) and 4 male patients (G6 n: 2, G0 n: 2), without significant differences in sample distribution ($p > 0.05$, Chi-Square test). The mean age was 32 ± 9.1 years old, in G6= 39.6 ± 8.8 and G0= 41.3 ± 9.7 years old (maximum: 59, minimum: 27) without statistically significant differences between groups ($p = 0.71$, t-test).

The intra individuals QualST test for qualitative sensory evaluations in the G6 group demonstrated significant differences for the proportion of hyposensitivity to the Prick G test (pressure with toothpick on the grafted area) ($p=0.04$), Therm P (cold with a cooled spatula on the palate donor site) ($p=0.04$) and Tact P (straight movement with a cotton swab on the palate donor site) ($p=0.04$). The G0 group was norm sensitive in all evaluations, showing an adequate inter individuals' reproducibility of performed measures (Table 1).

No significant differences were observed for the values of the Two-point sensory acuity test between different individuals (G6 versus G0 groups) and in intra individuals' evaluation (comparison in G6 group between recipient/donor sites with HNO areas) (ANOVA, $p> 0.05$).

A descriptive evaluation was performed for the DN4 test with McGill descriptors. No participant, either in G6 or G0 groups, reported feeling pain in the evaluated areas. In the grafted area, all patients responded with normal sensitivity. However, in the donor site, 36.66% of participants reported a feeling of numbness. The duration of these sensations was described as indeterminate, and the frequency of sensations was mainly reported when the participant touched the tongue in the region and during brushing, feeding, and drinking water. The degree of sensation changes in disabled oral functions as chewing, speaking, and brushing teeth was reported in more than 90% of participants with no disability in daily oral functions (rated this intensity as 0).

Table 1. Results of QualST self-reported by patients in relation to study group.

G6 group*								
N	Tact G	Brush G	Prick G	Ther G	Tact P	Brush P	Prick P	Ther P
1	0	0	0	0	0	0	0	-
2	0	0	-	-	-	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	-
5	0	0	0	0	-	-	0	0
6	0	0	-	0	0	0	0	0
7	0	0	0	-	0	0	0	0
8	0	-	-	-	-	-	-	-
9	-	0	-	0	-	0	0	-
p value intragroup analysis(!)	NS	NS	(p=0.04)	NS	(p=0.04)	NS	NS	(p=0.04)
G0 group#								
N	Tact G	Brush G	Prick G	Ther G	Tact P	Brush P	Prick P	Ther P
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
p value intragroup analysis(!)	NS	NS	NS	NS	NS	NS	NS	NS

* Results acquired by comparison with HNO (homologous non-operated areas) with surgical sites in palatal (P) and grafted (G) areas; #results acquired in the similar and most common areas of the G6 group by comparison with both non operated sites; -: hyposensitivity; 0 normosensitivity; + hypersensitivity; (!): chi-square association test; NS: without statistical difference (p>0.05).

Discussion

This preliminary study evaluated possible somatosensory differences in recipient and donor sites after root coverage procedures with SCTG associated with CAF. The SCTG recipient and palatal donor sites clinically evaluated by the QualST test revealed superior tendency to hyposensitivity in the Prick G (needle prick), Therm P (cold), and Tact P (cotton swab friction) tests. There is lack of evidence about this type of assessment in periodontal plastic surgeries. In general, most participants in the G6 group did not report feeling sensorial differences in the recipient and donor sites in the DN4 questionnaire. The minority reported sensitivity alteration in palatal donor area, characterizing this sensation as a slight numbness that did not incapacitate the oral functions.

Similarly, to the present study, hyposensitivity was observed in 50% of patients when investigating nerve recovery in 15 areas with SCTG after 12 months of healing (Aimet *et al*., 2010), this can be explained by changes in Merkel cells, observed after histopathological analyses of hyposensitive patients (Aimet *et al*., 2010). To evaluate the loss of sensation in the palate at 8 weeks (Del Pizzo *et al*., 2002) and 14 days (Roman *et al*., 2012) after graft removal, the similar classification system used in the present study was performed by a verbal patient description. Both studies reported absence or minimal change in sensitivity (Del Pizzo *et al*., 2002; Roman *et al*., 2012), thus corroborate with the association of results of QualST and DN4 questionnaire obtained.

The G0 group exhibited normal sensitivity for all tests, an expected result for areas not submitted to surgical procedures. These results reinforce the accuracy and validation of assessments since they were not submitted to surgical procedures and thus did not show changes in the somatosensory profile.

In the G6 group, the sensory acuity of the grafted area appears to be equal to HNO areas, as described by the Two-point test, in both directions horizontal and vertical. Two-point test demonstrated good efficacy to verify the sensory acuity (Costa et al., 2020). However, in protocols performed on the tongue, lip, cheeks, and region innervated by the lingual nerve (Stevens and Choo, 1996), the distances between the compass tips vary from 0.2 to 1 mm, one important factor to

be considered when execute this test (Calhoun *et al*., 1992; Stevens and Choo, 1996; Fukunaga *et al*., 2005; Wickremaratchi and Llewelyn, 2006; Carr *et al*., 2016; Won *et al*., 2017). There is lack of evidence about the use of Two-point and QualST tests in SCTG recipient and donor sites (Aimet *et al*., 2010) as a real patient related outcome in their protocols. Therefore, this preliminary study contributes with significant information about somatosensory profile in areas undergoing periodontal plastic surgery.

In this study, there were no significant differences in the age of participants, which is an important factor since age can be associated with a lower sense of touch (Won *et al*., 2017) and differences in immune response (Gwaltney, 2010). However, as observed previously, these results are controverse because different protocols detected decreased touch on the tongue in older patients (Wickremaratchi and Llewelyn, 2006), while another did not find differences in the decreased sensitivity for the tongue and anterior palate but found differences for cheeks and lips (Fukunaga *et al*., 2005). Also, a loss in taste, but not in tongue sensitivity was observed related to patient age (Carr *et al*., 2016). Factors that can be observed in the Two-point test are the differences in results according to the applied region, gender, and the application technique (Won *et al*., 2017). Sensitivity was decreased from the forehead to the cheek, chin, upper lip, lower lip, tongue tip and index finger. Females showed superior sensitivity to the test, as well as measurements with sharp-tipped compared to blunt-tipped calipers (Won *et al*., 2017). Due this sensitivity, the present study compared the execution of stimuli in a group that do not receive surgical procedure (G0 group) to standardize and demonstrate the validation of the protocol used. And despite the difficulties of obtaining standardization in different individuals, the results showed that the protocol presented a good level of agreement centered on the main calibrated examiner.

Recently, the discussion of the root coverage qualitative data about PROMS is increasing in literature, considering PROMS as the real scientific endpoints and the professional evaluations, the surrogate outcomes, after periodontal plastic procedures (Mounssif *et al*., 2018). Besides that, the questionnaires are wildly used in studies to evaluate these parameters, but it's difficult to observe one of these instruments that fulfilled all requirements (Mounssif *et al*., 2018). Taking this into consideration, the application of the DN4 questionnaire in grafted patients was unprecedented in literature and allowed the patient to directly answer their sensations in the grafted region, using McGill's descriptors. Results reported directly by the participants are important, since there is no interpretation of the response by a clinician or anyone else, only their original complaint (Gwaltney, 2010). Another original aspect of this study was the application of QualST and Two-point tests. Although these tests present a subjective nature that depend on the patient profile and the examiner experience, they are practical and low-cost methods, due to their rapidly evaluation and cost-effectiveness. It should be considered that the standardization of neurological diagnosis is difficult so the importance of comparison with homologous areas and control group.

One important limitation of this study was that the analysis was retrospective and without information on the preoperative evaluation in the case group. However, the inclusion of a control group without history of periodontal plastic surgery allowed analysis of the somatosensory profile in normal conditions. Other limitations are the absence of the gingival health, the graft thickness, and gingival phenotype data. Thus, the results of this study incorporated an important assessment of the absence of major complications and sequelae after performing procedures with SCTG, contributing with information about the postoperative response and providing adequate patient guidance.

Considering the limitations of this preliminary study, the recipient and donor sites of SCTG seem to present normal somatosensory results for most tests performed. A longitudinal study is suggested to better understand the effects of periodontal plastic surgical procedures on the somatosensory profile of graft recipient and donor sites.

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