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Electrical stimulation effect on coronally advanced flap for the treatment of single gingival recession: randomized controlled clinical trial

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ClinicalTrial.org- NCT02987231

São José dos Campos, São Paulo - Brazil

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STUDY PROTOCOL

This investigation was a parallel, double-blind, randomized clinical trial. The study protocol (ClinicalTrial.org- NCT02987231) was approved by the Institutional Review Board at College of Dentistry – São José dos Campos, State University of São Paulo (1.679.369-UNESP). Each subject provided informed consent after a thorough explanation of the nature, risks, and benefits of the clinical investigation.

Study Population

Sixty patients from the Periodontology Clinic, UNESP—State University of São Paulo (São José dos Campos, Brazil), presenting 60 maxillary buccal gingival recessions in their canines and premolars were included in the study. The subjects were selected in the period between October 2016 and December 2017, according to the following eligibility criteria: 1) ≥18 years 2) periodontally and systemically healthy 3) FMPS and FMBS ≤20% 4) single Cairo's class RT1 gingival recession (Cairo et al., 2011) in the maxillary canines or premolars (≥3mmin depth) at the buccal 5) presence of identifiable CEJ 6) no previous periodontal surgery at the affected teeth. Study exclusion criteria: 1) systemic problems that would contraindicate the surgical procedure; 2) patients taking medications known to interfere with the wound healing process or that contraindicate the surgical procedure; 3) smokers and pregnant women; 4) patients who underwent periodontal surgery in the area of interest; 5) patients undergoing orthodontic treatment.

Before surgery, Patients received oral hygiene instructions (roll technique) with a soft-bristled toothbrush to correct wrong habits related to the etiology of the recession. All participants received a session of prophylaxis and scaling. The surgical treatment was performed only when patients achieved adequate plaque control (full-mouth plaque score ≤15%)

Sample size

The sample dimension was calculated using $\alpha = 0.05$ and the power of 80%. For the standard deviation (SD), the value of 0.8 mm obtained in a previous paper (Jepsen et al. 2013). Thus, study was powered to detect a minimum clinically significant difference in root coverage of 0.8 mm between the test and control treatments for root coverage after 6 months. On the basis of these data, the minimum needed number of

patients to be enrolled in this study resulted 28 for the test group (CAF) and 28 for the control group (CAF+ES).

Randomization, allocation concealment, and blinding

The randomization process was performed by an external person who did not participate in the study. Patients were assigned to one of the two treatment groups with the use of computer-generated randomization sequence. Allocation concealment was obtained using sealed coded opaque envelope containing the treatment to the specific subject. The sealed envelope containing the treatment assignment was opened immediately after the surgical procedure by the investigator responsible for the electrical stimulation protocol. The surgeon, patients and investigators who performed clinical measurements were blinded to procedures.

Surgical procedure

All surgical procedures were performed by a single operator (FLSN). With a blade 15c (Surgi Blade® - Miami, FL - USA) two horizontal incisions were made at the bases of the mesial and distal papillae of the tooth, towards the adjacent teeth, taking care not to touch the adjacent teeth. From the end of the incisions, two divergent relaxing incisions were performed vertically in the apical direction that surpassed the mucogingival line so that the flap had mobility for coronary traction. A sulcular incision was performed in order to unite the releasing incisions and a split-full-split thickness flap was raised beyond the mucogingival junction (MGJ) (de Sanctis & Zucchelli 2007). After the root planing procedures were performed, the epithelial layer was removed from the incised papillae exposing the connective tissue. Before the flap was positioned coronally, the region was washed copiously with physiological solution to remove the clot allowing intimate contact of the flap with the recipient bed. Sling sutures (5.0 Vicryl™; Ethicon Inc, São José dos Campos, Brazil) were placed to stabilize the flap 2 mm coronal to CEJ, followed by interrupted sutures to close the releasing incisions.

Electric Stimulation Protocol

The patients allocated in the CAF+ES group received the protocol for electrical stimulation. A unit consisting of a signal generator (Keysight Technologies., Inc., Santa Rosa-CA, USA), a power supply and circuit board, was assembled. Conductive electrodes for electrical current application were positioned on each side of the flap, at a

mean distance of 3 mm from the relaxing incisions and an alternating current with intensity of 100 μA at 9 kHz, was distributed in order to traverse the operated region. Patients received the stimulus applications for 120 seconds, once a day for 5 consecutive days. The electrical current was optimized through an oscilloscope (Keysight Technologies., Inc., Santa Rosa-CA, USA). All applications were performed by a single researcher involved in the study (CAS). Patients randomized to the CAF group received the simulation of the electrical stimulation process.

Clinical Assessments

Clinical parameters were evaluated at baseline, 3 and 6 months postoperatively as follows: 1) full-mouth visible plaque index (FMPI) (Ainamo & Bay 1975), and presence or absence of visible plaque accumulation at the site included in the study (plaque index [PI]); 2) full-mouth sulcus bleeding index (FMBI) (Muhlemann & Son 1971), and presence or absence of bleeding on probing (BOP) at the site included in the study; 3) PD: measured in millimeters with a periodontal probe (XP23/UNC-15, Hu-Friedy, Chicago, IL); 4) relative GR (RGR): distance from the gingival margin to the incisal border of the tooth; 5) RCAL: measured as PD+RGR; 6) GR: measured at the mid-buccal aspect of the tooth (both RGR and GR quantified using a caliper with a precision of 0.01mm and a pair of dividers); 7) keratinized tissue width (KTW); and 8) keratinized tissue thickness (KTT): measured at the midpoint location between the gingival margin and mucogingival junction as previously described (Fernandes-Dias et al., 2015). A masked and calibrated investigator (MMVM) carried out all clinical evaluations. The examiner calibration was performed for PD and RGR using Kappa statistic and intra-class correlation (k = 0.88 and ICC = 0.81, respectively).

Esthetic evaluation

To assess the final aesthetic outcome, two analyses were performed, one professional e one patient-centered. The professional evaluation was assessed using the Root Coverage Esthetic Score (RES) (Cairo et al. 2009). For this, two examiners performed the evaluations (JMB and IFM) 6 months after surgeries. The examiners presented previous experience in cosmetic dentistry, were blinded for the treatments and were previously calibrated (intra and inter-examiners K>0.8). Esthetic outcomes were also evaluated from the patient's point of view, using a VAS scale pre- and 6 post-postoperative.

Patient-centered outcomes

At the end of the first postoperative week, the patients completed a questionnaire about the occurrence of discomfort and postoperative pain. The pain was rated by the patient on a VAS scale of 0 to 10 (0 = no sensitivity and 10 = extreme pain). In addition, the patients were asked to report the number of analgesic pills they consumed that week. Dentin hypersensitivity was assessed by 5 s air blast from a triple syringe, applied to the exposed buccal cervical area. A visual analogue scale (VAS) scale (0 = no pain, 10 = extreme pain) was used to record DH related to stimulus. DH was recorded at baseline, 3 and 6 months postoperatively.

Sampling and immunological evaluation

The samples were collected as previously described (Santamaria et al., 2013). Briefly, gingival crevicular fluid (GCF) was collected from the vestibular region of the site that received the CAF. Sampling was performed by introducing filter paper strips (Periopaper; Proflow Inc., Amityville, NY, USA) into sulci until mild resistance was felt, removed after 20 s, and collected fluid volume immediately determined by calibrated instrument (Periotron 8000; Ora Flow Inc., Plain View, NY, USA). The strips were placed in sterile tubes containing 300 ml of phosphate buffered saline (PBS) with 0.05% Tween-2. Strips contaminated with visible blood were discarded. All GCF samples were stored immediately at -80° C. Samples were collected at baseline (before surgery), and 7, 14, 30, 60 and 90 days after surgery.

Levels of Cito / chemokines were determined by a multiplex immunoassay. Aliquots of each GCF sample were tested using a commercial human kit (Millipore Corporation, Billerica, MA, USA), in order to evaluate levels of the following inflammatory markers: interleukin (IL)-1β, IL-6, IL-10, tumoral necrosis factor α (TNFα), macrophage inflammatory protein-1α (MIP-1α), and chemotactic protein for monocytes-1α (MCP-1α). In addition, matrix metalloproteinases (MMP)-2, MMP-9, tissue inhibitor of metalloproteinases (TIMP)-1, TIMP-2 and VEGF were also evaluated. Sample concentrations (antigens in gingival crevicular fluid samples) were estimated from the standard curve using standard kit proteins following manufacturer's instructions and expressed as pg / mL.

STATISTICAL ANALYSIS PLAN

Descriptive statistics were performed using mean ± standard deviation (SD), and normality was tested using Shapiro-Wilk tests. The PD, RGR, CAL, KTT, KTW, and DH values and also inflammatory markers / growth factors were examined by two-way repeated measures ANOVA to evaluate the differences within and between groups, followed by a Tukey test for multiple comparisons when the Shapiro-Wilk p value was ≥ 0.05. Data presenting Shapiro-Wilk p values < 0.05 were analyzed using Mann-Whitney tests (for intergroup comparisons) and a Friedman test (for intragroup comparisons). Postoperative discomfort, patient's esthetics measured through VAS and intergroup RES comparisons were analyzed by T-tests. The frequency of complete root coverage (CRC) was compared using $\chi 2$ tests. For all tests a significance level of 0.05 was adopted.