Connective tissue graft associated or not with low laser therapy to treat gingival recession: randomized clinical trial


Abstract
Background: To evaluate the treatment of gingival recession with a connective tissue graft (CTG) alone or in combination with low-level laser therapy (CTG + L).
Methods: Forty patients presenting 40 Miller Class I and II gingival recessions were included. The defects were randomly assigned to receive either CTG (n = 20) or CTG + L (n = 20). A diode laser (660 nm) was applied to the test sites immediately after surgery and every other day for 7 days (eight applications).
Results: The mean percentage of root coverage was 91.9% for the test group and 89.48% for the control group after 6 months (p > 0.05). The test group presented more complete root coverage (n = 13, 65%) than the control group (n = 7, 35%) (p = 0.04). Dentine sensitivity decreased significantly after 6 months in both groups (p < 0.001). The two groups showed improvement in aesthetics at the end of treatment.
Conclusions: Low-level laser therapy may increase the percentage of complete root coverage when associated with CTG.

Conflict of interest and source of funding statement
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Gingival recession is defined as the apical positioning of the gingival margin in relation to the cemento-enamel junction, which results in root exposure. Several studies have indicated that traumatic brushing and inflammation caused by biofilm are the main aetiological factors for this condition (Löe et al. 1992, Serino et al. 1994). The presence of gingival recession may bring many negative effects, such as dentin hypersensitivity, aesthetic complaints, and predisposition to root caries and non-caries cervical lesions (Toffenetti et al. 1998, Goldstein et al. 2002).

As a result of the clinical problems caused by the presence of gingival recession, more than 750 clinical studies in the literature use various techniques to elucidate better treatment options for gingival recessions. The coronally positioned flap (CAF) and connective tissue graft techniques (CTG) are the most predictable techniques, reaching up to 100% root coverage. Systematic reviews have confirmed these results and concluded that the CAF alone or combined with biomaterials (enamel matrix derivative) and a connective tissue graft are the most predictable techniques for root coverage in cases of gingival recession-type defects (Buti et al. 2013, Cairo et al. 2014, Sanz & Simion 2014, Tonetti & Jepsen 2014).
However, even the most predictable techniques demonstrate some variability in their results among studies, with averages ranging between 60 and 96% of root coverage. This variability in the results is common, even in trials presenting strict inclusion and exclusion criteria to standardize the recession defects and the participants’ profiles, and highly expedient surgeons, in order to eliminate the possible influence of factors other than the effect of the tested therapies. It may be explained by several factors: (1) different training levels of operators; (2) local anatomic factors of recessions (such as the height and width of the papilla, and tissue thickness); and (3) differences in the wound healing potential of each patient (Santamaria et al. 2010, Cortellini & Pini Prato 2012, Vignoletti et al. 2014). Only the degree of experience of the operator can be solved, thus leaving the other two factors that may influence the results of periodontal plastic surgery procedures. Therefore, the use of new techniques and therapeutic strategies, as well as devices that accelerate wound healing, could improve the results of procedures for root coverage and allow more predictable results.

Low-level laser therapy (LLLT) has been successfully used to photo-biostimulate and to accelerate wound healing in humans. The wound healing after surgery procedures involves several biological events that can be improved by LLLT, such increased motility of human keratinocytes, enhanced neo-vascularization of tissue, increased fibroblast proliferation, maturation, and attachment. These effects may contribute to the higher tensile strengths of gingival flap margins, which may subsequently minimize the soft tissue recession, increasing the results of procedures for root coverage (Khadra et al. 2005, Ozturan et al. 2011). Several studies have indicated a wavelength between 600 and 840 nm to be the most effective for biostimulation (Karu 1998). However, only a few studies in periodontal plastic surgery have evaluated the benefits of applying low-level lasers for root coverage. Ozturun evaluated the additional benefits of LLLT for the CAF technique. The authors observed that laser irradiation made a statistically significant difference in terms of root coverage (Ozturan et al. 2011). To the best of our knowledge, there are no other studies that used LLLT associated with periodontal plastic surgical techniques for root coverage and its effects regarding aesthetic improvement or patient-centred outcomes. Thus, the aim of this study was to evaluate the 6-month outcomes of applying low-intensity laser therapy associated with connective tissue grafts to treat gingival recession.

Material and Methods

This investigation was designed as a parallel, double-blind, randomized clinical trial. The study protocol (ClinicalTrial.org-NCT02118155) was approved by the Institutional Review Board of State University of São Paulo (CEP-UNESP). Informed consent was signed by each subject after a thorough explanation of the nature, risks, and benefits of the clinical investigation.

Study population

Forty patients from the Periodontology Clinic of UNESP – State University of São Paulo (São José dos Campos, Brazil), presenting 40 maxillary buccal gingival recessions in their canines and premolars were included in the study.

The subjects were selected from October 2011 to June 2013, according to the following eligibility criteria: presenting Miller Class I or II gingival recession in the maxillary canines or premolars; visible cemento-enamel junction (CEJ) with pulp vitality; patients presenting no signs of active periodontal disease and full-mouth plaque and bleeding score ≤20%; patients older than 18 years; probing depth <3 mm in the included teeth; and patients who agreed to participate and signed an informed consent form. If patient presented multiple recessions, only the deepest recession was treated.

The criteria for exclusion were as follows: patients with systemic problems that would contraindicate the surgical procedure; patients taking medications known to interfere with the wound-healing process or that contraindicate the surgical procedure; smokers or pregnant women; patients who underwent periodontal surgery in the area of interest; and patients with orthodontic therapy in progress.

Initially, the participants received information about the aetiology and treatment of gingival recession. All of the patients were included in a pre-treatment programme to eliminate possible aetiologic factors related to gingival recession. Oral hygiene instructions were given, along with a non-traumatic brushing technique and a soft toothbrush. All participants received a session of prophylaxis and scaling. The surgical treatment was performed only when patients achieved adequate plaque control.

Clinical assessments

The primary endpoint of this trial was the percentage of root coverage at 6 months. After the initial therapy, the following parameters were recorded: (1) full-mouth visible plaque index (FMPI-Ainamo & Bay 1975) and the presence or absence of visible plaque accumulation at the site included in the study (plaque index (PI)); (2) full-mouth sulcus bleeding index (FMBI-Mühlemann & Son 1971) and the presence or absence of bleeding on probing (BOP) at the site included in the study; (3) probing depth (PD), measured in millimetres with a University of North Carolina periodontal probe; (4) relative gingival recession (RGR), measured as the distance from the gingival margin to the incisal border of the tooth; (5) relative clinical attachment level (CAL) as PD + RGR; (6) gingival recession (GR), measured at the mid-buccal of the tooth (the GR and RGR were measured using a pair of dividers and quantified by a digital caliper with 0.01-mm precision); and (7) keratinized tissue width (KTW), distance from the gingival margin to the mucogingival junction; (8) keratinized tissue thickness (KTT) was measured at the mid-point location between the gingival margin and mucogingival junction; (9) dentin sensitivity (DS) was assessed by an air blast from a triple syringe. The air blast was applied to the exposed buccal cervical area for 5 s. A VAS scale
(0 = without pain, 10 = extreme pain) was used to record the DS related to the stimuli. The presence or absence of plaque, BOP, PD, RGR, CAL, and DS was measured at the baseline, 3 and 6 months after surgery. KTW and KTT were measured at baseline, 3 and 6 months after surgery. RGR, CAL, and DS was measured or absence of plaque, BOP, PD, related to the stimuli. The presence pain) was used to record the DS comes were also evaluated from Kerner et al. 2009). Aesthetic out-

Aesthetic evaluation

Three aesthetic evaluations were performed: two professional and one patient-centred. The professional aesthetic evaluation was made using the photographs of the baseline and 6 months after surgery set in a before-and-after panel and performed by three professionals who were previously calibrated (K > 0.8) and blinded to the treatments (two periodontists – MANJ and SBF, and one orthodontist – MSJ). The professional evaluation was conducted using the qualitative cosmetic scale (QCE) and the root coverage aesthetic score (RES) (Cairo et al. 2009, Kerner et al. 2009). Aesthetic outcomes were also evaluated from the patient’s point of view, using a VAS before and after 6 months postoperative.

Patient discomfort

At the end of the first week after surgery, the patients completed a questionnaire in which questions about the occurrence of discomfort and postoperative pain were asked using a VAS scale. In addition, the patients were asked to report the number of analgesic pills they consumed that week. Additionally, tissue oedema (TE) was evaluated after 7 day of the surgical procedure using the score: 1 = absent, 2 = slight, 3 = moderate, or 4 = severe (Sanz-Moliner et al. 2013).

Randomization, allocation concealment, and blinding

Randomization was performed by a person who did not participate in the study and who generated a random allocation sequence in a computer program. This sequence was placed in opaque, sealed envelopes. Each envelope included the number of patients for each treatment, which was only revealed after surgery. In addition to the allocation concealment, the patients and the investigator responsible for the surgical procedures did not know which treatment each subject received.

Surgical procedure

The surgical procedures were performed by only one operator (MPS) who was blinded for the treatments. The gingival recession defects were treated by either connective tissue graft alone (CTG: control group; n = 20) or CTG plus application of LLLT (CTG + L: test group; n = 20). Briefly, after local anaesthesia, the surgical procedure was the trapezoidal type of CAF (de Sanctis & Zucchelli 2007) fully covering a CTG taken form palate area. The exposed root surface was gently scaled and planed to remove any possible irregularities. Afterwards, a thin and small connective tissue graft was sutured over the root surface, in such a way that covered the CEJ and the recession. Then, the flap was coronally positioned and sutured to completely cover the graft.

Laser protocol

The patients allocated for the test group received the following protocol for laser application: Five points of irradiation were performed (Fig. 1). The irradiation was performed with a GaAlAs (TheraLase-DMC®-Brazil) diode laser that continuously emitted a wavelength of 660 nm. 30 mW was used for 20 s, and the total applied energy density (fluence) was 15 J/cm² (3 J/cm² per point and an application time of 4 s per point). The applications were performed using punctual contact with the tip perpendicular to the gingival tissue. Laser therapy was initiated in the immediate post-operative period (just after sutures) and was followed by seven more applications performed every other day. The patients allocated to the control group received sham irradiation.

Postoperative care

After the surgery, the participants were instructed to take 500 mg of sodium dipyrone every 4 h for 3 days, in case of pain, and to discontinue tooth brushing around the surgical sites during the initial 14 days after surgery. During this period, plaque control was performed using 0.12% chlorhexidine rinse used twice a day. The sutures were removed after 7 days.

Statistical analysis

Sample size

The analyses were performed by a blind examiner. The sample size calculation was performed to detect 0.5 mm differences between the test and control treatment for root coverage after 6 months. The sample size was calculated using a power of 80%, α = 0.05, and, as previously described (Cairo et al. 2012), a standard deviation of 0.46 mm. Based on these data, a sample of 14 recessions per group would be required to achieve this purpose. With a sample of 20 recessions in each group, the power values were confirmed to be >80%.

Descriptive statistics were expressed as mean ± standard deviation (SD), and normality was tested using Shapiro–Wilk tests. The PD, RGR, CAL, KTT, KTW, and DS values 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. Those presenting Shapiro–Wilk p-values <0.05 were analysed using a Friedman test (for intra-group comparisons) and Mann–Whitney tests (for inter-group comparisons). Patients’ aesthetics and discomfort
measures using VAS were analysed by t-tests. The frequency of sites that were scored as very good or excellent in each group by QCE analysis, the frequency of complete root coverage, BOP, and the presence or absence of plaque at the site were compared using $\chi^2$ tests. Inter-group RES comparisons were performed with a t-test. Difference between groups regarding TE was evaluated using Mann–Whitney rank sum test, and the proportion of patients presenting absence or slight TE was compared by Fisher exact test. A significance level of 0.05 was adopted.

Results

All 40 patients completed the study. No dropouts occurred, and no adverse events were reported during the follow-up period (Fig. 2; Table 1). FMPI and FMBI were maintained below 20%, indicating that the patients controlled their supragingival plaque effectively during the study period.

Gingival recession

The gingival recession averages were 3.09 ± 0.67 mm for test group and 3.33 ± 0.72 mm for control ($p = 0.2$). There was a statistically significant reduction in the RGR for both groups; this parameter changed from 12.28 ± 1.58 mm to 9.44 ± 1.10 mm, with a gain of 2.84 mm in root coverage for the test group and from 12.36 ± 1.21 mm to 9.38 ± 0.94 mm, with a gain in root coverage of 2.98 mm ($p < 0.05$) for the control group. The percentage of root coverage was 91.9 ± 22.5% for the test group and 89.48 ± 22.38% for the control group ($p > 0.05$) (Table 2). Thirteen of 20 sites (65%) in the test group and 7 of 20 sites (35%) in the control group achieved complete root coverage after 6 months of observation; this difference between the groups was statistically significant ($p = 0.04$) for this parameter.

Probing depth and clinical attachment level

There was a statistically significant increase in the PD after 6 months for both groups ($p > 0.05$). This parameter changed from 1.05 ± 0.22 mm to 1.4 ± 0.49 mm in the test group and from 1.05 ± 0.22 mm to 1.34 ± 0.66 mm in the control group. This difference between groups was not statistically significant ($p = 0.550$). Both groups showed statistically significant CAL gains: 2.49 ± 0.91 mm for the test group ($p < 0.001$) and 2.69 ± 1.00 mm for the control group ($p < 0.001$). The difference between the groups was not statistically significant ($p = 0.38$) (Table 3).

Keratinized tissue

The two groups presented a statistically significant increase in KTT ($p < 0.05$). However, the difference between groups was not statistically significant ($p > 0.05$). The intra-group and inter-group analysis of KTW showed that the differences were not statistically significant after 6 months for both groups ($p > 0.05$). Table 2 shows the values.

Postoperative pain, DS, and TE

The assessment of pain during the first seven postoperative days showed no statistically significant

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difference between groups ($p = 0.333$). The number of analgesic pills ingested also did not show a significant difference ($p = 0.651$). The TE was $2.8 \pm 0.9$ for the test group and $3.14 \pm 0.7$ for control group ($p = 0.29$). When the proportion of patients presenting absence or slight TE at day 7 was evaluated, the test group presented 9 of 20 (45%) patients, whereas control group showed 3 of 20 (15%) ($p = 0.08$) (Table 3).

In the study sample, 65% of the subjects from the test group and 75% from the control group reported DS at baseline ($p > 0.05$). After the procedure, both groups presented statistically significant DS reduction. After 6 months, the frequency was 10% for both groups.

Aesthetics

The results of aesthetic evaluation by the patient through the VAS scale demonstrated a statistically significant improvement in aesthetics for both groups after 6 months ($p < 0.05$). The inter-group analysis revealed no significant differences between the groups after 6 months ($p = 0.59$). The results of the professional aesthetic evaluation using the RES scale showed no statistical difference between the test and control groups ($p = 0.480$). Qualitative cosmetic evaluation (QCE) also showed no statistical difference between groups ($p = 0.740$). The values of the three aesthetic evaluations are presented in Table 4. Figure 3 shows the final clinical appearance of the test and control groups.

Discussion

The aim of this study was to evaluate the 6-month outcomes of CTG associated with LLLT application for the treatment of gingival recessions. The present data indicate that LLLT may increase the predictability of CTG, with significantly higher percentages of CRC. However, caution should be taken to interpret this data. The number of shallow (2.5–3 mm) recessions allocated to the test group was greater than in the control group (10 and 5, respectively). This may have contributed to this result. Additionally, the literature shows a considerable variation regarding the complete root coverage achieved by CTG, which ranges from 10% (Wilson et al. 2005) to 88–89.5% (Salhi et al. 2013, Zucchelli et al. 2014). Our results show that the CTG + L group achieved 65% of CRC which is not better than the results of previous cited studies.

Because of this great variability regarding the CRC shown in the literature and the greater number of shallow defects allocated in the test group in the present study, attention should be given to the percentage of root coverage, which difference was not statistically significant (91.84 ± 22.5% for test and 89.38 ± 22.38% for control group). This result shows that LLLT may have minimal positive influence on root coverage when compared to CTG alone. Despite this, our findings are in accordance with a previous study that described the positive adjunctive effect of low-laser biostimulation associated with a coronally advanced flap for root coverage. In this study, Ozturan showed that irradiation using a diode laser with a 588 nm wavelength and 4.0 J/cm² of power density after the CAF procedure statistically produced more complete root coverage than in the control group. However, our study presents some differences in terms of the LLLT protocol and other results, when compared to Ozturan’s study (Ozturan et al. 2011).

In our study, 3 J/cm² of density of energy was applied per point, and 15 J/cm² was applied in each session. Although some authors recognize 4 J/cm² to be the optimal dose for tissue repair, the doses used in other studies range from 0.1 to 100 J/cm² (Karu 1998). Studies have shown that the use of low doses (3–6 J/cm²) stimulates wound healing by increasing fibroblast proliferation and collagen synthesis, and decreasing inflammation and oedema (Medrado et al. 2003, Almeida et al. 2009, Costa et al. 2010). The clinical data of the present study suggest that 15 J/cm² of energy density may also be suitable for periodontal root coverage surgery.

In addition to the density of energy, the chosen wavelength was based on the literature. Most studies utilizing LLLT in tissue repair employ wavelengths within the red visible spectrum (622–780 nm) and...
show significant benefits in tissue repair within that spectrum. Garcia suggested that the effect of laser biostimulation on wound healing may be due to the high absorption of 660-nm-wavelength lasers by cytochrome oxidase (the primary photoreceptor of the respiratory chain), resulting in increased production of ATP in cellular metabolism and in DNA synthesis (Garcia et al. 2012). Furthermore, there is an increase in fibroblastic proliferation and the early formation of granulation tissue.

Besides the biological effects, the growing interest in LLLT is based on the patients’ desire for less invasive and painful treatments. Some studies have suggested that LLLT also decreases inflammation and oedema, providing a more comfortable postoperative experience to the patient with less oedema and lower levels of pain (Enwemeka et al. 2004, Sanz-Moliner et al. 2013). In periodontics, LLLT may be used with periodontal surgeries to accelerate the healing process, promote analgesia, and reduce postoperative discomfort. However, the present study did not show additional benefits neither regarding patient discomfort nor tissue oedema. Despite the proportion of patients that showed absence or slight oedema was greater in the test group (45% versus 15%), the difference was not statistically significant. The mean VAS pain score for the first seven postoperative days was 2.45 for CTG + L and 1.68 for CTG, with no statistical difference between the groups \( \ (p = 0.33) \) regarding the presence of pain in the recession area. Additionally, the numbers of analgesic pills taken after the procedures were not statistically different between the groups. One possible explanation for this result is that, despite the frequent presence of pain after surgery, this pain is usually not intense. Wessel and Tatakis showed the results of postoperative pain obtained by a 0–10 VAS scale very similar to that presented in this study (Wessel & Tatakis 2008). Thus, a statistically significant difference would be difficult to detect in these low values of pain in this sample.

In the present study, the results showed that the applied LLLT protocol did not enhance the aesthetic outcome. Despite the fact that the CTG + L showed slightly better results in all three aesthetic assessments, the differences between groups were not statistically significant. The application of LLLT after the surgical procedures would stimulate wound healing by increasing

| Table 3. Changes in clinical parameters after 6 months, the postoperative pain, and the number of analgesic pills after 7 days |
|--------------------------------------------------|------------------|------------------|------------------|
| RGR reduction (mm)                               | CTG + L (2.85 ± 0.95) | CTG (2.74 ± 0.74) | 0.4              |
| Percentage root coverage                         | 91.84 ± 22.5%     | 89.38 ± 22.38%   | 0.661            |
| Frequency complete root coverage                 | 13 (65%)*         | 7 (35%)          | 0.04             |
| CAL gain (mm)                                    | 2.47 ± 0.91       | 2.59 ± 1.00      | 0.38             |
| Postoperative pain (VAS)                         | 2.45 ± 2.50       | 1.68 ± 2.13      | 0.333            |
| Analgesic pills                                  | 1.8 ± 1.73        | 2.26 ± 2.92      | 0.651            |
| Percentage of absence or slight TE              | 45%               | 15%              | 0.08             |

| Table 4. Mean values and standard deviation of aesthetic evaluation |
|--------------------------------------------------|------------------|------------------|
| Aesthetic | Initial | Final | Initial | Final |
| VAS       | 4 ± 1.62 | 9.2 ± 1.05* | 4.31 ± 1.60 | 8.61 ± 1.75* |
| RES       | 8.05 ± 1.09 | 7.85 ± 0.95 | 3.75% |
| QCE       | 44.4% | 37.5% |

RGR, relative gingival recession; CAL, clinical attachment level; VAS, visual analogue scale; TE, tissue oedema.
*Inter-group statistically significant difference – \( \chi^2 \)
fibroblast activity and epithelization (Ozcelik et al. 2008). Therefore, it would decrease scar formation and, as a consequence, improved the aesthetic outcomes. However, our results did not show this possible adjunctive effect of LLLT, and other studies with different laser protocols may be required to test this hypothesis.

The presence of DS was also evaluated by the patients before and during the treatment period. The results revealed a statistically significant reduction in DS between the baseline and the subsequent evaluated periods in both groups. However, no statistically significant difference could be observed between the groups. Few studies have assessed DS after root coverage procedures. Some of these studies did not find statistically significant differences after root coverage (Cortellini et al. 2009). However, other authors (de Oliveira et al. 2013, Santamaria et al. 2009) have observed that the surgical procedures for root coverage may reduce DS and improve patient quality of life; this may be related to the fact that most of the exposed roots achieve complete root coverage, thus sealing the exposed dentinal tubules and reducing the chances of symptoms. Based on this hypothesis, if LLLT, as an adjunctive treatment, can improve complete root coverage alongside the CTG technique, it may have some impact on DS and patient quality of life after surgery. However, more studies are required to test this hypothesis.

The present study may contribute to the understanding of the LLLT effect on periodontal soft tissue and its cost-benefit. As mentioned before, LLLT may improve the CRC of root coverage procedures (Ozturan et al. 2011) and decreased postoperative pain and oedema (Ozcelik et al. 2008, Sanz-Moliner et al. 2013). However, disadvantages as the cost of the equipment and the higher number of appointments that are required for laser application should be considered. Within the limits of this study, it can be concluded that LLLT may have minimal additional benefits in terms of CRC when compared to the CTG alone to treat Miller Class I and II gingival recession. However, these results should be interpreted with caution based on the following considerations: First, the results of using LLLT as an adjunctive therapy in periodontal surgeries depend on the characteristics of the laser protocol employed, including the variables that might be directly related to the effects of tissue biostimulation, such as appliance power, wavelength, energy density, and number and frequency of applications. Second, longitudinal observation is also necessary to evaluate the stability of the results and to establish the long-term success of this combined approach. Third, the clinical effects of LLLT may be better understood through examinations and quantification of inflammatory markers, which will allow us to observe the effects of LLLT at the cellular level. Thus, more clinical studies are needed to determine the exact benefits of LLLT after periodontal plastic surgery procedures.

References


Clinical Relevance

Scientific rationale for the study: Low-level laser therapy (LLLT) has been successfully used to photobiostimulate and to accelerate dermal wound healing in humans. The literature lacks controlled studies evaluating the use of this therapy in the field of periodontal plastic surgery. Principal findings: The present study shows that when connective tissue graft is associated with LLLT more frequency of complete root coverage can be achieved in the treatment of gingival recession. Practical implications: More randomized clinical trials are required to test whether the LLLT have an adjunctive effect on root coverage procedures. The present results suggest that this combination may be useful.