J Clin Periodontol 2008; 35: 250–254 doi: 10.1111/j.1600-051X.2007.01194.x

Journal of Clinical Periodontology

Improved wound healing by low-level laser irradiation after gingivectomy operations: a controlled clinical pilot study

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Ozcelik O, Haytac MC, Kunin A, Seydaoglu G. Improved wound healing by low-level laser irradiation after gingivectomy operations: a controlled clinical pilot study. J Clin Periodontol 2008; 35: 250–254. doi: 10.1111/j.1600-051X.2007.01194.x.

Abstract

Aim: Low-level laser therapy (LLLT) may induce morphological, molecular and cellular processes, which are involved in wound healing. The aim of this split-mouth controlled clinical trial was to assess the effects of LLLT on healing of gingiva after gingivectomy and gingivoplasty.

Material and Methods: Twenty patients with inflammatory gingival hyperplasias on their symmetrical teeth were included in this study. After gingivectomy and gingivoplasty, a diode laser (588 nm) was randomly applied to one side of the operation area for 7 days. The surgical areas were disclosed by a solution (Mira-2-tones) to visualize the areas in which the epithelium is absent. Comparison of the surface areas on the LLLT-applied sites and controls were made with an image-analysing software.

Results: Despite the prolonged time needed for application, patients have tolerated LLLT well. While there were no statistically significant differences between the stained surface areas of the LLLT applied and the control sites immediately after the surgery, LLLT-applied sites had significantly lower stained areas compared with the controls on the post-operative third, seventh and 15th day ($p<0.001$ for each).

Conclusions: Within the limitations of this study, the results indicated that LLLT may enhance epithelization and improve wound healing after gingivectomy and gingivoplasty operations.

Key words: gingivectomy; gingivoplasty; low-level laser/therapy; wound healing

Accepted for publication 27 November 2007

Lasers have increasingly been used in modern dentistry for more than 30 years. A wide range of lasers such as CO_2, Nd:Yag, and Er:Yag are used in the field of periodontology for soft and hard tissue ablation, detoxification of root surfaces, pocket debridement, bacterial elimination and various surgical approaches (Cobb 2006). Despite the common use of these high-power surgical lasers, there is an other less known type of lasers called low-level lasers. These lasers work in the milliwatt range with wavelengths in the red or near-infrared spectrum (400–900 nm) (Qadri et al. 2005). Low-level lasers do not cut or ablate the tissues. The basic principle of low-level laser therapy (LLLT) is based on the biostimulation or the biomodulation effect (Walsh 1997, Damante et al. 2004a), which consists of the fact that irradiation at a specific wavelength is able to alter cellular behaviour (Hopkins et al. 2004, Posten et al. 2005). This effect is achieved by acting on the cellular mitochondrial respiratory chain (Silveria et al. 2007) or on membrane calcium channels (Alexandratou et al. 2002). This action subsequently promotes an increase in cell metabolism and proliferation (Khadra et al. 2005). In vitro and in vivo data suggest that LLLT facilitates fibroblast and keratinocyte cell motility (Yu et al. 1996, Walsh 1997, Kreisler et al. 2003), collagen synthesis (Pinheiro et al. 2005), angiogenesis and growth factor release (Tuby et al. 2006), which lead to increased wound healing.

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.
The study was self-funded by the authors and their institution.

Various light sources, including helium–neon, ruby, diode and gallium arsenide, have been used to deliver LLLT under different conditions such as for treatment of mucositis (Lara et al. 2007), paresthaesia (Khullar et al. 1996) and TMJ disorders (Venancio et al. 2005). In addition, LLLT has been used for promoting wound healing and reducing pain after gingivectomy (Damante et al. 2004b, Amorim et al. 2006), endodontic surgery (Kreisler et al. 2004), orthodontic treatment (Turhani et al. 2006) and as an adjunct after non-surgical periodontal treatment (Kreisler et al. 2005, Qadri et al. 2005). However, the use of LLLT has still not been widely accepted by the medical and dental community due to the lack of sufficient number of controlled clinical trials.

The aim of this controlled clinical pilot study was to assess the effects of LLLT on wound healing of gingiva after gingivectomy and gingivoplasty operations.

Material and Methods

This pilot study had an examiner- and patient-blinded, placebo-controlled and split-mouth design.

Study population

The study sample was selected from patients who area referred to the Department of Periodontology, Faculty of Dentistry, Cukurova University, between January and April 2007. In order to be included, the patients had to be systemically healthy and non-smokers. The study protocol was reviewed and approved by the Institutional Review Board. Informed written consent was obtained from all patients.

Clinical procedures and LLLT application

All patients received oral hygiene instructions and scaling and root planning at the beginning of the study. After 3 weeks, the physiological gingival contours of the patients were re-evaluated for gingivectomy and gingivoplasty requirements. Thus, patients (nine women and 11 men with a mean age of 24.3) who had symmetrical over-contoured gingiva on their maxillary or mandibular anterior region with at least six affected teeth (Fig. 1a) were included in the study. During the gingivectomy and gingivoplasty operations, the scalloped external bevel incision was accomplished with a Kirkland knife and a #15 blade. Then, a sulcular incision was performed and the release of the interproximal tissue was achieved with an Orban knife. Following excision of the enlarged tissue with curettes, gingivoplasty was performed by periodontal knives (Fig. 1b). After these operations, one of the symmetrical surgical sites was randomly assigned to receive LLLT by the periodontist with the toss of a coin. After haemostasis, LLLT was applied to one side of the surgical area with slight contact with to the tissue for 5 min. while the adjacent symmetrical area (control site) did not receive laser. In order to prevent the scattering of light to the non-LLLT side, pre-prepared individualistic impressions were used (Fig. 1c). The low-level laser used in this study was a diode laser (ULOCKS, VSMA Lab, Voronezh, Russia) with a wavelength of 588 nm and an output power of 120 mW, and the irradiance for 5 min. was 4.0 J/cm^2, delivered by applying in the continuous wave mode for 5 min. For the control sites, the laser application was simulated, without pushing the start button. Post operatively, LLLT was repeated on the test sites for 5 min. daily for 7 days while the control sites again underwent laser simulation. No periodontal dressings were used and the patients were prescribed naproxen sodium as needed for analgesia. All surgical procedures and LLLT application were performed by the same periodontist (O. O.) in order to prevent inter-operator variations.

Surface area determination

After each LLLT application, the surgical area was disclosed by a plaque-disclosing solution (Mira-2-tone, GMBH & Co., Duisburg, Germany) to better visualize the areas in which the gingival epithelium is absent, abraded or lacking sufficient keratinization and to distinguish these areas from normal gingiva. This solution has been used previously for detecting even minor areas of gingival abrasion, which would otherwise be largely undetectable, and this method has been suggested to be a sensitive tool for the identification of areas lacking epithelium (Van der Weijden et al. 2004). Surface area determination was performed by the method of Esen et al. (2004). The surface analysis was performed by one of the authors (M. C. H.) who was blinded to the treatment techniques and to the test and control sites in order to assure an unbiased determination. The calibration

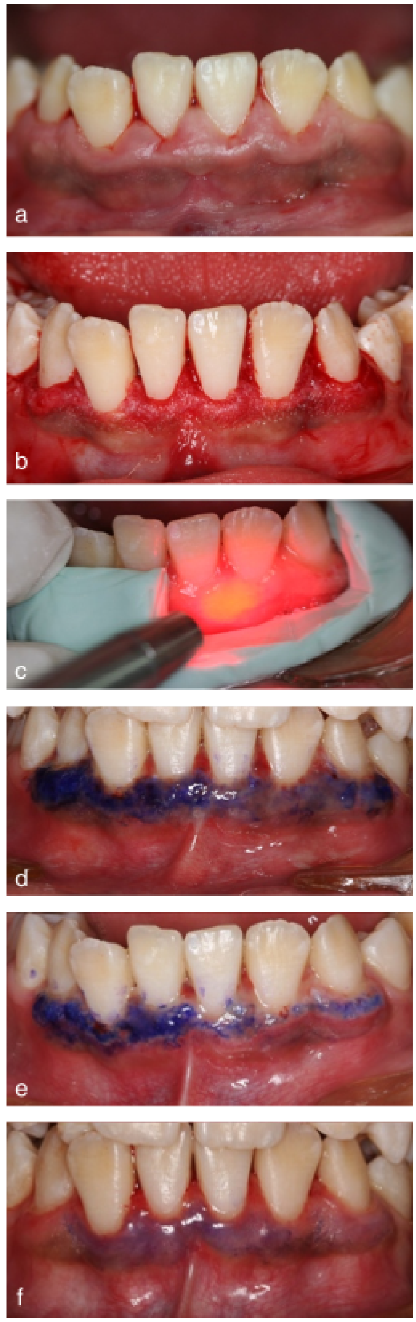

Fig. 1. Clinical view of the patient requiring gingivectomy and gingivoplasty before (a) and immediately after the surgery (b). Application of low-level laser therapy (LLLT) to the randomly selected site (c). Note the pre-prepared individualistic impression, which was used to prevent the scattering of laser to the control site. The stained areas of the LLLT applied and control sites on the post-operative third (d), seventh (e) and 15th (f) days.

of the examiner was performed by the previous evaluation and calculation of the stained surgical fields on two occasions on the images of 10 patients who had undergone gingivectomy and gingivoplasty operations and who were not enrolled in the study. Calibration was accepted if the calculations were similar to the millimetre square at the 90% level on these two separate evaluations. Digital images of the stained gingiva were obtained immediately after the operations and on all post-operative visits. In an attempt to standardize the images, each patient's head was positioned and stabilized by a panoramic imaging unit according to the routine instructions used for orthopantomography. A digital camera was mounted on a tripod and the images were obtained at standard magnification and distance (four optical zoom, 20 cm). The light source in the room and the flash level of the camera were also standardized in order to prevent bias of the luminosity of the images. The examiner traced and measured the total area of stained gingiva with the aid of an image-analysing software (Image J 1.31o, National Institutes of Health, Bethesda, MD, USA). Actual intra-oral dimensions of the maxillary right central incisor (mesial to distal) were measured in each patient and were then compared with the dimensions of the incisor in the images, and the proportion between the actual and photographic size was used to calibrate the images.

The darkly stained fields were considered as sites still undergoing wound healing with the lack of enough layers of epithelium. Surface areas of stained fields on the LLLT-applied sites and the controls were measured on the third (Fig. 1d), seventh (Fig. 1e) and 15th (Fig. 1f) days after surgery and were then compared by statistical analysis.

Statistical methods

Normality was checked by the Shapiro–Wilks test of continuous variables. Non-parametric tests were chosen because the data were not distributed normally. Intra-group time-dependent data were analysed by Friedman's test, and Wilcoxon's rank sum test was used to evaluate the differences within groups at each time point. Data were expressed as median (minimum–maximum). A p value of <0.05 was considered to be significant. Statistical analysis was performed using the statistical package SPSS v 12.0.

Sample size was calculated with an expected parameter estimate based on a previous analysis that was performed on six patients. Taking into consideration the results of this trial [a mean of 0.41 mm^2 of stained surface areas in LLLT-applied groups and a mean of 0.64 mm^2 in the control sites (SD = 0.32) on the 15th day], the minimum required sample size was found to be approximately 19 in the paired study groups within a 95% confidence interval and 80% power.

Results

All patients completed the study course and complied with the post-operative LLLT application appointments. No post-operative complications such as swelling, bleeding or oedema were observed in any patient and three patients used analgesics for 4 days.

The results of the study are summarized in Table 1. While there were no statistically significant differences between the stained surface areas of the LLLT-applied and the control sites immediately after the surgical procedures, LLLT-applied sites had significantly lower stained surface areas compared with the control sites on the post-operative third, seventh and 15th days ($p<0.001$ for each), indicating faster surface epithelization at all time periods.

In the LLLT-applied sites, complete wound healing and absence of gingival staining were observed between 18 and 21 days, while the wound healing in control sites was completed between 19 and 24 days.

Discussion

The use of LLLT for oral and periodontal purposes has been the subject of numerous in vitro and in vivo studies. The increasing interest in the field of LLLT is based on the perceptions of patients who desire minimally invasive and painless treatments. However, there is insufficient evidence in the peer-reviewed literature for the effectiveness of LLLT in oral soft tissue applications.

Within the limitations of this study, the findings revealed that LLLT promotes wound healing after gingivectomy and gingivoplasty operations in humans. The wound-healing after these operations mainly includes fibroblasts, keratinocytes and immune cells. Within a few days following surgery, epithelial cells start to migrate over the wound surface from the margins, while fibroblasts proliferate and lay a new connective tissue underneath the epithelial seal (Stahl et al. 1968a, b). During this period, cytokines and growth hormones expressed by the immune cells such as neutrophiles and macrophages orchestrate the wound healing process (Stahl et al. 1968a, b). Previous studies suggest that LLLT application may accelerate wound healing by increasing the motility of human keratinocytes and promoting early epithelization, by increasing fibroblast proliferation and matrix synthesis and by enhancing neovascularization. It has also been shown that the expression of fibroblast growth factors by macrophages and fibroblasts is increased after LLLT application (Tuby et al. 2006). Another effect of LLLT on wound healing is to increase the revascularization rate as it is known that successful wound healing following periodontal surgery is strongly influenced by the revascularization rate (Donos et al. 2005).

Despite the listed beneficial effects of LLLT, there are very few clinical studies about gingival surgery, which makes the comparison of our results impractical. While Amorim et al. (2006) have reported that LLLT (at 685 nm) significantly promoted gingivectomy wound

Table 1. The comparison of the wound surface area analysis of LLLT applied and control sites on the baseline and post-operative third, seventh and 15th days

	LLLT median (minimum–maximum)	Control median (minimum–maximum)	p Wilcoxon's test
Baseline		2.05 (0.95–3.01)	0.145
Third day	1.53 (0.53–2.32)	1.60 (0.71–2.41)	0.001
Seventh day	0.61 (0.17–1.48)	1.02 (0.38–1.93)	0.001
15th day	0.17 (0.06–0.41)	0.33 (0.16–0.56)	0.001
p Friedman's test	0.001	0.001	

LLLT, low-level laser therapy.

healing, Damante et al. (2004a, b) have found that LLLT with a diode laser (at 670 nm) did not improve healing after gingivoplasty both clinically and histomorphometrically. In Damante et al.'s studies, the laser was applied at 48-h intervals for four sessions; however, it has been suggested that daily treatment with LLLT is required to achieve the maximal benefit. (Walsh 1997) In addition, Damante et al. (2004a) have used subjective criteria such as gingival colour, texture and contour for the clinical evaluation parameters. The daily application of LLLT for 7 days and the use of staining solution for the detection of the abraded surgical sites, together with the digital analysis of surface area determination on standardized clinical photos, may explain the differences in the present results and the previous ones.

Important factors in the effectiveness of LLLT include dose, wavelength and the amount of energy applied (Khadra et al. 2005). Extreme variations of these parameters are used in previous studies with LLLT (Tuner & Hode 1998). Most experimental and clinical studies on LLLT were performed using semiconductor diode lasers with wavelengths in the range of 635–830 nm. The wavelength used in this study was 588 nm, and the total applied energy for each session was 4 J/cm^2. Although the biological effectiveness of irradiation of 588 nm is not well documented in the literature, it has been suggested that the best absorption of light, which leads to LLLT-induced cellular mitochondrial respiratory chain activation and subsequent fibroblastic activity, occurs between 562 and 600 nm (Weiss et al. 2004, 2005). Recently, Ozcelik et al. (2008) have demonstrated that LLLT at 588 nm, when used together with Emdogain, had resulted in less gingival recession, less swelling and less post-operative pain scores in the treatment of intra-bony defects compared with Emdogain alone. In addition, Almeida-Lopes et al. (2001) have shown that lasers of equal power output present a similar biological effect i.e. cell growth independently of their wavelengths. Therefore, there is currently an urgent need for the standardization of specific wavelength and dose for each procedure and for each case. In addition, the application mode of LLLT is also an important factor, as some laser devices do not have appliance tips for intra-oral use.

This pilot study has a series of methodological limitations. Firstly, the small sample size of the study may affect the reproducibility of our results and therefore these results should be interpreted with caution. Secondly, healing after a gingivectomy operation is a rapid and simple process, which is usually uneventful even when diverse techniques are used. In addition, the young age of the study population and lack of any systemic diseases may also have affected the outcome of the study. In addition, although an image-analysing programme was used to determine the stained gingival surface, this method is still strongly operative sensitive. Therefore, further clinical, histological and/or immunohistological studies with larger study populations including diverse clinical conditions are required in order to evaluate the exact benefits of LLLT on gingival healing and to correlate the clinical alterations with the findings at the cellular level.

Because the LLLT-applied and the control surgical fields were adjacent in our study, it is important to mention the ''systemic effect'' of laser therapy (Damante et al. 2004b). It has been suggested that LLLT promotes the release of growth factors into the blood stream, which can reach adjacent and distant sites of the body. Therefore, it can be theorized that the application of LLLT to one site of the surgical field may also be beneficial for the adjacent site in our study. In addition, as it is well known that the keratinocytes migrate until they come in contact with another keratinocyte (i.e. contact inhibition), the already excitable keratinocytes on the LLLT-applied sites might have rapidly migrated to the control sites. Although these interpretations require further elucidation, these issues might have caused the underestimation of effectiveness of LLLT.

Besides LLLT application, there are several other approaches for reducing the post-operative morbidity such as minimally invasive flaps (Cortellini et al. 1999, 2001, Cortellini & Tonetti 2007). In the present study, the time needed for LLLT application (intra-surgically and once a day for 7 days after surgery) added up to 40 min. This is a considerable amount of time that is required in addition to the time of the conventional operation and complicates the justification of the use of LLLT with the small additional benefits of this technique. However, the study has revealed that the patients could tolerate the required time for LLLT application. Patients who are informed about the potential beneficial effects of painless, minimally invasive nonablative LLLT may feel that they are being treated in the best manner and they are receiving a high-quality treatment technique. All these factors can make the LLLT well tolerable.

In conclusion, within its limitations, the findings of this pilot study have shown that LLLT may improve the wound healing by increasing the surface epithelization after gingivectomy and gingivoplasty operations. A better understanding of the novel treatment strategies will shed light on the periodontal treatment of the new era (Tonetti 2002). Thus, the more we improve our knowledge of LLLT, the better we will benefit from this technique.

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Clinical Relevance

Scientific rationale for the study: Numerous studies have reported that low-level laser therapy on tissues has a bio-stimulatory effect, which promotes an increase in cell metabolism and proliferation and improves wound healing.

Principal findings: Despite a small sample population, it was found that LLLT-applied sites had an increased surface epithelization rate after gingivectomy and gingivoplasty operations compared with the control sites.

Practical implications: Within the limitations of the study, these findings may be of clinical relevance, in that LLLT may be considered to be an adjunctive tool in the immediate post-operative healing period after gingival surgery by improving wound healing