

CLINICAL AND HISTOLOGICAL EVALUATION OF GINGIVAL HEALING AFTER GINGIVECTOMY AND LOW-LEVEL LASER THERAPY

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ABSTRACT

Background: Low-level laser therapy (LLLT) may induce morphological and cellular processes, which are involved in wound healing. Thus, increasing interest in the matter of LLLT is based on its ability to provide minimally invasive and painless treatments for the patient as well as increasing his comfort following surgery.

Objective: The purpose of this study was to assess the clinical and histological effect of LLLT on healing of gingiva after gingivectomy.

Methods: Twenty patients with inflammatory gingival enlargement on their maxillary or mandibular anterior region were included in this study. Patients were divided randomly into two groups (Test group=ten patients) after gingivectomy a diode laser (660 nm) was applied to the wound area immediately post-surgery (day 1) and at days 3, 5 post-surgery. The control group (ten patients) did not receive laser irradiation. Healing was evaluated clinically using Healing score and Visual analogue scale VAS. For histological evaluation, mucosal defect measuring 2 mm in diameter and 0.2 mm in wall thickness was created into the hard palate of eighteen male Dawley rats. For the test group (9 rats) LLLT was applied with the same settings and intervals used in the clinical part of the study, while for the control group (9 rats) no laser irradiation was applied.

Results: The laser-treated group had significant improvement regarding the healing score at 5, 7 and 14 days p.s., and significant improvement in VAS score was observed at day 3 and 5 p.s., than the control group. The histological sections revealed a thin layer of epithelium covering the wound after 4 days in the test group in contrast to the control group. Also, complete epithelization of the wound area with keratin formation and revascularization after 7 days was observed in the test group.

Conclusion: LLLT can be used as an effective adjunctive treatment following gingivectomy procedure to promote healing of oral mucosa.

KEY WORDS: Gingivectomy, Low level Laser, Healing, Clinical, Histological evaluation.

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INTRODUCTION

Over than 40 years Lasers have been investigated as an alternative or adjunctive tool to conventional procedures commonly used in periodontology. Many lasers such as CO₂, Er:YAG, Nd:YAG, and Diode have been used for soft and hard tissue ablation, detoxification of root surfaces, pocket debridement, bacterial elimination and various surgical approaches (Cobb 2006).

Although the frequent use of these high-power lasers, another type of laser called low-level laser have been the subject of numerous in vitro and in vivo studies. These lasers wavelengths are in the red or near infrared spectrum (400–900nm) and there are used in the milliwatt range (Qadri *et al.* 2005).

The increasing interest in the matter of LLLT is based on the appraisals of patients who desire minimally invasive and painless treatments as well as increasing his comfort following surgery. (Javier *et al.* 2013) Low-level lasers do not cut the tissues. However, their action is based on the biostimulatory effect they exert on various cells. (Walsh *et al.* 1997, Damante *et al.* 2004). It has been demonstrated that the biologic effects of LLLT on tissues are processed in different ways. By acting on the mitochondrial respiratory chain (Silveria *et al.* 2007) or on membrane calcium channels (Alexandratou *et al.* 2002). Subsequently, increase in cell metabolism and proliferation are induced (Khadra *et al.* 2005).

Previous In vitro and in vivo studies observed that LLLT promotes fibroblast and keratinocyte cell motility (Yu *et al.* 1996, Walsh *et al.* 1997, Kreisler *et al.* 2003), collagen synthesis (Pinheiro *et al.* 2005), angiogenesis and growth factor release (Tuby *et al.* 2006), that lead to enhancement of wound healing.

Different laser systems such as helium–neon, diode and gallium arsenide, have been used to deliver LLLT for different oral conditions. Treatment of mucositis (Lara *et al.* 2007), paresthesia (Khullar *et al.* 1996) and TMJ disorders (Venancio *et al.* 2005) have been investigated. Furthermore, LLLT has been

used for sponsoring wound healing and reducing post-operative pain after gingivectomy (Damante *et al.* 2004, 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).

The purpose of this study was to assess the effect of LLLT on gingival healing following gingivectomy procedure.

MATERIALS AND METHODS

A. Clinical section:

The study sample was selected from patients who are referred to the Department of Periodontology, Faculty of Dentistry, Alexandria University, between September and December 2015. In order to be included in the study, the patients had to be suffering from chronic inflammatory gingival enlargement, systemically healthy, non-pregnant and non-smoker.

Gingivectomy had been planned for each patient to involve maxillary or mandibular anterior region with at least six affected teeth. Each subject signed a detailed informed consent form, and ethical approval was granted by the Ethics Committee of Faculty of Dentistry, Alexandria University.

In all cases, pre-surgical preparation consisted of supra and sub- gingival scaling, oral hygiene instructions and occlusal adjustments if needed. Using a periodontal probe with William's calibration (Hu-Friedy) at pretreatment, initial probing depths were recorded for the mid-buccal, and mesial and distal interproximal surfaces of each affected tooth. After 4 weeks, the gingival condition of the patients was re-evaluated prior to the gingivectomy procedure.

Procedure:

The patients were divided into two groups; the test group (ten patients) in which LLLT was applied after the gingivectomy procedure. While in

the control group (ten patients) no LLLT was used following the gingivectomy procedure.

The gingival pockets were first examined with a periodontal probe and marked with a pocket marker, then Kirkland knife was used for external bevel incision and Orban knife was used for releasing the interdental tissue.

After hemostasis, the surgical site of the test group was irradiated immediately after surgery and at days 3, 5 p.s. with a diode laser (Photon Laser Comp. 660 nm) using 50-mW output power, continuous wave, and beam diameter of 3 mm. The irradiation was made at 1 cm distance in scanning mode of an area about 1 cm² during 80 sec corresponding to an energy density of 4 J/cm², while holding the delivery tip perpendicular to the tissue surface (Fig. 1).

Subsequent to the gingivectomy procedure, control sites were not exposed to laser irradiation (Fig. 2). At conclusion of the prescribed treatments, all surgical sites were covered with a periodontal

dressing (Coe-Pak). A Titanium foil was placed in the inner surface of the Coe-Pack to prevent mechanical injury to the tissues while healing due to pack removal for further laser applications every 48 hour during the first week. All surgical procedures and LLLT application were performed by the same periodontist (M.M) in order to prevent inter-operator variations.

Photographs of the treatment sites were taken pre-surgery, immediately post-surgery, and at 3, 5, 7, and 14 days p.s. The photographs were used by two examiners to score three clinical parameters. These examiners were blinded to test versus control sites. The clinical parameters that were scored consisted of the following: tissue color (pink, red, bluish); tissue contour (normal, hyperplastic, or atrophic); and clinical status of the healing wound for the specific time interval (complete healing, partial healing). For the purpose of analysis, a three-point scale was used to score healing of the surgical wound:



Fig. (1) Clinical view of the patient requiring gingivectomy (Test group) before (a) and immediately after the surgery (b) Application of low-level laser therapy (LLLT) to the surgical site (c) and after 2 weeks follow-up (d)



Fig. (2) Clinical view of the patient requiring gingivectomy (control group) before(a) and immediately after the surgery (b)and after 2 weeks follow-up

B. Healing score

Score 0: indicating that laser-treated test sites and control sites exhibited the same degree of healing response

Score 1: indicating superior healing of control sites compared to laser-treated wounds

Score 2: indicating superior healing of laser-treated wounds compared to control sites

To assess agreement of scoring among the two examiners, the nonparametric kappa test was used. The kappa index is interpreted as follows: <0.40, weak agreement; 0.40—0.75 interval, from reasonable to good agreement; >0.75, excellent agreement.

B- Visual analogue scale scores VAS (Huskisson, 1976):

Patients were given log diaries and instructed to record the intensity of pain, the period of healing. The recordings were made just immediately after treatment, 3, 5 and 7 days.

Experimental section (Hashemipour, 2013):

18 male Dawley rats, weighing 200 g–250 g and between 6–7 weeks of age, were purchased from the research Institute, Alexandria Egypt. The animals were housed under standard conditions (23°C–25°C; 12 hours of light/dark cycles) and given laboratory food and water throughout the study period.

In this study, adult male rats were divided into 2 groups (n = 9 per group):

Control group:

Wound created without any surface management

Test group:

Wound created plus low level laser applications

A wound in form of mucosal defect measuring 2mm in diameter and 0.2 mm in wall thickness was created into the hard palate of each rat. The wounds were created by the same researcher. For the test group, LLLT was applied with the same settings

used in the clinical part of the study immediately after surgery and at days 3, 5 p.s., while for the control group a sterile gauze was placed over the wound to control bleeding then removed without applying any surface treatment.

For histological observations, three rats from each group were sacrificed using inhalant anesthetics at 2, 4 and 7 days after surgery. The palate was dissected and specimens were fixed in 10% neutralized formalin for 1 week. Decalcification was carried out then samples were embedded in paraffin wax. Sections of about 5 µm thickness were cut and stained with haematoxylin-eosin.

Light microscopy was used to evaluate the histological changes and comparisons were carried out between the test and control groups. (Sainte-Marie, 1962)

Statistical analysis

The comparisons between control and treated groups were done using the nonparametric Kruskal-Wallis test. The Friedman test was used to compare wound healing of the control and laser groups at several time points. Significance was accepted at $p < 0.05$.

RESULTS

Clinical results

1. Healing score:

During the immediate post-operative period (day 1) both groups were similar. At day 3 p.s., there was better improvement in healing of the wounds treated with laser compared to the control wounds. At 5, 7 and 14 days p.s., the laser-treated group had significant better results in healing than did the control group. The agreement of scoring among the examiners was calculated with a 0.671 kappa index value considered significant ($p > 0.05$). (Fig. 3)

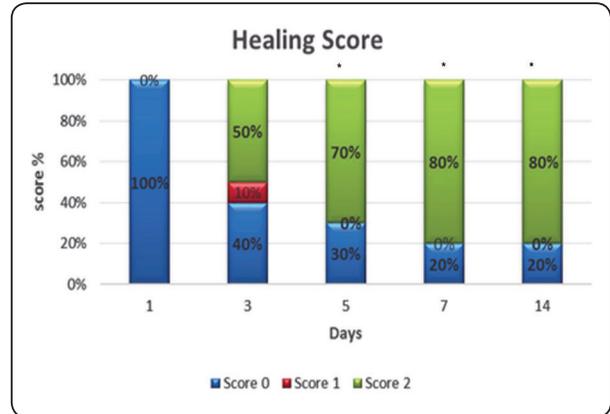


Fig. (3) Gingival wound healing for laser and control groups during the study period. (*significant difference, $p > 0.05$)

2. VAS:

During the immediate post-operative period (day 1) both groups were similar. At day 3 and 5 p.s., there was a statistically significant reduction in VAS score in the laser group compared to the control group ($p=0.001$, $p= 0.003$). At 7 days postoperatively the test group showed reduced VAS score compared to the control group but no statistical significant was observed. ($p=0.581$). (Fig. 4).

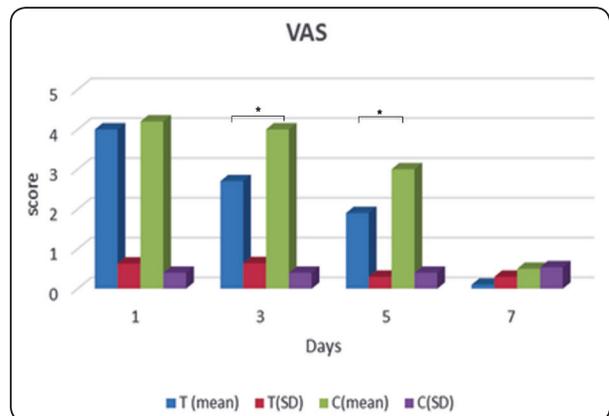


Fig. (4) Visual analogue scale scores VAS for laser and control groups during the study period. A statistically significant reduction in VAS score in the laser compared to the control group at day 3 and 5 p.s., ($p=0.001$, $p= 0.003$). (* significant difference, $p > 0.05$)

Histological results:

Control group:

At 2 days postoperatively: The incision area at the palatal mucosa of rats revealed loss of epithelial thickness. The area at the base of the incision showed areas of exposed connective tissue. Many inflammatory cells were seen in the lamina propria. Irregular distribution of collagen fibers were observed in the underlying lamia propria. (Fig. 5 a)

At 4 days postoperatively:

The two epithelial edges of the wound area were not completely incorporated together. However, re-epithelization started to be observed as a thin continuous layer of epithelium bridging the incision area. V-shaped invagination still could be seen at the area of incision. Mild inflammatory reaction could be seen in the lamina propria. (Fig. 5 b)

At 7 days postoperatively:

The two epithelial edges of the wound were completely incorporated and covered by thin parakeratinized epithelial layer. Persistence of a mild inflammatory reaction could be seen in the underlying connective tissue.(Fig.5 c)

The Test group:

At 2 days postoperatively:

The incision area in the palatal mucosa revealed loss of keratinized surface epithelium and cutting of the basement membrane. The lamina propria at the incision site showed disruption of the connective tissue , irregular distribution of the collagen fibers and dilated blood vessels.(Fig.6 a)

At 4 days postoperatively:

The site of incision showed discontinuity of both keratinized epithelium and basement membrane. Many dilated blood vessels were seen at the base of the incision area. (Fig.6 b)

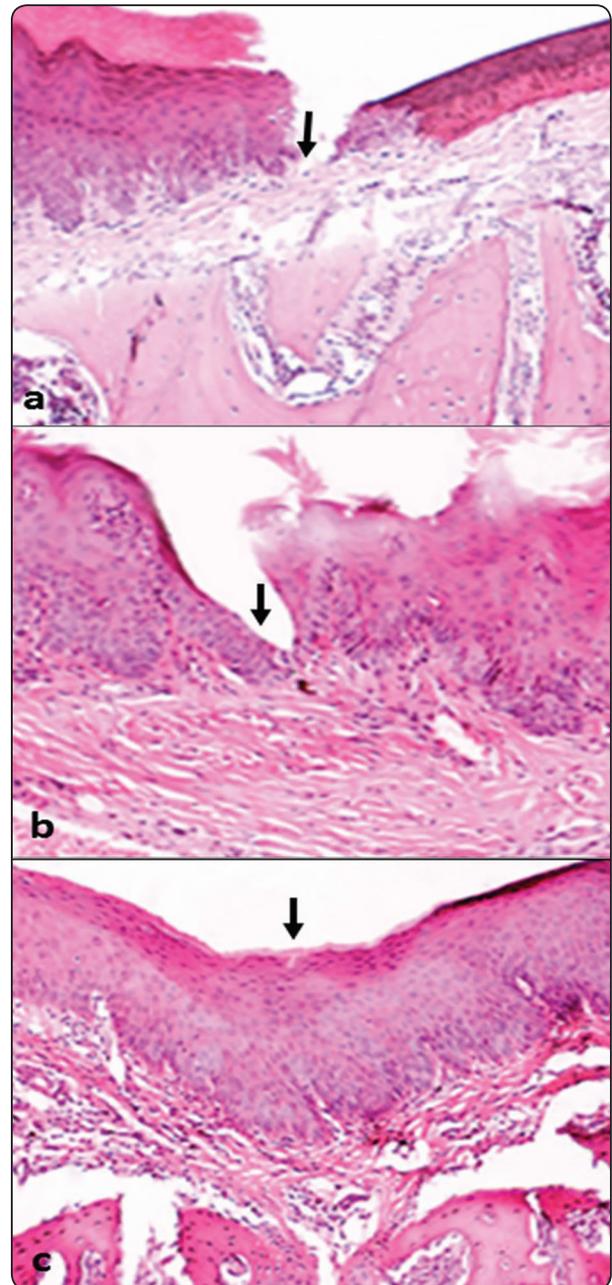


Fig. (5) Light photomicrograph of control group

- (a) 2 days postoperatively showing discontinuity of the keratinized epithelium. Inflammatory cell infiltration were observed in the incision area. Note: bare connective tissue at the base of the incision area.
- (b) 4 days postoperatively showing incomplete incorporation of the epithelial edges with a thin layer of epithelium bridging in the incision area. Mild inflammatory reaction are also seen in the lamina propria.
- (c) 7 days postoperatively showing thin parakeratinized epithelial layer covering the incision with mild inflammatory reaction is seen in the lamina propria (H&EX100).

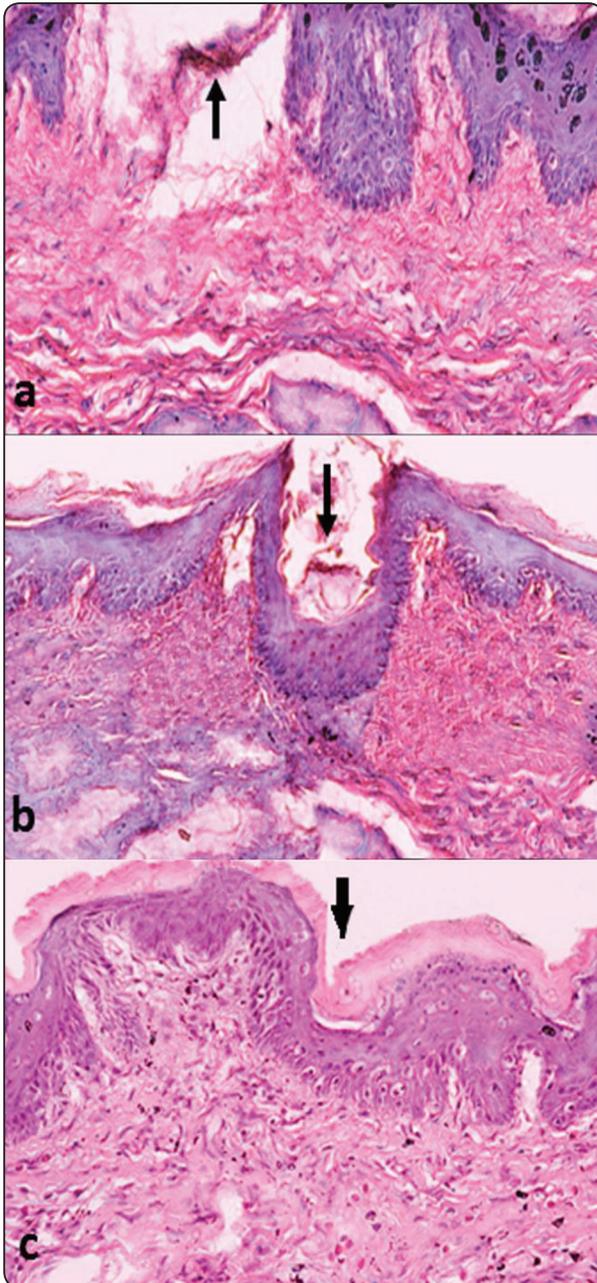


Fig. (6) Light photomicrograph of test group

- (a) 2 days postoperatively showing cutting of the basement membrane and keratinized epithelium at the incision area (black arrow) with irregular distribution of collagen fibers in the lamina propria.
- (b) 4 days postoperatively showing discontinuity of the keratinized surface epithelium and continues basement membrane at the incision area. Dilated blood capillaries at the lamina propria at the base of incision area.
- (c) 7 days postoperatively showing complete incorporation of the epithelial edges of the incision with normal layer of keratin. Continuity of the basement membrane with a thin layer of epithelium at the incision area also revascularization in the connective tissue was noted. (H&E X100)

At 7 days postoperatively:

The histological section revealed complete incorporation of the epithelial edges at the wound area with normal keratin layer on its surface. The basement membrane was continuously formed with normal appearance. The lamina propria showed revascularization with many newly formed blood vessels. (Fig.6 c)

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. (Ozcelik *et al.* 2008)

Many studies demonstrate that low-level laser therapy (LLLT) modulates many biochemical processes, increase ATP synthesis and accelerate the healing process. (, *Silveira et al.* 2009, *Karu et al.* 2010).

However, many researchers using different parameters have investigated the effect of LLLT on wound healing. Although lasers have shown effectiveness in inducing changes within the cultured cells and, as a consequence, an increased healing effect, the optimal parameters to achieve this end have yet to be determined. (Baxter 1994) _

Within the limitations of this study, the findings revealed that LLLT promotes wound healing after gingivectomy operations in humans. There are very few clinical studies about gingival surgery, which makes the comparison of our results impractical. Similar to our findings Amorim *et al.* (2006) have reported that LLLT (at 685 nm) significantly promoted gingivectomy wound healing.

In this study, the selected parameters were based on Schindl *et al.*(2000) (dose of 4 J/cm², exposure time of 80 sec, and power of 50 mW), and these were applied immediately after surgery and repeated every 48h for 1 week. Such parameters

were considered to be effective for the gingival healing following gingivectomy.

The present findings are in contrast to Damante *et al.*, which demonstrated that LLLT did not accelerate the healing of oral mucosa after gingivoplasty. This could be attributed to the different laser settings that the previous study used. Damante *et al.* (2004) used a 15-mW GaAlAs laser in a punctual mode on the wound. On the other hand, in the present study a 50-mW GaAlAs laser was applied in scanning mode over the injured area. Our results show that these parameters were adequate to trigger a faster healing process in laser treated wounds when compared to control wounds.

In the current study, the test group histologically showed better earlier epithelization and more vascular formation than the control group. These findings are in accordance with previous study (Cankat *et al.* 2013) that suggest that LLLT used for soft tissue operations provides better and faster wound healing as well as enhancing epithelization. Similarly, Bibikova *et al.* (1994), reported that LLLT stimulates the microcirculation and new blood vessels formation from the pre-existing vessels.

Previous studies (Steinlechner *et al.* 1993, Tuby *et al.* 2006) suggested that LLLT application may accelerate wound healing by increasing the motility of human keratinocytes and promoting early epithelization and enhancing neovascularization. It has also been shown that the expression of fibroblast growth factors by macrophages and fibroblasts is increased after LLLT application.

In the present study we used control/test individuals to avoid any systemic overlapping effect of the laser therapy to the control group. 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 (Damante *et al.* 2004). Therefore, it can be theorized that the application of LLLT to one site of the surgical field may affect the adjacent surgical site.

Regarding the healing score, the test group showed significant better results than the control group at 5, 7 and 14 days p.s. This could be explained by the favorable effect of LLLT on wound healing through increasing the revascularization rate. (Donos *et al.* 2005). The histological findings come in line with the clinical findings in which many dilated blood vessels were seen at the base of the incision area at 4 and 7 days p.s.

In the current study, the highest % reduction of VAS and wound healing scores were observed in test group (LLLT group) compared to control group. These results are in agreement with Ozcelik *et al.* who found a statistically significant reduction in pain values for the laser group during the first 2 days after surgery compared to conventional treatment. Also, Chukuka *et al.* (2004) in their meta-analysis concluded that laser phototherapy can be considered as a highly effective therapeutic modality for tissue repair and pain relief.

Our findings are in agreement with the results of previous study (De Souza *et al.* 2010). They observed that 75% of the patients suffering from recurrent aphthous ulcer RAS reported a reduction in pain in the same session after laser application patients treated with topical corticoid.

Hersheal *et al.* (2014) also found similar benefits of quicker healing and reduced pain after using low levels of laser treatment on RAS. Hence, they concluded and validated that LLLT is the most appropriate treatment modality for minor RAS. However, long-term comparative studies are needed to further substantiate the advantages of LLLT in the treatment of oral lesions.

Histologically LLLT can improve wound healing through increase the production of ATP, fibroblast proliferation, collagen synthesis, phagocytosis of macrophages, and acceleration of the inflammatory phase of wound healing. All these mechanisms can result in cellular proliferation and acceleration of the wound healing process. (Kreisler *et al.* 2003, Khadra *et al.* 2005)

Silveira *et al.* (2009) suggested that the treatment with low-level laser may induce an increase in ATP synthesis, and that may accelerate the muscle healing process.

After gingivectomy, collagen formation and a better gingival tissue organization occur gradually within 3–4 weeks as the inflammation and the vascularity of the granulation tissue decrease, even if the gingival surface appears to be completely healed clinically 2–3 weeks after the surgical procedure. Collagen production on the granulation tissue occurs after fibroblast proliferation, which originates locally around the vascular, bone, and lamina propria areas. (Ramfjord *et al.* 1991) Acceleration of the wound healing process with laser can be explained by a higher collagen synthesis in fibroblast and vascular proliferation on the connective tissue, coupled with higher mitotic activity in epithelial cells.

CONCLUSIONS

Low Level Laser as an adjunctive therapy to gingivectomy procedures can be used clinically in the coming future to promote healing of oral mucosa, to reduce re-epithelialization time and to minimize post-operative pain.

RECOMMENDATIONS

Further studies with fixed laser settings are needed in the future to add more clarification on the effect LLLT on gingival healing.

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