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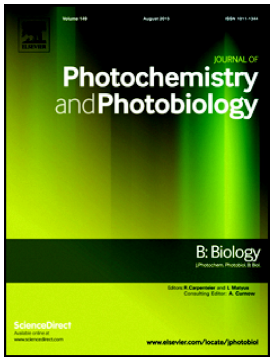

Effect of laser photobiomodulation on wound healing and postoperative pain following free gingival graft: A split-mouth triple-blind randomized controlled clinical trial

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Title: Effect of laser Photobiomodulation on Wound Healing and Postoperative Pain following Free Gingival Graft: A split-mouth triple-blind randomized controlled clinical trial

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Abstract:

Background and aim: Free gingival graft (FGG) is one of the most predictable techniques for gingival augmentation. However, patient's discomfort and pain during healing period are significant concerns. The aim of this study was to assess if laser photobiomodulation (PBM) was effective in terms of enhancing wound healing and reducing postoperative pain.

Methods and Materials: Twelve patients participated in this split-mouth randomized controlled clinical trial. Each patient had a 30-day interval between the two procedures. In the test group, donor and recipient sites received diode laser (660nm, 200mW, continuous mode, time of irradiation:32 seconds, energy density: 4J/cm^2, spot size:0.5cm) immediately after FGG surgery, and 1,2,4 and 7 days later. The control side received the same sequence of irradiation with the laser-off. Complete wound epithelialization of donor site and clinical wound healing and visual analogue scale (VAS) pain score of donor and recipient sites were evaluated after surgery.

Results: At 14 and 21 days after surgery, the number of donor sites with complete epithelialization was greater in laser group compared to the placebo. After 21 days, all donor sites in the test group were epithelialized completely, while at the same time, only eight donor sites in the control group showed complete epithelialization (P value=0.05). In terms of clinical healing of the recipient and donor sites, the test and control groups did not show any significant difference during the 45-day period, except at days 1 (for recipient site) and 14 (for donor site), when the test group showed better results (P values: 0.01 and 0.03, respectively). The VAS pain score did not show statistically significant difference between two groups during the study period, except for the first three hours after procedure when laser group showed greater VAS pain score (P values <0.05).

Conclusion: PBM following FGG procedure with the parameters used in this study could accelerate the rate of epithelialization at the donor site. However, it did not reduce postoperative pain.

Key words: Photobiomodulation therapy; Low-Level Laser Therapy; Re-Epithelialization; Wound Healing; Pain, Postoperative

Introduction

Gingival defects with a lack of keratinized tissue can be effectively treated with free gingival graft (FGG) to create an adequate zone of attached gingiva.(1) This approach is inevitably accompanied with postsurgical discomfort related to the exposed connective tissue.(2) Numerous substances have been employed to accelerate wound healing like application of chlorhexidine digluconate (3) the plant extracts (4, 5), ozonated oil,(6), and platelet concentrate. (7)

The effect of photobiomodulation (PBM) on cellular proliferation, collagen synthesis, and release of growth factors from various cells has been well documented.(8, 9) Little is known about the mechanism of action of PBM; however, it was found that PBM can alter cellular metabolism, especially through interfering with mitochondrial-membrane potential and ATP synthesis.(10, 11) Due to such capabilities, PBM may induce a significant impact on the course of biological events that take place during wound healing. (12)

Limited number of clinical trials has addressed the effect of PBM on postoperative oral wound healing. The results of a clinical study showed that application of low-intensity diode laser (685 nm) following gingivectomy was effective in promoting clinical healing of the gingiva. (13) When PBM was used after root coverage procedure, periodontal parameters such as clinical attachment level and width of keratinized tissue showed improved results. (14) On the other hand, Almeida et al. used PBM in order to accelerate healing (780 nm) and achieve analgesia (660 nm) at the recipient site after FGG surgery. They did not find any benefit from the use of PBM. (15)

Due to the lack of adequate evidence, the aim of this clinical trial was to evaluate if application of PBM after FGG surgery could accelerate healing process of donor and recipient sites. The null hypothesis was that of no difference in the outcome variables in patients undergoing FGG using PBM or placebo.

Materials and Methods:

Patient population

The present study was conducted according to the guidelines of Helsinki Declaration of 1975, as revised in 2008 (ClinicalTrials.gov ID: NCT02711501). The study protocol was approved by ethical committee of Tehran University of Medical Sciences (NO: 91-01-97-16886, IRCT registration code: IRCT201206121150N5). Patient selection was performed among those referred to the department of Periodontics, Tehran University of Medical Sciences for management of lack of keratinized gingiva. The inclusion criteria consisted of healthy patients with lack of keratinized gingiva affecting two adjacent mandibular teeth, bilaterally. Patients with poor oral hygiene, low cooperation, smoking habit, general contraindication for laser therapy, existence of infection at the site of surgery, presence of pain at the time of surgery, and pregnant or lactating females were excluded from the study. After explaining about the study protocol, all participants signed the informed consent form.

Sample size calculation

The sample size of the study was planned based on published data on gingival healing after PBM.(13) Considering significance level of 5% and power of 80%, the minimum sample size would be 12 samples per group.

Study design

This triple-masked split-mouth placebo-controlled randomized controlled clinical trial was performed from September 2014 to August 2015. The participants were consecutively recruited. Oral hygiene instruction, polishing, scaling, and elimination of causative factors were carried out, according to the needs of individual patients. The patients were randomly assigned to the test (laser) or control (placebo) groups with a 1:1 allocation ratio. An epidemiologist who was not aware of the treatment modalities generated the random allocation sequence using a computerized block randomization process. An investigator, who was not involved in the surgical procedure and laser/placebo therapy, performed the enrolment of patients and their assignments into interventions (N. M.). Allocation concealment was obtained by sealed non-transparent coded envelopes. Just before surgery, a practitioner who was not involved in the surgical procedures opened the envelopes (H. N.). Patients did not receive information regarding the type of treatment that was performed in each session.

Surgical procedure

One skilled masked surgeon performed all surgeries (M. H.).The surgical process was the same for both groups and was carried out according to the method described by Sullivan and Atkins.(16)

Graft harvesting: After injection of local anaesthesia, the graft (length 20 mm × height 10 mm) was harvested from the palate with a thickness of approximately 1mm. The recipient site was located between mesial aspect of first premolar to distal aspect of first molar. The donor site was secured using cross mattress silk sutures.

Graft placement: The graft was adapted to the recipient site and secured by two periosteal and two C-type sutures. The apical periosteal sutures were anchored to the teeth under surgery.

Thirty days after the first surgery, the contralateral site was operated following the same procedure as described above.

Laser irradiation

All patients remained masked to the treatment modality throughout the study period. Regardless of treatment group, both patients and operator wore safety glasses in all sessions. The test group received diode laser (THOR® Laser, London, UK) at donor and recipient sites with the following parameters: wavelength: 660 nm, power: 200mW, continuous mode, total dose per session: 32 J/cm^2 (energy density per point:4 J/cm^2), total time of irradiation: 32 seconds (application time of 4 seconds per point), spot size:0.5Cm. The laser beam was directed perpendicularly with slight contact with the tissue. Laser was irradiated immediately after surgery and 1, 2, 4 and 7 days later at the donor and recipient sites. Before each application, the power of equipment was calibrated. The control group received placebo laser therapy with laser off state.

Post-operative protocol

After surgery, all patients received a non-steroidal anti-inflammatory medication (Gelofen 400mg, Jaber EbneHayan, Iran). Patients were asked to take additional medication whenever they felt pain. Chlorhexidine 0.2% was prescribed twice daily for one week. Patients were instructed to eat only soft food during the first week and avoid mechanical trauma to the treated sites for four weeks. The sutures of the donor and recipient sites were removed one and two weeks after surgery, respectively.

Examined parameters:

Epithelialization of donor site: The donor site was dried with a gauze sponge. Then, 3% hydrogen peroxide (H2O2) was applied to the wound, seeking to observe bubbling (oxygen liberation) in the wound area. (17) This test was carried out at 7, 14, 21, and 30 days after surgery. The degree of epithelialization was classified as follows: *complete epithelialization*: bubble was not seen; *incomplete epithelialization*: bubbles were seen in the donor site.

Clinical Healing (CH): Photographs were taken from donor (D-CH) and recipient (R-CH) sites at 1, 2, 7, 14, 21, 30, and 45 days after surgery (PowerShot SX200 IS Canon, Japan). Three expert and masked periodontists evaluated the photographs (N. M., M. P., and R. F.). Two photographs (test and control sites) corresponded to the same elapsed postsurgical time were placed side by side. The examiners were asked to choose the photograph exhibiting the better healing based on shade, texture, and morphology.(15) The agreement was evaluated by means of 2 day-interval between the observations (Kappa=0.85).

Postoperative pain: Postoperative pain was measured using visual analogue scale [no pain(0) to unbearable pain(100)].(18) One masked examiner asked the patients to point out the pain severity (H. N). The degree of pain perception at donor (D-VAS) and recipient (R-VAS) sites was recorded at the day of surgery (first eight hours after surgery) and then daily till day 12.

NSAID intake: The number of NSAIDs used by the patients during the first postoperative week was recorded.

Epithelialization of donor site and clinical healing were defined as the primary outcome variables. Postoperative pain and number of NSAID intake were considered as the secondary outcome variables.

Statistical analysis:

Kolmogrov-Smirnov test showed that data regarding “epithelialization of donor site” and “clinical healing” were not normally distributed. Therefore, Wilcoxon signed ranks test was used to compare test and control sides during the study period in terms of these two parameters. To compare treatment groups regarding “postoperative pain” and “NSAID intake” over time, two-way repeated measures analysis of variance (ANOVA) was performed. P-value of less than 0.05 was set as statistically significant.

Results:

Twelve patients (8 women and 4 men; 24 to 55 years; mean age: 40.2 ± 9.2 years) including 48 sites (24 donor and 24 recipient sites) completed the study. Postoperative period was uneventful except for bleeding of the donor site in three patients (two patients at the test sites

and one patient at the control site) that occurred immediately after the surgery. The bleeding was resolved by re-suturing the site.

Epithelialization of donor site:

At day 7, no donor site exhibited complete epithelialization (Table 1). At day 14, 25% of donor sites in the laser group showed complete epithelialization, while at the same time none of the sites in the control group were completely epithelialized (P value= 0.05).

After 21 days, 100% of test sites and 66.6% of control sites demonstrated complete epithelialization. The difference between two groups was statistically significant (P value=0.02).

Clinical Healing:

Regarding D-CH, laser-treated group showed better results than did the placebo group at 14^{th}day (P value=0.01) (Figure 1-A). At first day after surgery, the R-CH wounds treated with laser was significantly better compared to the control wounds (P value=0.01) (Figure 1-B).

Postoperative pain:

Day of surgery:

D-VAS: A two-way repeated measures ANOVA with Greenhouse-Geisser showed that in the test group, the mean D-VAS pain score was significantly higher in the first three hours after surgery compared to other time points (Figure 2-A). Between-group analysis revealed that during the first 3 hours after surgery, the mean value of D-VAS was significantly greater in the test group compared to that of control group (P values=0.04, 0.003, 0.02, respectively).

R-VAS: A two-way repeated measures ANOVA with a Greenhouse-Geisser correction determined that the mean R-VAS did not differ significantly between time points at the first 8 hours after surgery (P value=0.164). Moreover, no significant difference was observed between the two groups in terms of the R-VAS (Figure 2-B).

Days 1 to12:

D-VAS: Bonferroni post-hoc test revealed that D-VAS was significantly greater in first 5 days compared to days 9-12 (all p values <0.05). The two groups did not show significant difference in terms of D-VAS during this period (p value= 0.533) (Figure 2-A).

R-VAS: Post hoc tests using the Bonferroni correction showed that the mean values of R-VAS at days 8-12 were significantly lower than that of first 4 postoperative days. No statistically significant difference was noted between the treatment groups regarding the mean value of R-VAS in the study period (Figure 2-B).

NSAID intake:

The average number of NSAID intake during the study period was 1.33 ± 1.52 in the test group and 1.29 ± 1.63 in the control group (Figure 3). Two-way repeated measures ANOVA showed an overall significant reduction of NSAID intake over time (P value<0.001), but between-group analysis did not show any significant difference between test and control groups (P value=0.93).

Discussion

The main aim of this triple-blind split-mouth randomized clinical trial was to evaluate the effect of PBM on FGG donor and recipient areas healing process. The results of the present study showed that the PBM protocol that was used (660 nm, 32 J/cm^2) can promote epithelialization of donor site and thus reduce wound repair time in the palate. From clinical point of view, laser-treated recipient and donor sites showed better healing at days 1 and 14, respectively.

Epithelialization is the most essential part of secondary wound healing and is used as a defining parameter of its success. In the absence of epithelial resurfacing, wound cannot be considered healed.(19) PBM with the protocol used in this study could promote re-epithelialization of FGG donor site, since at day 21, 100% of laser-treated donor sites presented complete epithelialization. In contrast, Vieira et al. did not find any difference between laser and control groups, in terms of rate of epithelialization of palatal donor site.(20) This controversy can be attributed to methodological differences, particularly laser protocol (650 nm, 4 J/cm^2 per point transcutaneously, immediately and 2 and 3 days after surgery). Moreover, limited number of participants (5 patients in each group) might hamper this effect and lead to insignificant result.

Using H2O2-bubble test, even thin epithelial barrier is detected, while simultaneously it appears as incomplete wound coverage in the clinical photograph. Moreover, other factors such as connective tissue maturation and neovascularization influence on the clinical judgment about palatal wound healing. In the present study, clinicians found that healing of laser-treated palatal donor wounds was significantly better than that of placebo sites at day 14. This finding is in accordance with that of Da Silva Neves et al. (21) (660 nm, 15 and 30 J/cm^2, at 0, 2,4,6,8,10,12 days after surgery) and Dias et al. (22) (660 nm, 3 J/cm^2, at 0,2,4,6,8,10,12,14 days after surgery). They used PBM in the donor palatine area after connective tissue graft removal

and found that palatal wound color pattern in the PBM group was better than the placebo group at day 14.This finding can be attributed to the influence of PBM on tissue erythema and angiogenesis during healing process.

The results obtained from clinician's assessment and H2O2-bubble test were consistent at day 14, when both tests showed better result in laser-treated sites compared to the placebo sites. Despite greater number of laser-treated donor sites presenting complete epithelialization at day 21, the clinicians did not find significantly better clinical healing at the same site and day, although a trend in favor of PBM was noted. This discrepancy between the results might be explained by the fact that healing of the palatal donor site was advanced at day 21 and better healing site might not be distinguishable by clinicians at this time.

In terms of clinical healing of FGG recipient site, the superiority of PBM over placebo was evident at first day after surgery. This effect was not observed later on. A similar study by Almeida et al. evaluated clinical healing of FGG recipient sites at days 7, 15, 30, and 60. They did not find better clinical healing in laser-treated FGG recipient sites compared to the control sites at any time points.(15) The ability of PBM to modulate inflammation could be contributed to its beneficial effects in early stage of wound healing. The inflammatory phase (first postoperative days) is a critical step in the process of wound healing, during which inflammatory cells play a key role in clearing tissue debris and promoting migration of keratinocytes and fibroblasts for healing to progress(23-26).

In our study, energy density of 4 J/cm^2 was applied per point in each session. Most authors recognized 4 J/cm^2 to be the optimal flounce for tissue repair.(27) However, comparing two energy densities (15 and 30 J/cm^2) of 660 nm diode laser, Da Silva Neves et al. revealed that

protocol of 30 J/cm^{2}provided faster palatal wound healing after removing the connective tissue graft (CTG) for root coverage.(21) It should be noted that there are some differences between the palatal wounds of FGG and CTG regarding depth of wounds and the healing process. Typically, FGG donor site is a shallow and open wound that heals by secondary intention. On the other hand, CTG donor site usually includes deeper palatal connective tissue without involving the superficial epithelium; thereby it allows healing of the donor site by primary intention. As a rule, higher energy density of laser is needed for deeper wounds compared to ones that are more superficial.

Besides energy density, laser wavelength was selected based on the literature. Most studies on tissue repair show significant benefits within the red visible spectrum (622–780 nm).(28) Garcia suggested that the influence of PBM on tissue repair might be due to the high absorption of 660-nm-wavelength lasers by cytochrome C oxidase.(29)

Although the exact mechanism of action of PBM on palatal donor wound healing after FGG harvesting is not clear, a recent clinical trial demonstrated that the level of TGF-β_1, PDGF-BB, and IL-8 in the palatal wound fluid increased after application of PBM. They suggested that PBM may accelerate palatal wound healing by stimulating production of these mediators.(30)

Despite findings regarding acceleration of donor site epithelialization, the beneficial effect of PBM on postoperative pain relief was not verified through this study. Consistently, Almeida et al. (660 and 780 nm) and Dias et al. (660 nm) did not report palliative effect after postoperative PBM (15, 22). On the contrary, Ozcelik et al. reported that the use of 810 nm diode laser for biostimulation after harvesting the FGG resulted in less postoperative pain and discomfort than the controls (31). This dissimilarity between the results can be due to the following factors:

collagen sponge and sutures were only used in the control sites which might cause postoperative pain and discomfort.(32). More importantly, infrared lasers (810 nm) can penetrate deeper in the tissue where nerve endings are located. Therefore, they seem to be suitable for pain relief. (33) In the present study, laser setting was adjusted for wound healing purposes, since the main goal of our study was to accelerate wound healing through PBM. The analgesic effect of laser was considered as a secondary outcome and result of wound healing progress.

We could not find the effect of PBM on the number of NSAID intake after FGG. The result of a systematic review on the effectiveness of PBM in acute pain showed equal effect size of PBM and NSAIDs. (34)We administered NSAID after surgery and upon patient's discretion, due to ethical concerns. Therefore, the effect of NSAID might hinder the analgesic effect of PBM.

Patients in the laser group experienced more pain than that of control group at the first 3 hours after surgery. This side effect has been reported before in the literature. (35) In a systematic review, Bjordal et al. reported that optimal analgesic effects of PBM will be achieved if it is administered at high doses, typically 7.5 J/cm^2, in the first 72 hours (34). From ethical point of view, it is essential to inform the patients about this transient adverse effect before initiating PBM.

More well-designed randomized clinical trials with larger sample size need to be performed in this area to find the optimal laser irradiation parameters in which promote healing and at the same time reduce patient's discomfort.

In conclusion, the results of this study showed that PBM with the parameters used in this study was helpful in accelerating complete epithelialization of palatal donor site after harvesting FGG.

However, the effect of laser on alleviation of postoperative pain was not confirmed through this trial.

References:

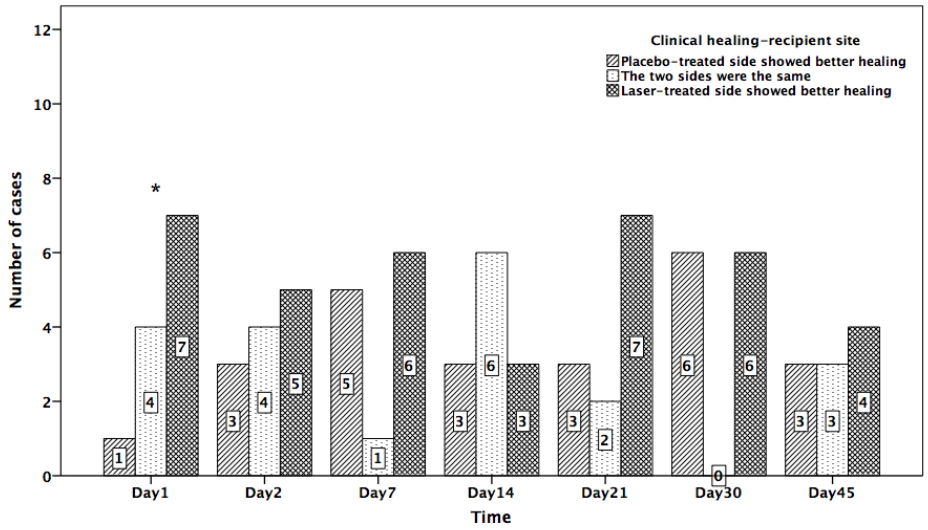
1. Cairo F, Nieri M, Pagliaro U. Efficacy of periodontal plastic surgery procedures in the treatment of localized facial gingival recessions. A systematic review. J Clin Periodontol. 2014;41 Suppl 15:S44-62.
2. Burkhardt R, Hammerle CH, Lang NP, Research Group on Oral Soft Tissue B, Wound H. Self-reported pain perception of patients after mucosal graft harvesting in the palatal area. J Clin Periodontol. 2015;42(3):281-7.
3. Kozlovsky A, Artzi Z, Hirshberg A, Israeli‐Tobias C, Reich L. Effect of local antimicrobial agents on excisional palatal wound healing: a clinical and histomorphometric study in rats. Journal of clinical periodontology. 2007;34(2):164-71.
4. Kosger HH, Ozturk M, Sokmen A, Bulut E, Ay S. Wound healing effects of Arnebia densiflora root extracts on rat palatal mucosa. European journal of dentistry. 2009;3(2):96-9.
5. Keceli HG, Aylikci BU, Koseoglu S, Dolgun A. Evaluation of palatal donor site haemostasis and wound healing after free gingival graft surgery. J Clin Periodontol. 2015;42(6):582-9.
6. Patel PV, Kumar S, Vidya GD, Patel A, Holmes JC, Kumar V. Cytological assessment of healing palatal donor site wounds and grafted gingival wounds after application of ozonated oil: an eighteen-month randomized controlled clinical trial. Acta Cytol. 2012;56(3):277-84.
7. Shayesteh YS, Eshghyar N, Moslemi N, Dehghan MM, Motahhary P, Ghobadi Z, et al. The effect of platelet-rich plasma on healing of palatal donor site following connective tissue harvesting: a pilot study in dogs. Clin Implant Dent Relat Res. 2012;14(3):428-33.
8. Karu TI, Kolyakov SF. Exact action spectra for cellular responses relevant to phototherapy. Photomed Laser Surg. 2005;23(4):355-61.
9. de Medeiros ML, Araujo-Filho I, da Silva EM, de Sousa Queiroz WS, Soares CD, de Carvalho MG, et al. Effect of low-level laser therapy on angiogenesis and matrix metalloproteinase-2 immunoexpression in wound repair. Lasers Med Sci. 2017;32(1):35-43.
10. Quirk BJ, Sannagowdara K, Buchmann EV, Jensen ES, Gregg DC, Whelan HT. Effect of near-infrared light on in vitro cellular ATP production of osteoblasts and fibroblasts and on fracture healing with intramedullary fixation. Journal of Clinical Orthopaedics and Trauma. 2016.
11. Amaroli A, Ravera S, Parker S, Panfoli I, Benedicenti A, Benedicenti S. An 808-nm diode laser with a flat-top handpiece positively photobiomodulates mitochondria activities. Photomedicine and Laser Surgery. 2016;34(11):564-71.
12. Medrado AP, Soares AP, Santos ET, Reis SR, Andrade ZA. Influence of laser photobiomodulation upon connective tissue remodeling during wound healing. J Photochem Photobiol B. 2008;92(3):144-52.

13. Faria Amorim JC, Sousa GRD, Silveira LDB, Prates RA, Pinotti M, Ribeiro MS. Clinical study of the gingiva healing after gingivectomy and low-level laser therapy. Photomedicine and Laser Therapy. 2006;24(5):588-94.
14. Ozturan S, Durukan SA, Ozcelik O, Seydaoglu G, Haytac MC. Coronally advanced flap adjunct with low intensity laser therapy: a randomized controlled clinical pilot study. J Clin Periodontol. 2011;38(11):1055-62.
15. Almeida AL, Esper LA, Sbrana MC, Ribeiro IW, Kaizer RO. Utilization of low-intensity laser during healing of free gingival grafts. Photomed Laser Surg. 2009;27(4):561-4.
16. Sullivan HC, Atkins JH. Freeutogenous gingival grafts. 1. Principles of successful grafting. Periodontics. 1968;6(1):5.
17. Silva CO, Ribeiro Edel P, Sallum AW, Tatakis DN. Free gingival grafts: graft shrinkage and donor-site healing in smokers and non-smokers. J Periodontol. 2010;81(5):692-701.
18. Yen CA, Griffin TJ, Cheung WS, Chen J. Effects of platelet concentrate on palatal wound healing after connective tissue graft harvesting. J Periodontol. 2007;78(4):601-10.
19. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. Epithelialization in wound healing: a comprehensive review. Advances in wound care. 2014;3(7):445-64.
20. Vieira J, Lopes CB, DeMarco AC, de Melo Filho AB, Jardini MAN. Clinical study of laser biomodulation (650λ) after free gingival grafts. J Oral Laser Appl. 2010;10:159-63.
21. da Silva Neves FL, Silveira CA, Dias SBF, Júnior MS, de Marco AC, Kerbauy WD, et al. Comparison of two power densities on the healing of palatal wounds after connective tissue graft removal: randomized clinical trial. Lasers in medical science. 2016;31(7):1371-8.
22. Dias SBF, Fonseca MVA, dos Santos NCC, Mathias IF, Martinho FC, Junior MS, et al. Effect of GaAlAs low-level laser therapy on the healing of human palate mucosa after connective tissue graft harvesting: randomized clinical trial. Lasers in medical science. 2015;30(6):1695-702.
23. Khan I, Arany P. Biophysical approaches for oral wound healing: emphasis on photobiomodulation. Advances in wound care. 2015;4(12):724-37.
24. Basso FG, Soares DG, Pansani TN, Cardoso LM, Scheffel DL, de Souza Costa CA, et al. Proliferation, migration, and expression of oral‐mucosal‐healing‐related genes by oral fibroblasts receiving low‐level laser therapy after inflammatory cytokines challenge. Lasers in surgery and medicine. 2016.
25. Noda M, Aoki A, Mizutani K, Lin T, Komaki M, Shibata S, et al. High‐frequency pulsed low‐level diode laser therapy accelerates wound healing of tooth extraction socket: An in vivo study. Lasers in surgery and medicine. 2016.
26. Silveira PC, Ferreira KB, da Rocha FR, Pieri BL, Pedroso GS, De Souza CT, et al. Effect of Low-Power Laser (LPL) and Light-Emitting Diode (LED) on Inflammatory Response in Burn Wound Healing. Inflammation. 2016:1-10.
27. Wagner VP, Meurer L, Martins MAT, Danilevicz CK, Magnusson AS, Marques MM, et al. Influence of different energy densities of laser phototherapy on oral wound healing. Journal of biomedical optics. 2013;18(12):128002-.
28. Suzuki R, Takakuda K. Wound healing efficacy of a 660-nm diode laser in a rat incisional wound model. Lasers in medical science. 2016;31(8):1683-9.

29. Garcia VG, Macarini VC, de Almeida JM, Bosco AF, Nagata MJ, Okamoto T, et al. Influence of low-level laser therapy on wound healing in nicotine-treated animals. Lasers Med Sci. 2012;27(2):437-43.
30. Keskiner I, Lutfioğlu M, Aydogdu A, Saygun NI, Serdar MA. Effect of Photobiomodulation on Transforming Growth Factor-β1, Platelet-Derived Growth Factor-BB, and Interleukin-8 Release in Palatal Wounds After Free Gingival Graft Harvesting: A Randomized Clinical Study. Photomedicine and Laser Surgery. 2016;34(6):263-71.
31. Ozcelik O, Seydaoglu G, Haytac CM. Diode laser for harvesting de‐epithelialized palatal graft in the treatment of gingival recession defects: a randomized clinical trial. Journal of clinical periodontology. 2016.
32. Hürzeler MB, Weng D. A single-incision technique to harvest subepithelial connective tissue grafts from the palate. International Journal of Periodontics & Restorative Dentistry. 1999;19(3).
33. Hawkins D, Abrahamse H. Phototherapy-a treatment modality for wound healing and pain relief. 2009.
34. Bjordal JM, Johnson MI, Iversen V, Aimbire F, Lopes-Martins RA. Low-level laser therapy in acute pain: a systematic review of possible mechanisms of action and clinical effects in randomized placebo-controlled trials. Photomed Laser Surg. 2006;24(2):158-68.
35. Pandeshwar P, Roa MD, Das R, Shastry SP, Kaul R, Srinivasreddy MB. Photobiomodulation in oral medicine: a review. J Investig Clin Dent. 2016;7(2):114-26.

Table 1- Number (percentage) of donor sites with complete/incomplete epithelialization in laser and control groups at 7, 14, 21, and 30 days after surgery.

Epithelialization		Laser [N (%)]	Placebo [N (%)]
Complete	Day 7	0 (0%)	0 (0%)
	Day 14	3 (25%)	0 (0%)
	Day 21	12 (100%)	8 (66.6%)
	Day 30	12 (100%)	12 (100%)
Incomplete	Day 7	12 (100%)	12 (100%)
	Day 14	9 (75%)	12 (100%)
	Day 21	0 (0%)	4 (0%)
	Day 30	0 (0%)	0 (0%)



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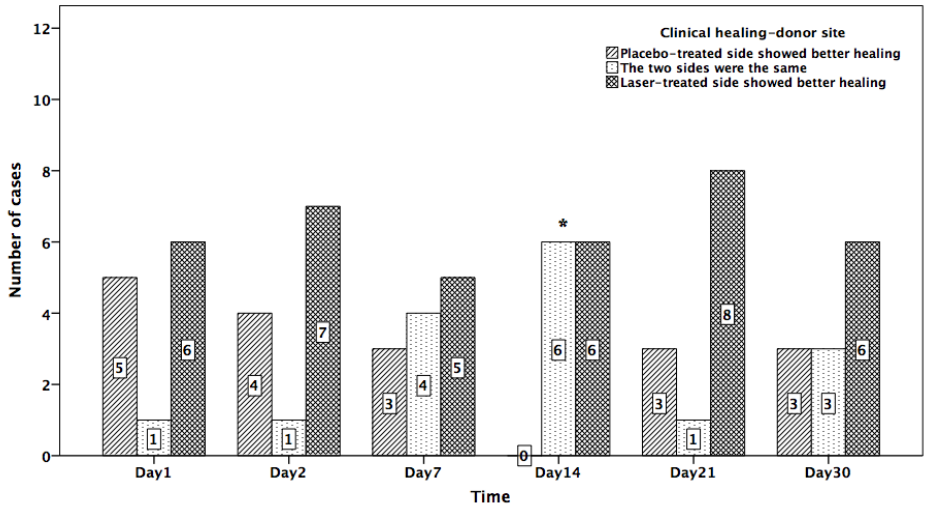
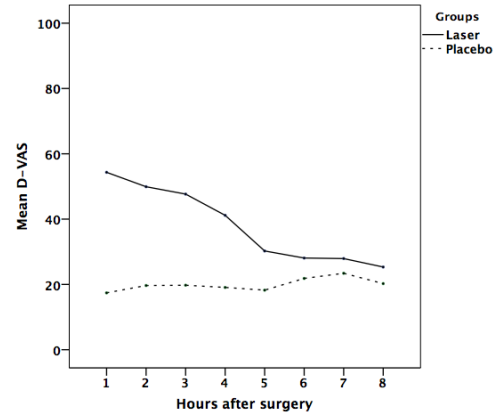


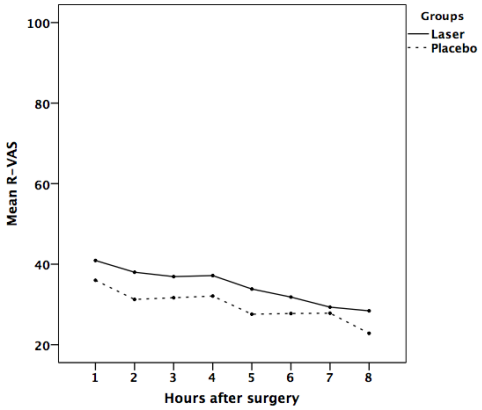
B

Figure 1- Clinical wound healing for laser and control groups at recipient (A) and donor (B) sites during the experimental period.

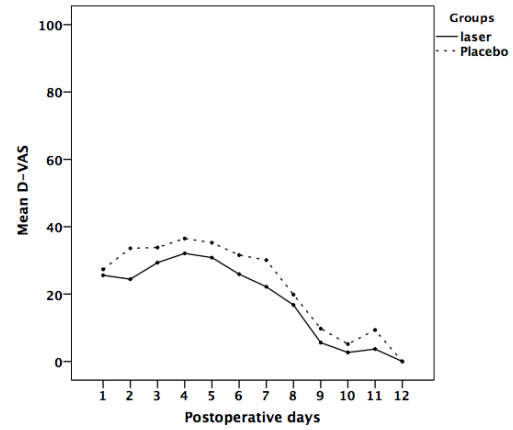
* denotes that clinical healing at laser group was significantly better than the placebo group.



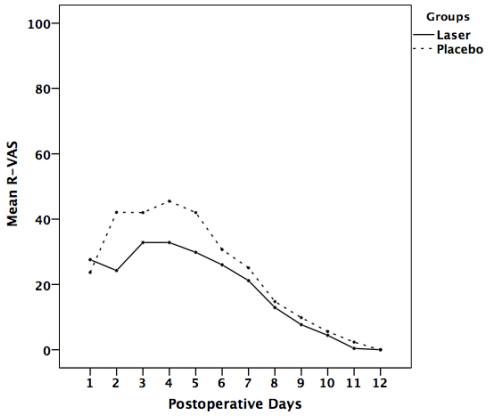
A



B



C



D

Figure2-Mean visual analogue scale (VAS) pain score from donor (D-VAS) (A and C) and recipient (R_VAS) (B and D) sites during first 8 postoperative hours (A and B) and 1st to 12th postoperative days (C and D) in laser and placebo groups.

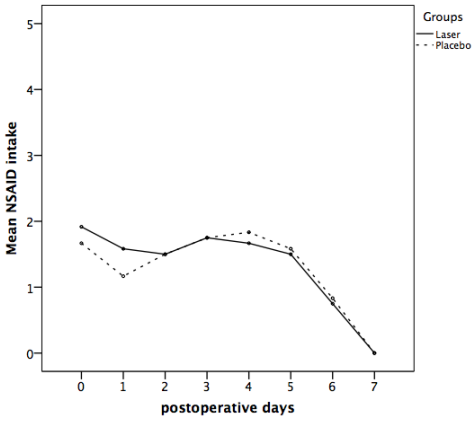


Figure 3- NSAID intake during the first postoperative week in laser and placebo groups.

Highlights

- Application of laser PBM accelerated epithelialization of palatal donor site.
- Use of laser PBM did not significantly affect the clinical healing outcomes.
- Laser PBM with this protocol was not effective for reducing the postoperative pain.