Effect of Photobiomodulation on Transforming Growth Factor-β_1, Platelet-Derived Growth Factor-BB, and Interleukin-8 Release in Palatal Wounds After Free Gingival Graft Harvesting: A Randomized Clinical Study

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Abstract

Objective: This study evaluated the impact of photobiomodulation (PBM) on the healing of the donor palatal area following free gingival graft (FGG) harvesting by examining changes in transforming growth factor (TGF)-β_1, platelet-derived growth factor (PDGF)-BB, and interleukin (IL)-8 levels in palatal wound fluid (PWF). *Material and methods:* Thirty patients were selected and randomly assigned to receive PBM (laser group) or PBM sham (sham group) in the palatine area after FGG harvesting. A neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (1064 nm) was applied to the test sites immediately after surgery and every 24 h thereafter for 4 days. PWF was collected on Days 7 and 12, and PWF TGF-β_1, PDGF-BB, and IL-8 levels were analyzed by enzyme-linked immunosorbent assays (ELISA). *Results:* PWF TGF-β_1, PDGF-BB, and IL-8 levels were significantly lower on Day 12 than on Day 7 for both groups. PWF TGF-β_1, PDGF-BB, and IL-8 levels of the laser group were significantly higher than those of sham group on Day 7 ($p < 0.05$). PWF TGF-β_1 levels were also significantly higher in laser group than in the sham group on Day 12; however, differences in PDGF-BB and IL-8 levels between groups on Day 12 were statistically nonsignificant. *Conclusions:* Observed increases in PWF TGF-β_1, PDGF-BB, and IL-8 levels suggest that PBM may accelerate wound healing by stimulating production of selected mediators.

Introduction

LASERS HAVE BEEN WIDELY USED IN MEDICINE for the past three decades. During this time, numerous scientific studies have demonstrated the biostimulative effects of photobiomodulation (PBM).[1,2] Both *in vitro* and *in vivo* studies have suggested that PBM may facilitate healing by stimulating fibroblast and keratinocyte motility and increasing collagen synthesis, angiogenesis, and growth-factor release.[3–8] Although a number of studies have reported improved wound healing with PBM,[9–12] others have reported no such improvements;[13–16] therefore, there is still no consensus on the effects of PBM on the wound healing process. It should be noted that most PBM studies have been conducted with helium-neon (He-Ne) or diode lasers,[3,4,17–20] whereas few studies have investigated the potential biomodulatory effect of the application of low-pulse energy neodymium:yitrium-aluminum garnet (Nd:YAG) laser light.[21–25]

Briefly, wound healing is a period of synthesis, deposition, and organization of a new extracellular matrix (ECM) that begins with the degranulation of platelets accompanied by the release of various inflammatory mediators, such as interleukin-8 (IL-8), and growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β, which stimulate the proliferation of the fibroblasts, keratinocytes, and endothelial cells required for wound

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healing.[26,27] During this initial phase, the wound matrix contains a rich cocktail of growth factors, proteinases, and inflammatory cytokines that play an important role in controlling molecular and cellular responses.[26,27] PDGF, along with proinflammatory cytokines such as IL-8, plays an important role in attracting neutrophils to the wound site to remove contaminating substances, whereas TGF-β acts to convert monocytes to macrophages, which play an important role in augmenting the inflammatory response and tissue debridement. Macrophages initiate the development of granulation tissue and release a variety of proinflammatory cytokines, including IL-8, and growth factors, including TGF-β and PDGF. TGF-β and PDGF subsequently promote fibroblast infiltration, thereby enhancing the production of the extracellular matrix (ECM), and depositing new matrix proteins.[28,29]

TGF, one of the major activators of ECM synthesis, and PDGF, an important fibroblast mitogen, are produced by platelets, macrophages, fibroblasts, and keratinocytes.[30] TGF-β_1, one of the isoforms of TGF-β, plays an important role in the inflammation, angiogenesis, re-epithelialization, and connective tissue regeneration that occur as part of the wound healing process. The communication between TGF-β_1 and keratinocytes; that is, their mutual stimulation, is important for quick re-epithelialization and successful wound healing.[29] PDGF-BB, one of the homodimeric forms of PDGF, plays different roles at different stages of wound healing, beginning with the release of PDGF from degranulated platelets in wound fluid to stimulate mitogenity and chemotaxis of neutrophils, macrophages, fibroblasts, and smooth muscle cells to the wound site,[31-33] and it is necessary for fibroblast and myofibroblast proliferation in order to comprise wound closure.[34,35]

Wound repair, inflammation, and fibrotic processes are governed by the same molecules and cellular events. Whereas TGF-β_1 and PDGF-BB are fibrogenic and anti-inflammatory mediators, IL-8 belongs to the α-subfamily of chemokines associated with proinflammatory and angiogenic properties.[30] Moreover, the presence of IL-8 receptors on resident cells suggests that IL-8 contributes to the regulation of re-epithelialization, tissue remodeling, inflammation, and angiogenesis.[34,35]

The use of PBM has been proposed as a means of stimulating cells in the wound matrix and accelerating healing of the palatal masticatory mucosa at the donor site of free gingival graft (FGG) used in periodontal plastic surgery.[17,18] The postoperative morbidity associated with the open palatal wound involves acute pain, excessive hemorrhaging, and bone exposure, and represents a major complication of this commonly used technique that tends not to resolve until the completion of wound epithelialization, which usually takes 2–4 weeks.[17,18,36] Recent data indicates that He-Ne lasers and diode lasers have a positive effect on the healing of experimental palatal wounds in rats and mice;[3,11,16] however, there is still no consensus about how different types of laser sources affect the wound healing processes in different tissues. Moreover, the very few studies examining the biomodulatory effects of Nd:YAG on the healing process of various types of periodontal wounds have had variable results, and, to the best of our knowledge, no clinical study has evaluated the effect of low-pulse energy Nd-YAG stimulation on the release of multiple growth factors in wounded palatal mucosa. Therefore, the present study aimed to evaluate the effect of PBM with Nd:YAG laser on the recovery of wounds at the palatal donor site following FGG harvesting by measuring TGF- β_1, PDGF-BB, and IL-8 levels.

Materials and Methods

Study population

This parallel, double-blind, randomized controlled clinical study was conducted with 30 out of 40 randomly selected patients who completed the study and the follow-up period [Fig. 1, Consolidated Standards of Reporting Trials (CONSORT)-patient flow chart]. All the patients were recruited from the Periodontology Department at the Ondokuz Mayis University Faculty of Dentistry in Samsun, Turkey between May 2013 and January 2014. The study protocol was approved by the Local and Ministry of Health Ethics Committees, and written informed consent was obtained from all study participants in accordance with the Helsinki Declaration.

Inclusion criteria were as follows: (1) age 18–35 years; (2) mucogingival problems indicating treatment by FGG, with no pathology or morphological alterations of the palatal mucosa; (3) no medication usage during the 6 weeks prior to data collection; and (4) no history of smoking. Exclusion criteria were as follows: (1) medical history of cancer, rheumatoid arthritis, diabetes mellitus, or cardiovascular disease; (2) compromised immune system; (3) pregnancy, menopause, or lactation; or (4) history of periodontal disease or periodontal surgery of teeth adjacent to the donor site.

Clinical treatment

Patients were randomly allocated to one of the following groups:

Laser group: PBM on donor site following FGG harvesting ($n = 15$; 8 male, 7 female; mean age: 25.20 ± 5.30)
Sham group: PBM sham on donor site following FGG harvesting ($n = 15$; 7 male, 8 female; mean age: 23.90 ± 4.40)

The patients, as well as the individual who performed the surgical procedures, were blinded to the groups. A nonparticipant in the study randomly allocated participants between the laser and sham groups using computer-generated random allocation sequencing, the results of which were placed in opaque, sealed envelopes that included the numbers of patients in each group and were not opened until after the surgery was performed.

Surgical procedure

All surgical procedures were performed by the same clinician (I.K.). Local infiltration anesthesia was performed using 2% lidocaine with 1:100.000 epinephrine. A graft of sufficient size was outlined in the palate by making a shallow incision ~1.5 mm in depth between the distal of a canine and the mesial of first molar at a distance of 2 mm from the gingival margins using a #15 surgical blade. As the blade was inserted to separate the graft, tissue forceps were used to hold onto the anterior edge of the graft and gently lift the tissue to provide visibility as the separation progressed. Graft thickness was measured at three points identified along the long axis of the graft (one at the center and the others at the ends, 2 mm from the midpoint of the anterior and posterior margins). The harvested graft was
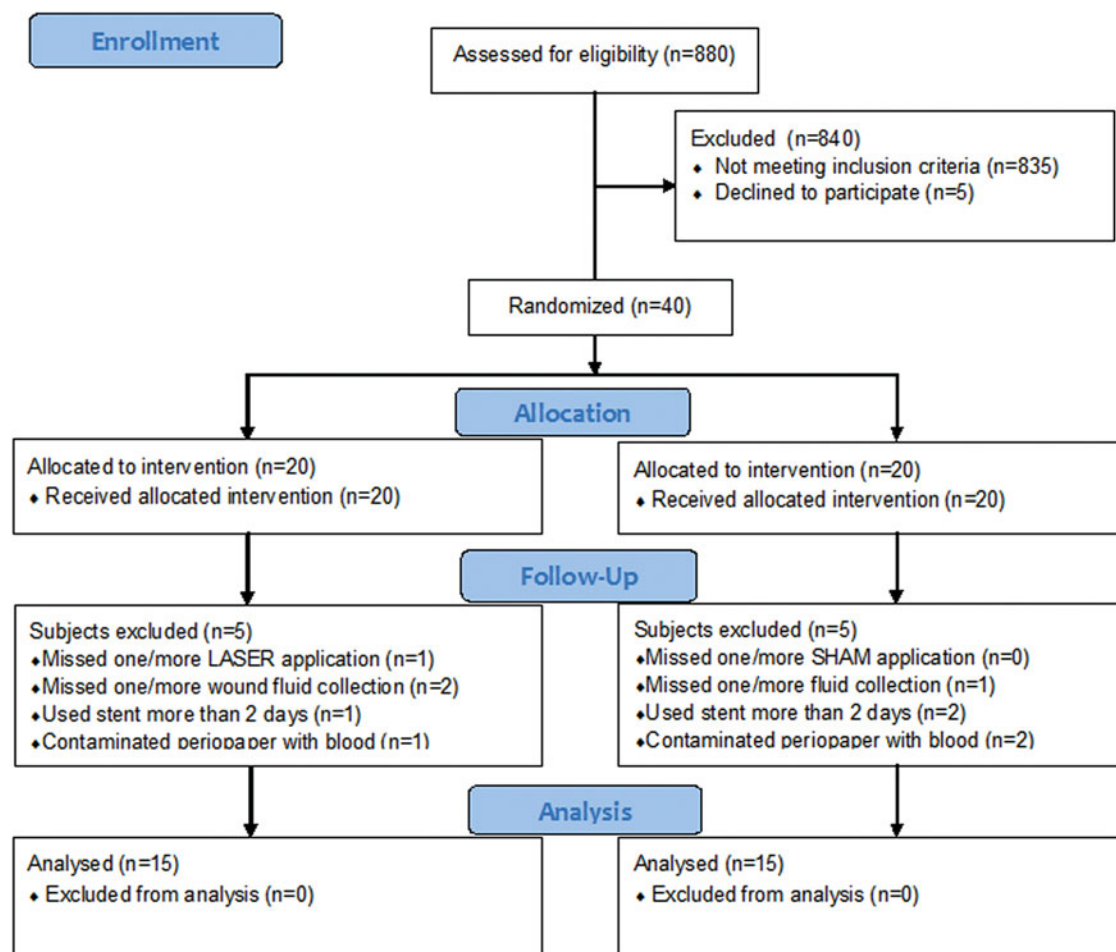
FIG. 1. Consolidated Standards of Reporting Trials (CONSORT) patient flow chart.

placed on a sterile microscope slide, and an endodontic reamer with a silicone stopper (No.20 Endodontic Reamer, Bahadır Dis Malz, Istanbul, Turkey) was placed on the selected points. The reamer was carefully removed, and palatal mucosa thickness was measured (mm) using a sterile caliper [40 mm Curved Castroviejo Bone Caliper (Hu-Friedy, Chicago, IL)]. The palatal donor site area was also measured from digital photographs of the graft taken immediately after harvesting, which included a periodontal probe as a reference scale. The photographs were exported to imaging software (ImageJ, National Institutes of Health, Bethesda, MD), adjusted to a standardized brightness, distance, and angle, and measurements were taken and recorded (cm^2). The abovementioned measurements (FGG thickness and wound area) were taken into account to maintain the standardization of the sampling sites.

Following surgery, no sutures, acrylates, or dressings were applied to the palatal donor site; however, the wound area was mechanically protected by covering it with a plastic surgical stent that had been fabricated prior to surgery, and the patient was instructed to wear the stent for 2 days.

PBM protocol

In laser group, irradiation was performed with an Nd:YAG (Fotona Fidelis III, Ljubljana, Slovenia) laser with a contin-uous wavelength of 1064 nm. Laser therapy was initiated in the immediate postoperative period, and applications were repeated four more times at 24 h intervals by the same clinician (A.A.). For each irradiation, 250 mW was applied for 10 sec, and the applied energy density (fluence) was ~ 1.6 J/cm^2 (total applied energy of 1.6 J/$cm^2 \times 5$) (Table 1). The laser probe (an R24 handpiece with a spot size of 600 μm optical fiber) was positioned perpendicularly, and laser irradiation was applied within a wound area using circular movements at

TABLE 1. LASER PARAMETERS

Manufacturer	Fotona,Slovenia
Model	Fidelis Plus III
Laser system	Nd:YAG
Photobiomodulation probe	R24
Wavelength	1064 nm
Power	250 mW
Probe spot size diameter	0.28 cm^2
Application mode	Continuous wave (CW)
Application distance	1 cm from tissue
Application type	Circular
Fluence	1.6 J/cm^2
Duration of each treatment session	10 sec/day
Frequency of treatment	5 times (24 h intervals)
Cumulative dose	~ 8 J/cm^2
Application area	~ 1.5 cm^2
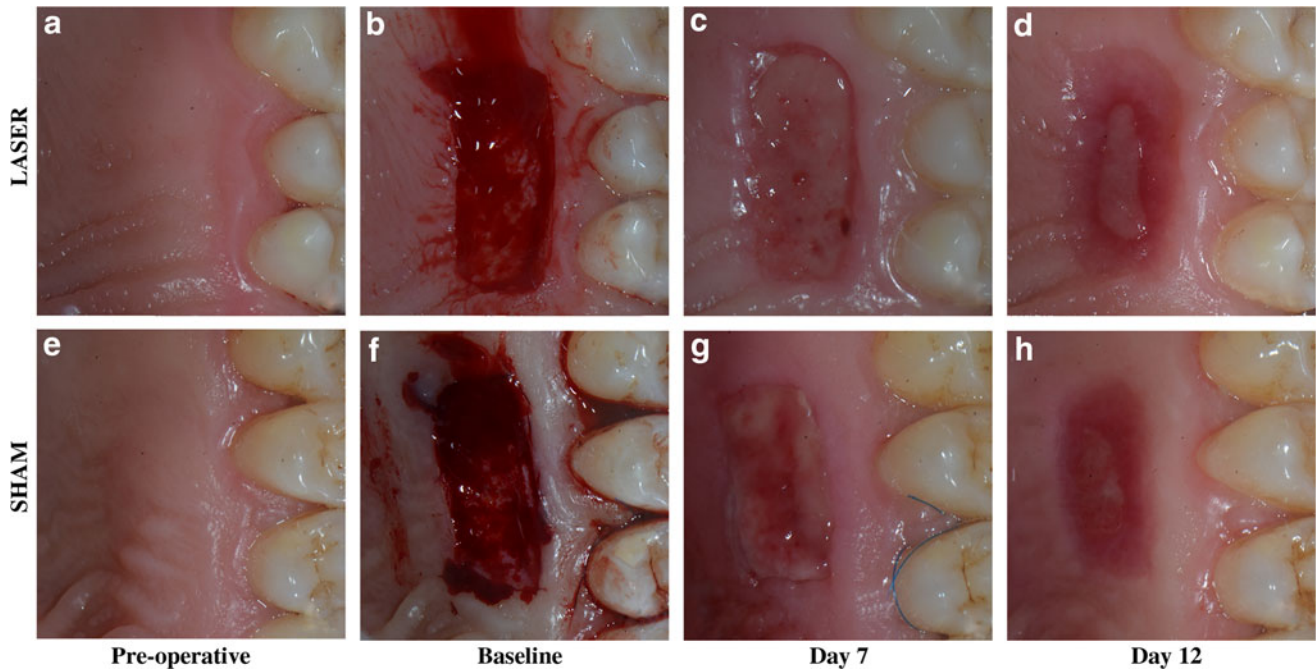
FIG. 2. Clinical views of wound area of the laser group: **(a)** before surgery, **(b)** after harvesting free gingival graft (FGG), **(c)** healing on Day 7, **(d)** healing on Day 12. Sham group: **(e)** before surgery, **(f)** after harvesting FGG, **(g)** healing on Day 7, **(h)** healing on Day 12.

a distance of 1 cm from the donor site. The patients allocated to the sham group received sham irradiation.

Palatal wound fluid (PWF) sampling and processing

PWF samples were collected from the palatal wound area of each subject on Days 7 and 12 post-surgery (Fig. 2), using periopaper strips (Fig. 3) (Oraflow Inc., NY). Prior to

FIG. 3. Palatal wound fluid sampling.

sample collection, the wound site was gently air dried, and the area was carefully isolated to prevent sample contamination by other oral fluids. Two strips were placed onto the center of the wounded area and were left in place for 15 sec. Care was taken to avoid mechanical injury of healing granulation tissue. (If a strip was contaminated with blood or other oral fluid, it was discarded, and another sample was taken.) The two strips were combined as a single sample for measurement. PWF sample volume (μL) was measured using a calibrated Periotron 8000 (Periotron® 8000; Oraflow Inc., NY). Samples were individually placed in 500 μL plastic Eppendorf microcentrifuge tubes that were labeled, sealed, and stored at −80°C until biochemical analysis. In total, 120 PWF (30×2 strips from each donor site wound area) samples were collected. All clinical examinations and PWF collection were performed by a single examiner (M.L.).

PWF elution was performed according to Curtis et al.'s procedure for the elution of gingival crevicular fluid from periopaper strips.[37] A total of 300 μL 2% bovine serum albumin (0.01M, pH 7.2) in phosphate-buffered saline (PBS) was added to each tube, and the samples were incubated at 4°C for 60 min. Following incubation, a sterile drill was used to bore a hole in the bottom of each tube, which was then placed inside a 1.5 mL tube, and the nested tubes were centrifuged at 10,000g for 10 min at 4°C.

TGF-β_1, PDGF-BB (catalogue number: BMS249/4, Human TGF-β1 Platinum ELISA; Catalogue number: BMS2071, eBioscience, Vienna, Austria) and IL-8 (catalogue number: KAP1301, DIAsource ImmunuAssays S.A., Belgium) levels in samples were evaluated using standard enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions. Enzyme-substrate reactions were terminated by the addition of an acid solution, and

TABLE 2. DESCRIPTIVE AND INTERGROUP COMPARISON STATISTICS OF WOUND AREA AND FGG THICKNESS

| | Groups | | p* Values |
	Laser	Sham	
Graft thickness (mm)	1.40 (1.30–1.50)	1.50 (1.30–1.60)	0.193
Donor site wound area (cm^2)	1.50 (1.42–2.05)	1.56 (1.39–1.70)	0.519

*Mann–Whitney U test was performed, values given as median (25–75 percentiles).
FGG, free gingival graft.

color change was measured spectrophotometrically at a wavelength of 450 nm. TGF-β_1, PDGF-BB, and IL-8 concentrations were identified using the standard curves. The standard detection limits of the TGF-β1, PDGF-BB, and IL-8 assays, as reported by the manufacturers, ranged from a minimum of 31, 31.3, and 40 pg/mL, to a maximum of 2000, 2000, and 1845 pg/mL, and also sensitivities of 8.6, 4.6, and 1.1 pg/mL, respectively. The intra-assay and interassay coefficients of variation were 3.2% and 4.9% for TGF-β1, 4% and 8.4% for PDGF-BB, and 3.6% and 13.1% for IL-8, respectively. After determining the amounts of TGF-β_1, PDGF-BB, and IL-8 collected from each sample in a 15 sec period, concentrations of TGF-β_1, PDGF-BB, and IL-8 per sample (pg/μL) were calculated by multiplying the results of ELISA assays by a dilution coefficient [PBS volume (μL) + PWF volume (μL)/PBS volume (μL)]. Total amounts of TGF-β_1, PDGF-BB, and IL-8 per sample (pg) were calculated by multiplying concentrations per sample (pg/μL) × PWF volume. All laboratory procedures were performed by a single researcher blinded to the study (M.A.S.).

Statistical analysis

Statistical analysis was performed using the statistical software program SPSS (SPSS v.21.0 Inc., Chicago, IL), and results are presented as medians and percentiles (25–75). A Shapiro–Wilk test showed non-normal data distribution; therefore, the Wilcoxon test was used for intragroup comparisons, and the Mann–Whitney U test was used for intergroup comparisons. Power analysis calculations indicated a minimum requirement of 15 participants per group in order to compare data between groups at $\alpha = 0.05$, with a power value of 95%.

Results

Significant differences were not observed in the distribution of age (laser group: 25.20 ± 5.30; sham group: 23.90 ± 4.40) or gender (laser group: 8 male, 7 female; sham group: 7 male, 8 female) between groups ($p > 0.05$).

Descriptive and intergroup comparison statistics of wound area and FGG thickness are summarized in Table 2. FGG thickness (mm), donor side wound area (cm^2) did not vary significantly between the groups ($p > 0.05$).

Intergroup comparisons of PWF TGF-β1, PDGF-BB, and IL-8 concentrations and total amounts on Day 7 and Day 12 for both laser and sham groups are given in Table 3. On Day 7, PWF TGF-β1, PDGF-BB, and IL-8 concentrations and total amounts were significantly higher in laser group than in the sham group ($p < 0.05$). On Day 12, TGF-β1 concentrations and total amounts were still significantly higher in laser group than in the sham group ($p < 0.05$). However, PDGF-

TABLE 3. THE INTERGROUP COMPARISONS OF PWF TGF-ß$_1$, PDGF-BB, AND IL-8 CONCENTRATIONS AND TOTAL AMOUNTS ON DAYS 7 AND 12

| | Day 7 | | | Day 12 | | |
| | Groups | | | Groups | | |
	Laser	Sham	p Value	Laser	Sham	p Value
TGF-ß1 (pg/μL)	189.05 (151.17–213.38)	124.62 (108.59–136.22)	**0.000***	107.43 (82.45–122.55)	63.85 (54.66–86.13)	**0.002***
TGF-ß1 (pg/15 sec)	89.49 (77.89–110.10)	65.02 (53.61–70.24)	**0.000***	42.21 (33.23–44.57)	26.13 (21.38–29.17)	**0.001***
PDGF-BB (pg/μL)	157.34 (148.97–174.46)	86.24 (78.01–101.7)	**0.000***	150.06 (141.20–172.38)	138.86 (132.92–166.68)	0.270
PDGF-BB (pg/15 sec)	83.53 (72.65–91.93)	42.36 (40.87–50.04)	**0.000***	68.17 (48.82–69.01)	52.40 (49.06–61.68)	0.169
IL-8 (pg/μL)	448.92 (366.01–486.49)	314.59 (262.25–385.36)	**0.015***	250.38 (206.76–275.49)	215.28 (128.03–266.43)	0.193
IL-8 (pg/15 sec)	232.39 (185.53–252.22)	161.63 (126.65–185.45)	**0.003***	96.78 (86.16–111.47)	80.97 (60.07–95.94)	0.095

Mann–Whitney U test was performed for intergroup comparisons, values given as median (25–75 percentiles).
*Statistical difference between groups if $p < 0.05$.
PWF, palatal wound fluid; TGF, transforming growth factor; PDGF, platelet-derived growth factor; IL, interleukin.

TABLE 4. THE INTRAGROUP COMPARISONS OF PWF TGF- ß₁, PDGF-BB,
AND IL-8 CONCENTRATIONS AND TOTAL AMOUNTS

	Comparisons	TGF-ß1 (pg/µL)	TGF-ß1 (pg/15 sec)	PDGF_BB (pg/µL)	PDGF_BB (pg/15 sec)	IL-8 (pg/µL)	IL-8 (pg/15 sec)
Laser n = 15	Day 7 vs. Day 12	0.011*	0.008*	0.001*	0.015*	0.008*	0.008*
Sham n = 15	Day 7 vs. Day 12	0.002*	0.002*	0.002*	0.015*	0.010*	0.003*

Wilcoxon test for intragroup comparisons.
*Statistical difference between groups if $p < 0.05$.
PWF, palatal wound fluid; TGF, transforming growth factor; PDGF, platelet-derived growth factor; IL, interleukin.

BB and IL-8 concentrations and total amounts were similar for both groups ($p > 0.05$). Intragroup comparisons between Day 7 and Day 12 for PWF TGF-ß1, PDGF-BB, and IL-8 concentrations and total amounts in the laser group were significant higher at Day 7 than Day 12 ($p < 0.05$). Also in the sham group, PWF TGF-ß1, PDGF-BB, and IL-8 concentrations and total amounts were significantly higher on Day 7 than on Day 12 ($p < 0.05$). All intragroup comparisons are detailed in Table 4.

Discussion

In this article, we describe the beneficial effect of PBM with low-pulse energy Nd-YAG laser stimulation of TGF-ß1, PDGF-BB, and IL-8 in palatal wounds after FGG harvesting. In this clinical proof of principle study, PBM significantly elevated PWF TGF-β_1, PDGF-BB, and IL-8 levels in laser group, particularly on Day 7, which is substantial evidence for improvement in wound healing. In addition, there was a significant decrease in PWF TGF-β_1, PDGF-BB, and IL-8 levels by sampling time points (Day 7 versus Day 12) in the laser group and sham group individually.

The wound healing process may be examined by performing a biopsy or by collecting wound fluid.[38–40] However, a biopsy is an invasive procedure that can be painful and unacceptable to patients, who are unlikely to give their consent for repeated biopsies, and, unless the entire area is excised, a biopsy can only assess the state of healing at a small, specific site and may not capture problems at other sites within the wounded area. In contrast, fluids collected from the wound healing area are representative of the entire process of healing, and can be used to identify problems anywhere within the area.[38–40] The present study relied on wound fluid collection, a noninvasive, easily tolerated method of assessing healing at the site of the palatinal wound, which allows for sequential sampling for analysis. To the best of our knowledge, this is the first study to utilize sample collection from the donor site wound to evaluate healing following the harvesting of FGG.

PBM appears to be a promising option for activating or modulating cell metabolism in periodontal tissue. The beneficial effects of PBM on periodontal wound healing have been demonstrated by both in vitro[4,6–8,12] and in vivo studies in animals[3,11,17,25,41,42] and humans.[18,20,43,44] Unfortunately, controlled clinical studies examining the effect of PBM from any kind of laser source on gingival wound healing following periodontal surgery are extremely scarce. Studies by Amorim et al.[45] and Ozcelik et al.[46] reported that PBM

for 7 days immediately following gingivectomy and gingivoplasty procedures enhanced epithelialization and improved wound healing. In another recent study, Fernandes-Dias et al.[44] claimed that low-level laser applied to a connective tissue graft used to treat gingival recession had a beneficial effect on healing and increased the percentage of root coverage. In contrast to these findings, Damante et al.[47] reported that low-level laser irradiation did not accelerate healing after gingivoplasty. Similarly, a photograph-based study by Almeida et al.[43] reported no beneficial effects from the application of low-intensity laser to the recipient site during healing of FGG. To the best of our knowledge, the literature contains only one randomized controlled clinical trial examining the effect of PBM on human hard palate gingival wound healing. That study, conducted by Dias et al.,[18] determined that low-level laser irradiation accelerated wound healing of the palatinal donor site following connective tissue harvesting based on wound area and tissue thickness measurements as well as changes in tissue color; however, the study did not assess biochemical and physiological parameters of wound healing.[18]

In the present study, both laser and sham groups showed significant decreases in TGF-β_1 and PDGF-BB expression on Day 12 when compared with Day 7. The reduction in growth factor expression is an usual event to be observed during the wound healing process.[29,48] Most importantly, the present study revealed that PBM stimulated the expression of TGF-β_1 and PDGF-BB, both of which play roles in the early phase of the wound healing process. Although these results were for Nd-YAG laser, they are in line with those of Safavi et al.,[41] who found that PBM using a He-Ne laser had a biostimulatory effect on healing of incisions created in rat gingiva, and resulted in significantly higher expressions of TGF-β and PDGF in gingival biopsy specimens. Another recent study conducted by Usumez et al.,[25] which compared the effects of low-level laser irradiation with four different laser wavelengths (660, 810, 980, and 1064 nm) on mucositis wound healing in an animal model by evaluating growth factor expression, showed that laser irradiation significantly increased PDGF expression, with the highest PDGF expression detected with Nd-YAG (1064 nm); however, low-level laser application had no effect on TGF-β expression. The different results reported by Safavi et al.[41] and Usumez et al.[25] regarding TGF expression could be the result of differences in the characteristics of fibroblasts and other matrix molecules found in mucosal and gingival tissue as well as differences in low-level laser application protocols and energy sources.[4,49] Further, there is currently a lack of consensus regarding low-level laser

application protocols, and the melange of laser application techniques, energy dosages, and treatment protocols currently employed make it difficult to compare findings regarding the effects of low-level laser irradiation on wound healing.

The initiation of ECM deposition in the granulation tissue located below newly formed epithelium has been reported to occur after ~ 7 days post-harvesting.[28] In the early phases of healing, TGF-β_1 and PDGF-BB initiate phenotypic changes in the cells within the ECM, converting fibroblasts into myofibroblasts that align themselves along the matrix borders to generate a constrictive force, thereby facilitating wound closure. Later on, the granulation tissue is removed and replaced by a framework formed from collagen and elastin fibers. TGF-β_1 and PDGF have also been shown to upregulate signals initiated by tension in the three-dimensional collagen matrix framework of newly deposited ECM and coordinated by specific collagen–integrin interactions.[50–52] Once the early phase of the healing process is complete, it is followed by tissue remodeling and maturation involving the TGF-β-mediated synthesis of new collagen and the breakdown of old collagen by PDGF.[50,53] In general, an ECM scaffold is constructed between 7 and 10 days, after which collagen synthesis continues so that the scaffold is able to acquire the strength and stiffness needed to sustain the proper ECM.[11,54] The increase in TGF-β_1 and PDGF-BB observed on Day 7 in the present study may be a sign of PBM's ability to facilitate quicker organization of the ECM collagen framework as a scaffold. Moreover, the persistent high levels of TGF-β_1 observed in the laser-irradiated wounds on Day 12 could contribute to faster saturation of the framework with additional matrix proteins (such as proteoglycans and glycoproteins).

IL-8 has been shown to be highly expressed on denuded wound surfaces at the exact point to which keratinocytes migrate to close the epidermal defect[35] and it has been claimed to be considerably effective on re-epithelialization.[55,56] Although IL-8 can have positive effects on healing, excessive amounts of IL-8 can retard wound closure by reducing fibroblast migration and engendering morphological changes in fibroblasts, which deteriorate and disrupt the focal adhesion and microfilaments of those cells aiming to organize compact collagen fibers into thicker fiber bundles.[35,57]

There is scant literature on the effects of PBM on IL-8 expression in oral tissue,[58–60] and none that is directly related to periodontal wound healing. In our study, decreases in IL-8 expression from Day 7 to Day 12 were observed in both groups, which is line with previous studies showing decreases in IL-8 tissue expression during the healing process.[29,57] The present study also found IL-8 expression in laser-irradiated wounds to be significantly higher than IL-8 expression in unirradiated wounds on Day 7, but not on Day 12. These findings suggest that the stimulation of IL-8 expression observed with PBM may be a sign of faster re-epithelialization related to laser biostimulation of epithelial cells. This is in line with the findings of Lee et al., who showed IL-8 expression of oral keratinocytes in a culture medium to increase significantly with the application of low-level laser.[7] On the other hand, not maintaining the increased levels on Day 12 is also suggested to be a favorable process in order to prevent the reported adverse effects of excessive IL-8 expression on retardation of wound healing.[35,57]

There are a number of limitations to the present study that need to be mentioned. First, PWF was collected at only two time points, whereas daily collection might have secured more meaningful data (although blood contamination of PWF in the first days following harvesting would likely have precluded the possibility of biochemical analysis). Second, not using biopsies, which provide a much clearer insight into the structure and reorganization of ECM, because of their being a rather invasive method, and not allowing us to make repeated samplings, could be considered another limitation of the present study.

Conclusions

The present study showed PBM with Nd:YAG laser to have a stimulative effect on the secretion of TGF-β_1, PDGF-BB, and IL-8 in donor site palatal wounds of patients undergoing FGG. Although there are few previous studies with which to compare these results, the limited information available supports these findings, suggesting that PBM may accelerate wound healing by stimulating the production of selected growth factors. Specifically, PBM may play an important role in periodontal wound healing by enhancing the production of the growth factors required during the early phase of matrix production to secure proper tissue healing.

Acknowledgments

Author Disclosure Statement

No competing financial interests exist.

References

1. Aoki A, Mizutani K, Schwarz F, et al. Periodontal and peri-implant wound healing following laser therapy. Periodontol 2000 2015;68:217–269.
2. Ishikawa I, Aoki A, Takasaki AA, Mizutani K, Sasaki KM, Izumi Y. Application of lasers in periodontics: true innovation or myth? Periodontol 2000 2009;50:90–126.
3. Firat ET, Dağ A, Günay A, et al. The effects of low-level laser therapy on palatal mucoperiosteal wound healing and oxidative stress status in experimental diabetic rats. Photomed Laser Surg 2013;31:315–321.
4. Saygun I, Nizam N, Ural AU, Serdar MA, Avcu F, Tözüm TF. Low-level laser irradiation affects the release of basic fibroblast growth factor (bFGF), insulin-like growth factor-I (IGF-I), and receptor of IGF-I (IGFBP3) from osteoblasts. Photomed Laser Surg 2012;30:149–154.
5. Saygun I, Karacay S, Serdar M, Ural AU, Sencimen M, Kurtis B. Effects of laser irradiation on the release of basic fibroblast growth factor (bFGF), insulin like growth factor-1 (IGF-1), and receptor of IGF-1 (IGFBP3) from gingival fibroblasts. Lasers Med Sci 2008;23:211–215.
6. Choi EJ, Yim JY, Koo KT, et al. Biological effects of a semiconductor diode laser on human periodontal ligament fibroblasts. J Periodontal Implant Sci 2010;40:105–110.
7. Lee JY, Kim IR, Park BS, et al. Effect of low-level laser therapy on oral keratinocytes exposed to bisphosphonate. Lasers Med Sci 2015;30:635–643.
8. Basso FG, Pansani TN, Soares DG, et al. Biomodulation of inflammatory cytokines related to oral mucositis by

low-level laser therapy. Photochem Photobiol 2015;91: 952–956.

9. Medrado AR, Pugliese LS, Reis SR, Andrade ZA. Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. Lasers Surg Med 2003;32:239–244.

10. Al-Watban FA, Delgado GD. Burn healing with a diode laser: 670 nm at different doses as compared to a placebo group. Photomed Laser Surg 2005;23:245–250.

11. Fahimipour F, Mahdian M, Houshmand B, et al. The effect of He-Ne and Ga-Al-As laser light on the healing of hard palate mucosa of mice. Lasers Med Sci 2013;28: 93–100.

12. Al-Watban FA, Andres BL. Laser biomodulation of normal and neoplastic cells. Lasers Med Sci 2012;27:1039–1043.

13. Cambier DC, Vanderstraeten GG, Mussen MJ, van der Spank JT. Low-power laser and healing of burns: a preliminary assay. Plast Reconstr Surg 1996;97:555–558.

14. Walker MD, Rumpf S, Baxter GD, Hirst DG, Lowe AS. Effect of low-intensity laser irradiation (660 nm) on a radiation-impaired wound- healing model in murine skin. Lasers Surg Med 2000;6:41–47.

15. Schlager A, Kronberger P, Petschke F, Ulmer H. Low-power laser light in the healing of burns: a comparison between two different wavelengths (635 nm and 690 nm) and a placebo group. Lasers Surg Med 2000;27:39–42.

16. Jahangiri Noudeh Y, Shabani M, Vatankhah N, Hashemian SJ, Akbari K. A combination of 670 nm and 810 nm diode lasers for wound healing acceleration in diabetic rats. Photomed Laser Surg 2010;28:621–627.

17. Firat ET, Dağ A, Günay A, et al. The effect of low-level laser therapy on the healing of hard palate mucosa and the oxidative stress status of rats. J Oral Pathol Med 2014;43: 103–110.

18. Dias SB, Fonseca MV, Dos Santos NC, et al. Effect of GaAIAs low-level laser therapy on the healing of human palate mucosa after connective tissue graft harvesting: randomized clinical trial. Lasers Med Sci 2015;30:1695–1702.

19. Aras MH, Güngörmüş M. Placebo-controlled randomized clinical trial of the effect two different low-level laser therapies (LLLT)—intraoral and extraoral—on trismus and facial swelling following surgical extraction of the lower third molar. Lasers Med Sci 2010;25:641–645.

20. Sezer U, Eltas A, Ustün K, Senyurt SZ, Erciyas K, Aras MH. Effects of low-level laser therapy as an adjunct to standard therapy in acute pericoronitis, and its impact on oral health-related quality of life. Photomed Laser Surg 2012;30:592–597.

21. Vescovi P, Meleti M, Merigo E, et al. Case series of 589 tooth extractions in patients under bisphosphonates therapy. Proposal of a clinical protocol supported by Nd:YAG low-level laser therapy. Med Oral Patol Oral Cir Bucal 2013; 18:680–685.

22. Vescovi P, Giovannacci I, Merigo E, et al. Tooth extractions in high-risk patients under bisphosphonate therapy and previously affected with osteonecrosis of the jaws: surgical protocol supported by low-level laser therapy. J Craniofac Surg 2015;26:696–699.

23. Luomanen M, Alaluusua S. Treatment of bisphosphonate-induced osteonecrosis of the jaws with Nd:YAG laser biostimulation. Lasers Med Sci 2012;27:251–255.

24. Chellini F, Sassoli C, Nosi D, et al. Low pulse energy Nd:YAG laser irradiation exerts a biostimulative effect on different cells of the oral microenvironment: "an in vitro study". Lasers Surg Med 2010;42:527–539.

25. Usumez A, Cengiz B, Oztuzcu S, Demir T, Aras MH, Gutknecht N. Effects of laser irradiation at different wavelengths (660, 810, 980, and 1,064 nm) on mucositis in an animal model of wound healing. Lasers Med Sci 2014; 29:1807–1813.

26. Susin C, Fiorini T, Lee J, De Stefano JA, Dickinson DP, Wikesjö UM. Wound healing following surgical and regenerative periodontal therapy. Periodontology 2000 2015; 68:83–98.

27. Polimeni G, Xiropaidis AV, Wikesjö UM. Biology and principles of periodontal wound healing/regeneration. Periodontol 2000 2006;41:30–47.

28. Häkkinen L, Uitto VJ, Larjava H. Cell biology of gingival wound healing. Periodontol 2000 2000;24:127–152.

29. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic–Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen 2008;16:585–601.

30. Bartold PM, Narayanan AS. Molecular and cell biology of healthy and diseased periodontal tissues. Periodontol 2000 2006;40:29–49.

31. Ray AK, Jones AC, Carnes DL, Cochran DL, Mellonig JT, Oates TW Jr. Platelet-derived growth factor-BB stimulated cell migration mediated through p38 signal transduction pathway in periodontal cells. J Periodontol 2003;74:1320–1328.

32. Mumford JH, Carnes DL, Cochran DL, Oates TW. The effects of platelet-derived growth factor-BB on periodontal cells in an in vitro wound model. J Periodontol 2001;72: 331–340.

33. Javed F, Al-Askar M, Al-Rasheed A, Al-Hezaimi K. Significance of the platelet-derived growth factor in periodontal tissue regeneration. Arch Oral Biol 2011;56:1476–1484.

34. Fujita T, Yoshimoto T, Matsuda S, et al. Interleukin-8 induces DNA synthesis, migration and down-regulation of cleaved caspase-3 in cultured human gingival epithelial cells. J Periodontal Res 2015;50:479–485.

35. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol 2001;69:513–521.

36. 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;81:692–701.

37. Curtis MA, Griffiths GS, Price SJ, Coulthurst SK, Johnson NW. The total protein concentration of gingival crevicular fluid. Variation with sampling time and gingival inflammation. J Clin Periodontol 1988;15:628–632.

38. Widgerow AD. Wound fluid intervention: influencing wound healing from the outside. Wound Healing Southern Africa 2011;4:12–15.

39. Drinkwater SL, Smith A, Burnand KG. What can wound fluids tell us about the venous ulcer microenvironment? Int J Low Extrem Wounds 2002;1:184–190.

40. Yager DR, Kulina RA, Gilman LA. Wound fluids: a window into the wound environment? Int J Low Extrem Wounds 2007;6:262–272.

41. Safavi SM, Kazemi B, Esmaeili M, Fallah A, Modarresi A, Mir M. Effects of low-level He-Ne laser irradiation on the gene expression of IL-1beta, TNF-alpha, IFN-gamma, TGF-beta, bFGF, and PDGF in rat's gingiva. Lasers Med Sci 2008;23:331–335.

42. Wang CY, Tsai SC, Yu MC, Lin YF, Chen CC, Chang PC. Light-emitting diode irradiation promotes donor site wound healing of the free gingival graft. J Periodontol 2015;86: 674–681.

43. 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;27:561–564.

44. Fernandes–Dias SB, de Marco AC, Santamaria M Jr, Kerbauy WD, Jardini MA, Santamaria MP. Connective tissue graft associated or not with low laser therapy to treat gingival recession: randomized clinical trial. J Clin Periodontol 2015;42:54–61.

45. Amorim JC, de Sousa GR, de Barros Silveira L, Prates RA, Pinotti M, Ribeiro MS. Clinical study of the gingiva healing after gingivectomy and low-level laser therapy. Photomed Laser Surg 2006;24:588–594.

46. Ozcelik O, Cenk Haytac M, 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.

47. Damante CA, Greghi SL, Sant'ana AC, Passanezi E. Clinical evaluation of the effects of low-intensity laser (GaAlAs) on wound healing after gingivoplasty in humans. J Appl Oral Sci 2004;12:133–136.

48. Dinh T, Braunagel S, Rosenblum BI. Growth factors in wound healing: the present and the future? Clin Podiatr Med Surg 2015;32:109–119.

49. Damante CA, De Micheli G, Miyagi SP, Feist IS, Marques MM. Effect of laser phototherapy on the release of fibroblast growth factors by human gingival fibroblasts. Lasers Med Sci 2009;24:885–891.

50. Meckmongkol TT, Harmon R, McKeown-Longo P, Van De Water L. The fibronectin synergy site modulates TGF-beta-dependent fibroblast contraction. Biochem Biophys Res Commun 2007;360:709–714.

51. Lin H, Chen B, Sun W, Zhao W, Zhao Y, Dai J. The effect of collagen-targeting platelet-derived growth factor on cellurization and vascularization of collagen scaffolds. Biomaterials 2006;27:5708–5714.

52. Rhee S, Grinnell F. P21-activated kinase 1: convergence point in PDGF- and LPA-stimulated collagen matrix contraction by human fibroblasts. J Cell Biol 2006;172:423–432.

53. Jinnin M, Ihn H, Mimura Y, Asano Y, Yamane K, Tamaki K. Regulation of fibrogenic/fibrolytic genes by platelet-derived growth factor C, a novel growth factor, in human dermal fibroblasts. J Cell Physiol 2005;202:510–517.

54. Niessen FB, Andriessen MP, Schalkwijk J, Visser L, Timens W. Keratinocyte-derived growth factors play a role in the formation of hypertrophic scars. J Pathol 2001;194:207–216.

55. Devalaraja RM, Nanney LB, Du J, et al. Delayed wound healing in CXCR2 knockout mice. J Invest Dermatol 2000;115:234–244.

56. Rennekampff HO, Hansbrough JF, Kiessig V, Doré C, Sticherling M, Schröder JM. Bioactive interleukin-8 is expressed in wounds and enhances wound healing. J Surg Res 2000;93:41–54.

57. Iocono JA, Colleran KR, Remick DG, Gillespie BW, Ehrlich HP, Garner WL. Interleukin-8 levels and activity in delayed-healing human thermal wounds. Wound Repair Regen 2000;8:216–225.

58. Basso FG, Pansani TN, Soares DG, et al. Biomodulation of inflammatory cytokines related to oral mucositis by low-level laser therapy. Photochem Photobiol 2015;91:952–956.

59. Qadri T, Bohdanecka P, Tunér J, Miranda L, Altamash M, Gustafsson A. The importance of coherence length in laser phototherapy of gingival inflammation: a pilot study. Lasers Med Sci 2007;22:245–251.

60. Saglam M, Kantarci A, Dundar N, Hakki SS. Clinical and biochemical effects of diode laser as an adjunct to nonsurgical treatment of chronic periodontitis: a randomized, controlled clinical trial. Lasers Med Sci. 2014;29:37–46.

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