Effect of Photobiomodulation on Transforming Growth Factor-$\beta_1$, 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)-$\beta_1$, 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-$\beta_1$, PDGF-BB, and IL-8 levels were analyzed by enzyme-linked immunosorbent assays (ELISA).

Results: PWF TGF-$\beta_1$, PDGF-BB, and IL-8 levels were significantly lower on Day 12 than on Day 7 for both groups. PWF TGF-$\beta_1$, 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-$\beta_1$ 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-$\beta_1$, 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 lasers3,4,17–20 whereas few studies have investigated the potential biomodulatory effect of the application of low-pulse energy neodymium:yttrium-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)-$\beta$, which stimulate the proliferation of the fibroblasts, keratinocytes, and endothelial cells required for wound

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healing. 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. 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.

TGF, one of the major activators of ECM synthesis, and PDGF, an important fibroblast mitogen, are produced by platelets, macrophages, fibroblasts, and keratinocytes. TGF-β1, 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-β1 and keratinocytes; that is, their mutual stimulation, is important for quick re-epithelialization and successful wound healing. 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; and it is necessary for fibroblast and myofibroblast proliferation in order to comprise wound closure.

Wound repair, inflammation, and fibrotic processes are governed by the same molecules and cellular events. Whereas TGF-β1 and PDGF-BB are fibrogenic and anti-inflammatory mediators, IL-8 belongs to the α-subfamily of chemokines associated with proinflammatory and angiogenic properties. 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.

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. 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. 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; 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-β1, 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

\[ n = 15; \quad 8 \text{ male, 7 female; mean age: } 25.20 \pm 5.30 \]

Sham group: PBM sham on donor site following FGG harvesting

\[ n = 15; \quad 7 \text{ male, 8 female; mean age: } 23.90 \pm 4.40 \]

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 \( \sim 1.5 \text{ mm} \) 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
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 (cm²). 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 continuous 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/cm² (total applied energy of 1.6 J/cm² × 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

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**FIG. 1.** Consolidated Standards of Reporting Trials (CONSORT) patient flow chart.

**Table 1. Laser Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Fotona, Slovenia</td>
</tr>
<tr>
<td>Model</td>
<td>Fidelis Plus III</td>
</tr>
<tr>
<td>Laser system</td>
<td>Nd:YAG</td>
</tr>
<tr>
<td>Photobiomodulation probe</td>
<td>R24</td>
</tr>
<tr>
<td>Wavelength</td>
<td>1064 nm</td>
</tr>
<tr>
<td>Power</td>
<td>250 mW</td>
</tr>
<tr>
<td>Probe spot size diameter</td>
<td>0.28 cm²</td>
</tr>
<tr>
<td>Application mode</td>
<td>Continuous wave (CW)</td>
</tr>
<tr>
<td>Application distance</td>
<td>1 cm from tissue</td>
</tr>
<tr>
<td>Application type</td>
<td>Circular</td>
</tr>
<tr>
<td>Fluence</td>
<td>1.6 J/cm²</td>
</tr>
<tr>
<td>Duration of each session</td>
<td>10 sec/day</td>
</tr>
<tr>
<td>Frequency of treatment</td>
<td>5 times (24 h intervals)</td>
</tr>
<tr>
<td>Cumulative dose</td>
<td>~8 J/cm²</td>
</tr>
<tr>
<td>Application area</td>
<td>~1.5 cm²</td>
</tr>
</tbody>
</table>
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 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 ($\mu$L) was measured using a calibrated Periotron 8000 (Periotron® 8000; Oraflow Inc., NY). Samples were individually placed in 500 $\mu$L plastic Eppendorf microcentrifuge tubes that were labeled, sealed, and stored at $-80^\circ$C until biochemical analysis. In total, 120 PWF (30 $\times$ 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. A total of 300 $\mu$L 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-$\beta_1$, PDGF-BB (catalogue number: BMS249/4, Human TGF-$\beta_1$ Platinum ELISA; Catalogue number: BMS2071, eBioscience, Vienna, Austria) and IL-8 (catalogue number: KAP1301, DIAsource ImmunoAssays 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
color change was measured spectrophotometrically at a wavelength of 450 nm. TGF-β₁, 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% 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-β₁, PDGF-BB, and IL-8 collected from each sample in a 15 sec period, concentrations of TGF-β₁, PDGF-BB, and IL-8 per sample (pg/L) were calculated by multiplying the results of ELISA assays by a dilution coefficient [PBS volume (L) + PWF volume (L)/PBS volume (L)]. Total amounts of TGF-β₁, PDGF-BB, and IL-8 per sample (pg) were calculated by multiplying concentrations per sample (pg/L) · 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 α=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 site wound area (cm²) 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 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-
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-β1, PDGF-BB, and IL-8 concentrations and total amounts in the laser group were significantly higher at Day 7 than Day 12 ($p < 0.05$). Also in the sham group, PWF TGF-β1, 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.

### Table 4. The Intragroup Comparisons of PWF TGF-β1, PDGF-BB, and IL-8 Concentrations and Total Amounts

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>TGF-β1 (pg/μL)</th>
<th>TGF-β1 (pg/15 sec)</th>
<th>PDGF_BB (pg/μL)</th>
<th>PDGF_BB (pg/15 sec)</th>
<th>IL-8 (pg/μL)</th>
<th>IL-8 (pg/15 sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser n = 15 Day 7 vs. Day 12</td>
<td>0.011*</td>
<td>0.008*</td>
<td>0.001*</td>
<td>0.015*</td>
<td>0.008*</td>
<td>0.008*</td>
</tr>
<tr>
<td>Sham n = 15 Day 7 vs. Day 12</td>
<td>0.002*</td>
<td>0.002*</td>
<td>0.002*</td>
<td>0.015*</td>
<td>0.010*</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

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.

### Discussion

In this article, we describe the beneficial effect of PBM with low-pulse energy Nd-YAG laser stimulation of TGF-β1, PDGF-BB, and IL-8 in palatal wounds after FGG harvesting. In this clinical proof of principle study, PBM significantly elevated PWF TGF-β1, 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-β1, 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. 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. The present study relied on wound fluid collection, a noninvasive, easily tolerated method of assessing healing at the site of the palatal 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 and in vivo studies in animals and humans. 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. and Ozcelik et al. 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. 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. reported that low-level laser irradiation did not accelerate healing after gingivoplasty. Similarly, a photograph-based study by Almeida et al. 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., 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.

In the present study, both laser and sham groups showed significant decreases in TGF-β1 and PDGF-BB expression on Day 12 when compared with Day 7. The reduction in growth factor expression is an unusual event to be observed during the wound healing process. Most importantly, the present study revealed that PBM stimulated the expression of TGF-β1 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., 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 Usuzume et al. 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. and Usuzume et al. 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. Further, there is currently a lack of consensus regarding low-level laser
application techniques, and the melting of laser application

The initiation of ECM deposition in the granulation tissue
located below newly formed epithelium has been reported to
occur after ~7 days post-harvesting.\textsuperscript{35} In the early phases of
healing, TGF-\( \beta_1 \) 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-\( \beta_1 \) 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 inter-
actions.\textsuperscript{50–52} Once the early phase of the healing process is
complete, it is followed by tissue remodeling and maturation
involving the TGF-\( \beta_1 \)-mediated synthesis of new collagen
and the breakdown of old collagen by PDGF.\textsuperscript{50,55} 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.\textsuperscript{11,54} The increase in TGF-\( \beta_1 \) 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-\( \beta_1 \) 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 mi-
grate to close the epidermal defect\textsuperscript{35} and it has been claimed to
be considerably effective on re-epithelialization.\textsuperscript{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.\textsuperscript{35,57}

There is scant literature on the effects of PBM on IL-8 ex-
pression in oral tissue,\textsuperscript{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.\textsuperscript{20,57} 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 find-
ings of Lee et al., who showed IL-8 expression of oral kerati-
nocytes in a culture medium to increase significantly with the
application of low-level laser.\textsuperscript{7} On the other hand, not main-
taining the increased levels on Day 12 is also suggested to be a
favorable process in order to prevent the reported adverse ef-
eff of excessive IL-8 expression on retardation of wound

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, be-
cause of their being a rather invasive method, and not al-
lowing 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-\( \beta_1 \), 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.

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Author Disclosure Statement
No competing financial interests exist.

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I (IGF-1), and receptor of IGF-1 (IGFBP3) from gingival
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therapy on oral keratinocytes exposed to bisphosphonate.
inflammatory cytokines related to oral mucositis by


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