Effects of Er:YAG Photobiomodulation Therapy on Wound Healing of Human Palatal Mucosa After Connective Tissue Graft Harvesting: A Pilot Study

by

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements of the degree of

MASTER OF DENTISTRY (PERIODONTOLOGY)

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Winnipeg

Abstract

Objective: The purpose of this pilot study was to evaluate the effects of photobiomodulation (PBM) with a 2940-nm Er:YAG laser on post-operative pain and wound healing at the palatal donor site of subepithelial connective tissue grafts (CTGs).

Methods: Ten systemically healthy patients who required CTG surgery completed this randomized clinical trial. Participants were assigned to receive PBM therapy or sham laser treatment of the palate following harvest of the graft. Analgesic consumption was recorded, and a visual analogue scale (VAS) pain questionnaires completed for the first post-operative week. At one week, patients completed an Oral Impact on Daily Performance (OIDP) questionnaire. At one and two weeks, healing was evaluated according to a modified early wound-healing index (MEHI) and the healing index of Landry, Turnbull and Howley (HI). Patients returned at six weeks for assessment of tissue thickness and for collection of a tissue sample.

Results: There were no significant differences in VAS scores, analgesic intake, or healing of the palate between the Test and Control groups. The Test group reported more frequent disruptions to doing light work, going out, sleeping, smiling, and interacting with others.

Conclusions: Within the limitations of this study, PBM therapy with an Er:YAG laser did not impart any beneficial effects.

Acknowledgements

Foremost, I would like to express my sincere gratitude to my research supervisor, Dr. Reem Atout, for her continued support, guidance, and willingness to contribute her time whenever I needed it. For that I am has truly grateful. Also, I would like to thank the other members of my advisory committee: Dr. Anastasia Cholakis, Dr. Robert Schroth, and Dr. Sylvia Todescan for their feedback and encouragement.

Dr. Vimi Mutalik, Dr. Veronique Benhamou, and Dr. Akira Aoki all consulted on this project. The generous donation of their time is greatly appreciated. As well, my thanks to Loring Chuchmach, his hard work and statistical expertise were invaluable.

I must thank my fellow residents, and specifically those that contributed to the clinical aspect of my research: Dr. Javier Cabrales, Dr. Carlo Sgarbanti, and Dr. Jay Vargas. Especially my co-resident, Javier, always willing to be a sounding board for my ideas and for my frustrations.

None of this would have been possible without the assistance and cooperation of the clinical and administrative staff, as well as the counsel of my clinical instructors. Dr. Daniel Price, Dr. Chrysi Stavropoulou, Dr. John Tsourounakis, Dr. Kevin Vint, Dr. Hoda Hosseini, Shelley McGavock, Christy Janis, Danielle Fontaine-Gagliardi, Celia Duarte-Trozzo, Matilde Kostiw, Michelle Dick, Tristyn Rodriguez, Sandra Iwankow, Sara Dueck, Hillary Smith, and Melissa Franzmann, I am deeply indebted to you all.

Last but not the least, I would like to thank my family: my parents, my siblings and their spouses, and all my nieces and nephews. Their unwavering emotional support gave me the resilience I needed. Thank you.

Table of Contents

List of Tables

List of Figures

Introduction

When evaluating the success of periodontal treatment, both surgical and non-surgical, one must consider the clinical results as well as patient-centred outcomes. Ensuring the healing process proceeds as optimally as possible is central to achieving success in both these realms. Finding new ways to improve post-operative healing and patient comfort continue to be areas of great interest in periodontology, from the evolution of new procedures to the implementation of minimally invasive and less traumatic surgical techniques to the development of new materials. All with the goal of minimizing morbidity and maximizing healing potential.

Literature Review

Wound healing in the human body encapsulates a wide spectrum of processes that vary greatly depending on factors such as the type of injury incurred, the severity of said injury, the immunocompetency of the patient, and the type of tissue injured, among others. Within the scope of this paper, the discussion of healing will be limited to that of the periodontal tissues.

Intraoral healing of periodontal tissues is considered more complex than epidermal wound healing. The periodontium includes a multitude of diverse tissue types: alveolar bone, gingiva, periodontal ligament, and cementum. The oral cavity is unique in that it is an "open system," which has teeth protruding from the underlying bone, up through the protective soft tissues, and establishing a communication with the external environment. Healing of these tissues attempts to establish a new connection between vascular soft tissues and mineralized hard tissues in the presence of a consistent and significant bacterial load. Thus, the outcomes of this process can be quite variable.

In the literature, many different sequences of healing have been described; on the tissue level, following a variety of different surgical procedures.[1–8] It was not until periodontal healing was later studied in the animal model with electron microscopy, that the overarching cellular events were described and adapted from the established model of healing observed in epidermal wounds.[9] Generally, the process can be broken down into four separate but overlapping phases. The initiation of the healing sequence is marked by the onset of the Clotting Phase. This begins immediately following wound closure. Bleeding is reduced due to vasoconstriction, facilitating formation of a fibrin clot, which acts as a scaffold for cell migration and proliferation from adjacent tissues. Gathering platelets promote the activation of coagulation proenzymes as well as releasing several growth factors, adhesive proteins, and chemotactic factors. These molecules signal the chemotaxis of osteoblasts, fibroblasts, leukocytes, monocytes, smooth muscle cells, and neutrophils.[10–12]

The next stage, referred to as the Inflammatory Phase, begins in the first hours following injury. There is an increase in vasodilation and capillary permeability as the inflammatory process dominates the site. Neutrophils and monocytes migrate to the site of injury, removing bacteria and necrotic tissue. Within three days, macrophages also migrate to the area, eliminating spent PMNs and erythrocytes. Granulation tissue formation then starts on approximately day four.[10–12]

Following this is the onset of the Proliferation Phase. Macrophages significantly outnumber neutrophils at this point. Macrophages stimulate the recruitment of fibroblasts, endothelial cells, and additional inflammatory cells to the wound via release of several growth factors and

inflammatory cytokines. There is an elevated metabolic demand at this stage, which is met by increased vascularity, stimulated by VEGF and other growth factors. Endothelial cells migrate into the provisional wound matrix where they proliferate and elongate, leading to formation of new blood vessels. By day seven, granulation tissue dominates the site of injury and there is formation of initial collagen fibers. Once the collagen matrix has been synthesized, some fibroblasts undergo transformation into myofibroblasts and express α-smooth muscle actin, which leads to wound contraction.[10–12]

As new vasculature develops, the wound matrix continues to mature. Within a few weeks of the initial injury, healing progresses into the Remodelling/Maturation Phase. Fibroblasts and endothelial cells will undergo apoptosis. Subsequently, tissue volume and vascularity are decreased. Maturation of the granulation tissue eventually results in regeneration or repair (i.e. scar formation).[10,12]

The patient's experience during the healing process, specifically their perception of post-operative pain or discomfort is another key component when evaluating treatment outcomes. How individuals perceive pain is an extremely nuanced and multi-faceted concept. It involves emotional and psychological aspects such as anxiety, previous experiences, control of one's environment, and anticipation of stress.[13,14] However, these factors are beyond the scope of this paper. The perceived level of post-operative pain also involves physical factors related to the surgery itself and the resultant physiological processes that occur. Such surgery-related factors include the complexity and duration of the procedure, the pre-operative thickness of the tissues at the surgical site, the experience of the surgeon, the extent of the surgical site, the amount of

anesthetic used, whether or not sedation was used, the post-operative analgesic regimen, and the specific surgical techniques employed.[13,15,16] The mechanism of post-operative pain is likely multifactorial, involving several of the steps that occur during the healing process. Initial perception of post-operative pain may be influenced by adequate hemostasis and formation of the fibrin clot which could act as a physical barrier for the underlying tissues containing mechanoreceptive nociceptive neurons.[15] Immune cells that migrate to the wound site secrete a variety of growth factors and these have been shown to directly excite primary afferent nociceptors.[17] Bone and periosteum are innervated with afferent sensory neurons that have acid-detecting ion channels which are likely activated by the decreased pH that is a result of inflammation at the site of injury.[18,19] The interacting effects of these activities which occur during healing, and likely several other processes which have not yet been fully elucidated, help illustrate why the experience of post-operative pain is so individualized.

As our understanding of the events involved in periodontal healing continues to expand, so too do the efforts to enhance the speed, comfort, and regenerative capacity of this process. Various modalities and techniques, aimed at improving different aspects of healing, have been advocated.

Synthetic biomaterials for wound closure have been developed to aid the healing process by minimizing the inflammatory response compared to traditional methods of wound closure (i.e. natural braided sutures). An example of this is the development of monofilament synthetic sutures fabricated from materials such as PTFE and polypropylene. Multifilament sutures allow penetration of bacteria into the inner compartment of the thread, between the filaments, impairing the immunological response of the host as well as facilitating migration of bacteria and

oral fluids along the suture, into the wound, via the wicking phenomenon.[20–23] Compared to synthetic monofilament sutures, natural multifilament sutures accumulate more plaque and have a higher bacterial adherence, induce a more pronounced inflammatory reaction, and display poorer overall healing of the soft tissues.[21–23] Furthermore, wound closure with tissue adhesives such as cyanoacrylate has demonstrated superior healing in the first post-operative week compared to traditional sutures, displaying less clinical signs of inflammation as well as less inflammatory cell infiltration.[24] Periodontal dressings have also been developed to help improve post-operative healing and patient discomfort however their value has been widely questioned and several studies demonstrate that they do not impart any significant benefit.[25–29]

Clinical and patient-centred post-operative outcomes may also be enhanced through modification and evolution of surgical techniques. This trend is well-illustrated by the multitude of connective tissue harvesting techniques that have been developed. From the trap-door (TD) technique to the parallel-incision (PI) technique to the single-incision (SI) technique, to name a few.[30–34] The rationale for development of such techniques is to reduce the number of incisions needed to harvest the graft, thus improving the blood flow to the remaining overlying tissue that remains at the donor site. This reduces the risk of flap necrosis, improving the potential for uneventful healing of the site and a better patient experience.[33] When SI techniques are used, this also allows primary closure of the palatal wound. Primary closure allows a thin, stable clot to form between the tissue margins with little to no local ischemia. Bacterial penetration into the wound is greatly reduced. Blood supply to the site is rapidly restored and there is little to no formation of scar tissue. In the context of healing, this can be described as regeneration as the tissues are essentially restored to their original condition.[35] Secondary wound healing,

conversely, illustrates repair of tissues. The body produces low-grade scar tissue in an attempt to quickly bridge the gap resulting from the injury.[35] These techniques, when applied to tissue-harvesting of the palate, likely have a very minor impact on the long-term clinical outcomes of surgery. However, they do illustrate how refinement of these principles in all aspects of periodontal surgery can improve healing and reduce patient morbidity.

Another step in the evolution of these tenets as applied to surgical techniques can be seen with the introduction of papilla-preserving incision designs, allowing for more predictable primary closure and wound healing. The papilla preservation incision design put forth by Takei in 1985 has since been adapted into the Modified Papilla Preservation Technique (MPPT) and the Simplified Papilla Preservation Technique (SPPT), both commonly employed today.[36–38] Results have demonstrated a trend of better regenerative outcomes associated with papilla preservation techniques.[39,40] Further building on these principles is the concept of minimally invasive surgery, which was introduced into periodontology by Harrel and Rees in 1995.[41] This approach is based on limited flap reflection and gentle handling of the soft and hard tissues. It has continued to evolve, incorporating new materials and instrumentation, all with the objectives of reducing surgical trauma, increasing wound and flap stability, reducing surgical time, attaining primary closure, and minimizing patient discomfort and morbidity.[37,38,42–54] The majority of studies on minimally invasive periodontal surgeries (MIPSs) report results that are comparable to conventional techniques and do not necessarily illustrate that these approaches provide clinically superior outcomes.[55] However, there is a paucity of studies that directly compare the clinical outcomes of MIPSs with those of "traditional" surgical approaches.[55] The evidence also shows that MIPSs do not benefit from the use of biomaterials or membranes, which have shown to

enhance clinical parameters when used with traditional regenerative techniques.[55] This may speak to the impact of the surgical principles, which are inherent in MIPSs. The positive effect of precise primary wound closure, adequate clot stability and maturation, good flap perfusion, and satisfactory space maintenance provided by a minimally invasive technique may overshadow any additional benefit that would be provided by membranes or biomaterials in a conventional surgical approach. Regarding patient-centered outcomes after MIPSs, generally patients report low VAS values for post-operative pain and discomfort.[55] Overall, the development and evolution of MIPSs illustrates the ongoing pursuit to improve patients' experiences and clinical healing following periodontal surgery.

In this pursuit, one area of interest that continues to gain momentum is the use of lasers in the field of periodontology. Experimentation with lasers and their effects on the oral tissues began in the 1960s.[56] By the late 1980s, the FDA had given approval for an Nd:YAG laser specifically designed for dental use.[57] Nd:YAG (neodymium-doped yttrium aluminum garnet) refers to the active medium within the optical cavity of the laser. Generally, lasers are named and differentiated based on the material of their active medium. The different lasers utilized in dentistry include carbon dioxide, Nd:YAG, Er:YAG, argon, Er,Cr:YSGG, and several types of diode lasers.[58,59] A defining characteristic of lasers is monochromaticity, meaning they emit one specific wavelength of light.[58,60] The wavelength of the laser determines the degree of scattering, tissue penetration, and the amount of energy absorbed by the tissues.[58] Thus it is wavelength that dictates how the laser will interact with its target tissues and the subsequent effects it will have. As such, lasers have been implemented for a variety of uses in dentistry, including treatment of dentinal hypersensitivity, aphthous ulcers, herpetic lesions, BRONJ and TMDs, osseous

resection, bacterial elimination, root and implant debridement, analgesia, periodontal regeneration, soft tissue incisions, as well as for restorative and endodontic purposes.[58–64]

The origins of laser treatment can be traced back to the use of sunlight for therapeutic purposes, which dates back several centuries. It has long been recognized that light can have a positive stimulatory effect on the growth and metabolism of organisms and their cells. Consequently, it was speculated whether or not lasers could impart similarly beneficial stimulation. This application of lasers was first in developed in 1967 by Endre Mester and has since been called by many names: low-level laser therapy, cold laser, low-intensity laser therapy, biostimulation, soft laser, low-power laser therapy, photobiomodulation, photobiostimulation, and perhaps others.[65,66] A nomenclature consensus meeting of the North American Association for Light Therapy and the World Association for Laser Therapy in 2014 has deemed photobiomodulation (PBM) to be the appropriate term. PBM has been defined as "a form of light therapy that utilizes non-ionizing forms of light sources, including lasers, LEDs, and broadband light, in the visible and infrared spectrum. It is a nonthermal process involving endogenous chromophores eliciting photophysical and photochemical events at various biological scales. This process results in beneficial therapeutic outcomes including but not limited to the alleviation of pain or inflammation, immunomodulation, and promotion of wound healing and tissue regeneration."[65]

PBM has been compared to photosynthesis in plants, whereby the light is absorbed and exerts chemical changes within the tissues.[67] However, the exact mechanism of action by which PBM exerts its therapeutic effects on healing is still not well established. The most widely accepted explanation has been the "CCO theory."[68] Within mammalian tissues, there are three major

photoacceptor molecule: hemoglobin, myoglobin, and cytochrome c oxidase (CCO).[69] CCO is a protein located in the inner membrane of eukaryotic cells and it is the terminal enzyme of the respiratory electron transport chain. CCO catalyzes the reduction of O_2 to H_20 and utilizes the energy of the redox reaction to transport protons across the membrane.[70] It has been proposed that PBM stimulates CCO, initiating secondary cell-signaling pathways, leading to increased ATP production and energy metabolism, reduced oxidative stress, and improved cell viability.[69,71–77] It is speculated that this occurs due to the removal of nitric oxide (NO) molecules that are bound to the CCO protein. NO competes for oxygen-binding sites on CCO and can displace O_2, inhibiting cellular respiration and decreasing ATP production. PBM may elicit its positive effects on wound healing by dissociating NO from CCO, reversing the mitochondrial inhibition of respiration and shifting the overall redox potential of the cell toward greater oxidation and increased generation of reactive oxygen species (ROS).[69,73] Redox-sensitive transcription factors are then activated, resulting in the expression of gene products that prevent apoptosis, modulate the inflammatory response, and stimulate fibroblast proliferation, collagen synthesis, angiogenesis, and tissue repair.[67,69,77–81]

There may be even more evidence to support the success of PBM in alleviating pain, but this mechanism of action is also more poorly understood than its effect on healing.[82–84] There are several hypotheses on how PBM reduces pain. Theories include modulation of nerve conduction and stimulation of endogenous opioid release, among others. The most plausible and well-supported theory proposes that PBM has an inhibitory effect on prostaglandins and pro-inflammatory cytokines, such as PGE_2, TNF-α, and IL-1β. These inflammatory mediators function either directly by activating peripheral nociceptors and causing spontaneous pain or

indirectly via stimulation of additional pain-inducing agents, such as bradykinin, which has shown to induce hyperalgesia when experimentally administered to humans.[84,85] However, the exact processes explaining how PBM could elicit this effect are not well established.

PBM may be administered with various different light sources, be they lasers, LEDs, or others. As research into this form of therapy progressed, a large predominance of studies seemed to be investigating its application with diode lasers.[86–93] Diode lasers can produce a spectrum of different wavelengths, depending on their active medium. As previously stated, a laser's interaction with tissues and the subsequent effects that are produced are dictated by its wavelength. Most of the diode lasers used in PBM research produce wavelengths in the domain of 810-1064 nm, which falls into the red and near-infrared (NIR) "optical window." Thus it is this range of wavelengths for which there is the majority of supporting literature demonstrating the effects of PBM.[62,94] However, research into PBM has ventured outside this range and investigated the potential effects Nd:YAG, Er:YAG, and CO_2 lasers with wavelengths of 1064 nm, 2940 nm, and 10600 nm, respectively.[62,69,77,79–81,95–100]

Studies into PBM with Er:YAG lasers have primarily consisted of *in vitro* experimentation, with a scarcity of research investigating its effects *in vivo*.[77,79–81,96,101] To this author's knowledge, there have been no human clinical trials assessing the effects of PBM on post-operative pain and healing using an Er:YAG laser. The aim of this pilot study was to investigate if PBM with Er:YAG laser treatment enhanced the post-operative patient experience and/or wound healing of the palatal donor site following subepithelial connective tissue graft surgery. The findings of this

study will help guide future research on this topic and provide insight into the feasibility of conducting larger clinical studies.

Materials and Methods

Subjects

Eleven systemically healthy patients of the Graduate Periodontics Clinic at the University of Manitoba who required a connective tissue graft (CTG) were recruited to participate. Informed consent was obtained from all patients. They received initial periodontal examination and treatment, if necessary, before proceeding with surgery. To be eligible for the study, participants had to be classified as ASA I or ASA II and have a Full Mouth Plaque Score (FMPS) <20% and a Full Mouth Bleeding Score (FMBS) <20%. This threshold was established based on the precedent set by previously conducted, similarly designed studies.[102–105] Patients were excluded from the study if they had any contraindications for periodontal surgery, were taking anticoagulants or corticosteroids, or smoked >10 cigarettes per day. Patients were assigned to one of two treatment groups using a computer-generated randomization table, either the Test group who received PBM or the Control group who received no additional treatment. The same randomization program was also used to evenly distribute and assign which resident would conduct which follow-up appointments. This study was independently reviewed and approved by the Biomedical Research Ethics Board of the University of Manitoba.

Surgical Procedure

Surgery was performed by one of four periodontal residents (BW, JC, CS, JV). The surgeons were calibrated on the study protocol in a training meeting prior to commencement of the study.

Each resident was informed at the time of surgery if their patient was in the Test group or Control group. The appropriate technique used for root coverage was dictated by the clinical situation and was at the discretion of the surgeon, as it did not impact the outcomes of the study. Prior to harvesting the CTG, transgingival probing was performed at the donor site to measure the initial thickness of the palatal tissue. The graft was then harvested using the Single-Incision technique described in detail in previous studies.[33] The length of the incision and the length and width of the graft were measured to the nearest 0.5 mm with a periodontal probe. The thickness of the graft and the thickness of the remaining palatal flap were measured to the nearest 0.1 mm with calipers. Hemostasis of the palatal wound was established with gauze and manual pressure. At the end of the surgery, the Test group received PBM with an Er:YAG laser and the Control group received sham laser treatment. The palatal incision was closed with cyanoacrylate.

Laser Protocol

In the Test group, irradiation was performed with an Er:YAG (Morita AdvErL Evo) laser that emitted a 2940 nm wavelength with an energy output of 80 mJ and at pulse rate of 25 Hz. PBM was administered for 30 seconds in a continuously sweeping motion over the site of graft harvest at a distance of 10 mm from the tissue. In the Control group, the same procedure was repeated with the exception that the "Ready" button on the laser unit was not activated and thus no laser was emitted from the handpiece. In both the Test and Control groups, during all sessions of laser or sham treatment, the manufacturer-recommended safety precautions were stringently adhered to. The clinician, patient, and anyone else inside the operatory wore protective glasses designated for use with Er:YAG lasers. Whenever possible, the individuals present within the operatory were limited to the patient and those directly involved with the procedure (i.e. surgeon and

assistant). The activated laser was never directed anywhere other than the intended site of treatment.

Post-Operative Instructions

After the surgery, all participants were administered 2 g of amoxicillin and 400 mg of ibuprofen to be taken immediately. In cases of allergy or sensitivity to these medications, 600 mg of clindamycin would be given instead of amoxicillin and 1 g of acetaminophen instead of ibuprofen. Patients were instructed to apply a cold pack alternating 15 minutes on and 15 minutes off for the first 24 hours, rinse twice daily with 0.12% chlorhexidine, avoid the surgical sites during oral hygiene, avoid hot foods and liquids as well as hard and crunchy foods for 24 hours after surgery, refrain from smoking for two weeks, and avoid sucking on straws or spitting. Participants were provided with a bag of 30 200 mg ibuprofen tablets (or 500 mg acetaminophen tablets), instructed to take them as needed, and bring the remaining tablets to their one-week follow-up appointment. They were also given a visual analogue scale (VAS) questionnaire to rate their post-operative pain from 0 (no pain) to 10 (unbearable pain), prior to intake of any analgesics, for each night of the first post-operative week.

Clinical Evaluations

Clinicians performing the one- and two-week follow-ups were blinded to which treatment group the patient belonged to. At the one-week post-operative appointment, participants returned their completed VAS questionnaires and any remaining ibuprofen tablets. Then they were asked to fill out a modified Oral Impact on Daily Performance (OIDP) questionnaire to assess the effect of the palatal wound on their oral health related quality of life (OHQoL) during the first week.

The OIDP has been translated into several languages and validated across many different populations.[106–112] It asked the participants to gauge the degree to which the palatal wound had disrupted ten different aspects of their daily life. For each category, subjects rated the frequency of disturbances from 0 (never) to 10 (constantly) and, when applicable, the usual severity of the disturbance from 0 (barely noticeable) to 10 (extremely disruptive). Next, the clinician assessed the donor site according to a modified early-wound healing index (MEHI) (Table 1) proposed by Fickl[113] (a variation of the EHI originally conceived by Wachtel)[114] as well as the healing index of Landry, Turnbull and Howley (HI)[115] (Table 2). It is important to note that the MEHI is a 5-point scale from 1 (complete primary wound closure) to 5 (complete flap necrosis) while the HI is also a 5-point scale, but its grading system runs in the opposite direction from 5 (excellent healing) to 1 (very poor healing). Assessment of the palatal wound via the MEHI and HI was repeated at the two-week follow-up. Sutures were also removed at this appointment. Participants returned at six weeks post-operatively for a follow-up with the original surgeon. The palate was anesthetized and the tissue thickness at the donor site was again measured with transgingival probing. Then a tissue sample was harvested with a tissue punch. However, the COVID-19 pandemic precluded the processing of specimens for histological analysis.

Table 1: Modified Early Wound Healing Index (MEHI)

Rating	Description
1	Complete flap closure without fibrin line at the palate
2	Complete flap closure with fibrin line at the palate
3	Complete flap closure with small fibrin clot(s) at the palate
4	Incomplete flap closure with partial necrosis of the palatal tissue (≤50% of the flap is involved)
5	Incomplete flap closure with complete necrosis of the palatal tissue (>50% of the flap is involved)

Table 2: Healing Index (HI) of Landry, Turnbull, and Howley

Rating	Quality	Criteria
1	Very Poor	**Tissue colour:** >50% of gingiva is erythematous **Response to palpation:** bleeding **Granulation tissue:** present **Incision margin:** not epithelialized, with loss of epithelium beyond margins **Suppuration:** present
2	Poor	**Tissue colour:** >50% of gingiva is erythematous **Response to palpation:** bleeding **Granulation tissue:** present **Incision margin:** not epithelialized, with connective tissue exposed **Suppuration:** absent
3	Good	**Tissue colour:** ≥25% and <50% of gingiva is erythematous **Response to palpation:** no bleeding **Granulation tissue:** none **Incision margin:** no connective tissue exposed **Suppuration:** absent
4	Very Good	**Tissue colour:** <25% of gingiva is erythematous **Response to palpation:** no bleeding **Granulation tissue:** none **Incision margin:** no connective tissue exposed **Suppuration:** absent
5	Excellent	**Tissue colour:** pink and healthy **Response to palpation:** no bleeding **Granulation tissue:** none **Incision margin:** no connective tissue exposed **Suppuration:** absent

Statistical Analysis

The primary outcome for this study was the patients' post-operative discomfort from the palatal donor site during the first week following surgery. This was assessed by VAS scores and analgesic consumption. Friedman's two-way ANOVA test was used to compare the change in VAS scores over time within each treatment group. Mann-Whitney U test was used to compare the difference in VAS scores between the two groups at each time point as well as the differences in total analgesic use between the two groups. The secondary outcomes were healing of the donor site and the palatal wound's effect on the patients' OHQoL. Healing was

assessed by MEHI and HI scores and by tissue thickness. Wilcoxon Signed Ranks test was used to compare the change in MEHI and HI scores from week one to week two within each treatment group. Mann-Whitney U test was used to compare the differences in MEHI and HI scores between the treatment groups as well as the differences in tissue thickness at six weeks post-operatively between the groups. OHQoL was assessed by OIDP scores. The differences in OIDP scores between treatment groups was compared with Mann-Whitney U test. The level of significance was established at $p \leq 0.05$. IBM SPSS Statistics for Windows (Version 25.0) was used for analysis.

Results

Participants

Ten patients (seven females and three males; ages 38 to 74 years old) completed the study. Enrollment and participation in this study was stopped earlier than originally planned due to the COVID-19 pandemic. The Test group (n=4) was composed of all females, the Control group (n=6) was three males and three females. The mean age was 62.3 for the Control group and 58.5 for the Test group. There were no statistically significant differences in the ages or in any of the initial clinical measurements between the groups (Table 3). One patient had to be removed from the study due to excessive post-operative bleeding from the donor site, which required sutures for satisfactory wound closure and hemostasis. No other treatment was required. One patient experienced a hematoma at the recipient site, and they were seen for an additional post-operative appointment at three weeks to monitor healing. No other measures were necessary. Two patients were allergic to penicillin and one patient had a sensitivity to ibuprofen. They were instead administered clindamycin and acetaminophen, respectively.

Table 3: Clinical measurements taken during harvesting of CTGs

Measurements (mm)	Control Group (mean)	Test Group (mean)	*p*-value
Pre-Op Palatal Tissue Thickness	3.08	4.00	0.10
Incision Length	18.17	19.25	0.56
CTG Length	15.33	16.50	0.45
CTG Width	6.33	7.75	0.24
CTG Thickness	2.42	1.65	0.65
Palatal Mucosal Flap Thickness	1.50	0.76	0.21

Post-Operative Pain

Comparison of the VAS scores within each group over the first post-operative week did not demonstrate any statistically significant changes over time for either group. Comparison of VAS scores between the two groups at each day during the first post-operative week did not show any statistically significant differences. However, the mean VAS scores for the Test group were consistently higher than the Control group at all time points (Table 4, Fig. 1). The mean number of analgesics taken for the Control group was 6.0 and for the Test group it was 10.5. This difference was not statistically significant.

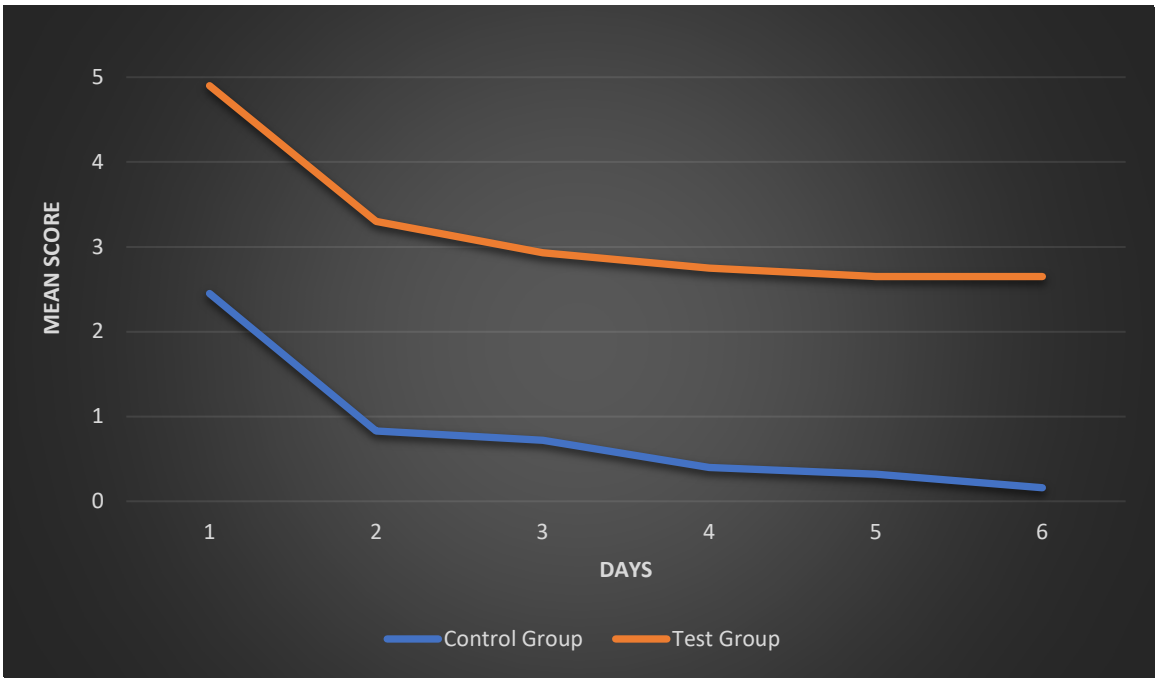
Table 4: Mean VAS scores recorded during the first post-operative week

Day	Control Group (mean)	Test Group (mean)	*p*-value
1	2.45	4.90	0.35
2	0.83	3.30	0.17
3	0.72	2.93	0.17
4	0.40	2.75	0.11
5	0.32	2.65	0.18
6	0.16	2.65	0.09

Oral Health Related Quality of Life (OHQoL)

The results of the OIDP questionnaire are presented in Table 5. Compared to the Control group, the mean frequency of disturbances was significantly higher in the Test group while doing light physical work, going out, sleeping, relaxing, smiling and laughing, and interacting with others.

Figure 1: Mean VAS scores recorded during the first post-operative week



The difference in mean frequency of disturbances was not statistically significant between the two groups for any of the other activities. The difference in mean severity of disturbances between the groups was not statistically significant for any category of the OIDP.

Table 5: Mean values of the OIDP questionnaire recorded at one-week post-operatively

	Frequency (mean)			Severity (mean)		
Oral Health Related Quality of Life	Control Group	Test Group	*p*-value	Control Group	Test Group	*p*-value
Eating Food	3.65	7.52	0.16	2.65	5.25	0.23
Speaking Clearly	1.87	4.73	0.16	0.55	3.28	0.34
Cleaning Teeth	4.22	5.18	0.46	4.98	4.40	1.0
Doing Light Physical Work	0.25	1.30	**0.03**	0.35	0.90	0.40
Going Out (e.g. shopping)	0.38	2.95	**0.02**	0.58	2.55	0.31
Sleeping	1.62	6.20	**0.05**	2.20	3.03	0.86
Relaxing	0.58	4.53	**0.05**	0.80	1.33	0.71
Smiling and Laughing	2.22	7.77	**0.05**	3.20	4.70	0.86
Emotional State or Mood	2.25	2.83	0.66	3.30	1.17	0.29
Interacting with Others	1.00	4.23	**0.05**	1.43	2.90	0.71

Healing

The mean MEHI scores for the Control group were 3.08 and 2.67 at the one- and two-week follow-ups, respectively. The mean MEHI scores at the one- and two-week follow-ups for the Test group were 4.25 and 2.50, respectively. The mean HI scores for the Control group were 2.67 and 2.83 at the one- and two-week follow-ups, respectively. For the Test group, the mean HI scores at the one- and two-week follow-ups were 2.25 and 3.25, respectively. Both healing indices demonstrated improvement from week one to week two within both groups. The amount of change for each group was not statistically significant, however the Test group did demonstrate a greater level of improvement between the two appointments compared to the Control group according to the MEHI and HI (Figs. 2 and 3). There were no statistically significant differences for the mean scores of both healing indices between the two groups at any time point. Comparison of the palatal tissue thickness of the Control group (3.50 mm) and the

Figure 2: Mean MEHI scores at one and two weeks post-operatively

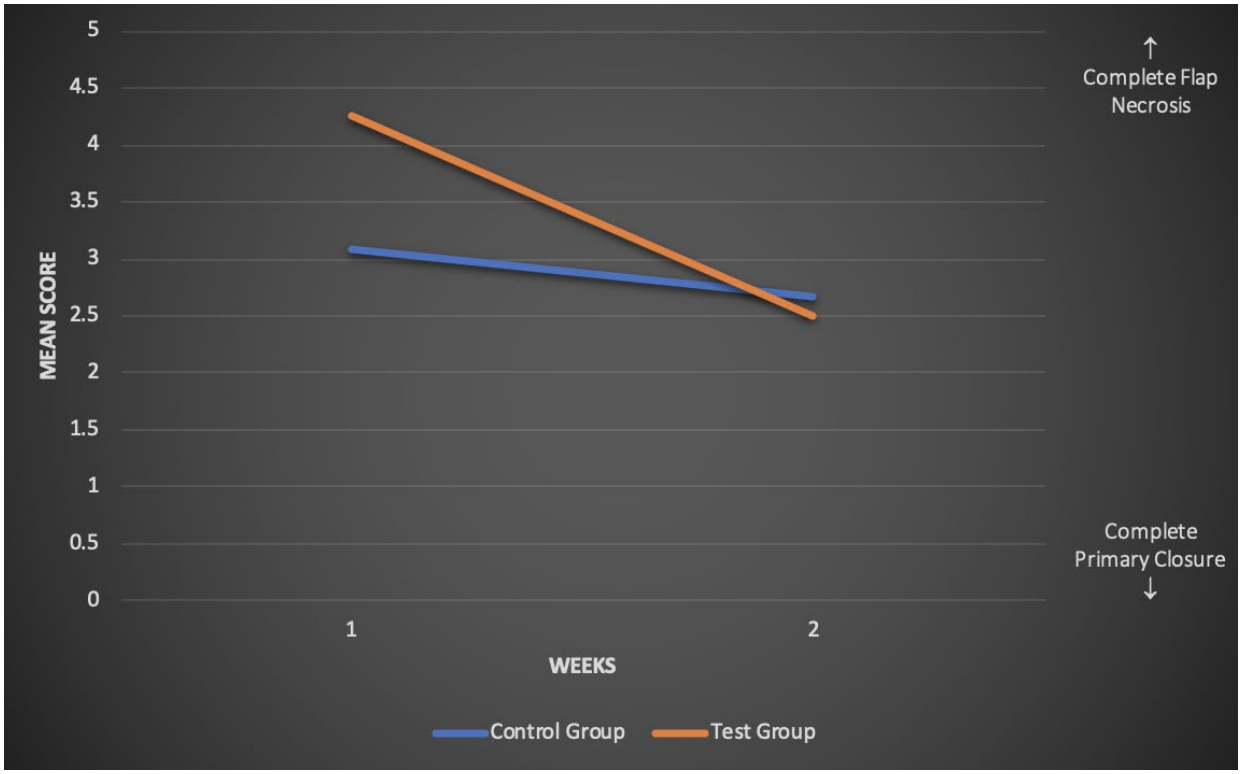
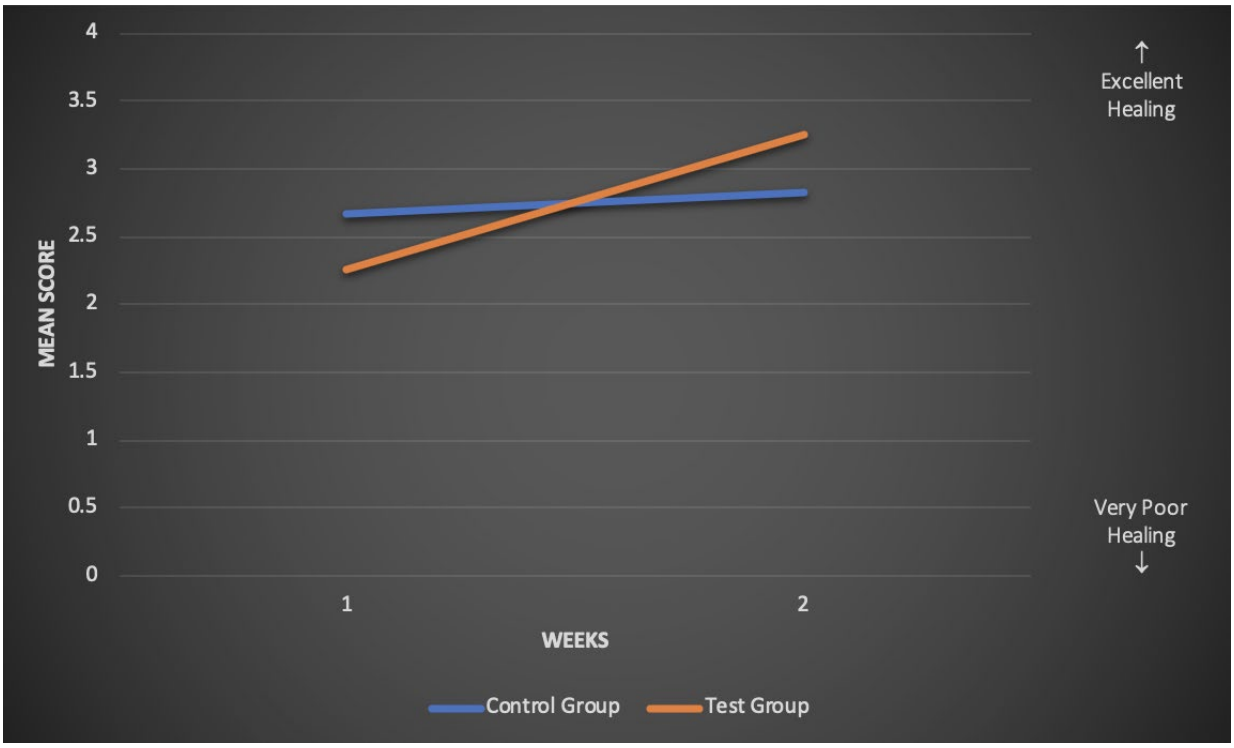


Figure 3: Mean HI scores at one and two weeks post-operatively



Test group (3.00 mm) at the six-week post-operative appointment did not demonstrate a statistically significant difference.

Discussion

This pilot study was the first clinical human trial investigating the effects of PBM with an Er:YAG laser on post-operative pain and intraoral wound healing. This field of study is relatively new and there is far from a consensus regarding the potential benefits, if any, provided by PBM therapy. The sequence of VAS scores over the first post-operative week display an almost identical pattern in both groups, with the highest score on day one, followed by a sharp decline on day two, and then gradually levelling off over the rest of the week (Fig. 1). The PBM-treated group demonstrated a mean VAS score of approximately 2.2-2.5 points higher than

the Control group on every post-operative day. Also, the relative difference in the mean VAS scores increased throughout the week. On Day 1, the Test group had a mean VAS score that was twice that of the Control group and on Day 6, the Test group reported a mean VAS score that was more than 16 times that of the Control group. However, given that the change in the absolute values of the differences in their scores is relatively small and the limited sample size of this pilot study, the importance of this increasing relative difference should be interpreted with caution. Statistically comparing the VAS scores of the subjects did not reveal any significant change in self-reported pain over the course of the first post-operative week or any significant difference between the groups. This is in opposition to several studies that report positive effects on post-operative pain following laser treatment.

A meta-analysis of nine human trials by Enwemeka et al[90] demonstrated that PBM had a positive analgesic effect, with the most significant results produced by a wavelength of 830 nm. However, there was significant heterogeneity between the included studies. There was no mention of how specific factors such as the type of wound/condition being treated or how different laser parameters (other than wavelength) affected the results. Heidari et al[116] investigated PBM therapy with a 940-nm diode laser on post-operative pain following MWF surgery. They found significantly less pain was experienced during the first post-operative week for the subjects receiving PBM, as indicated by lower VAS scores on days two through seven and reduced intake of analgesics on days three through seven. Sanz-Moliner[93] et al reported similar results using 810-nm diode laser. Subjects receiving PBM in conjunction with MWF surgery reported lower VAS scores and reduced analgesic intake compared to the control group.

The analgesic effect of PBM has not been universally observed, however. Heidari et al[86] compared post-operative pain following free gingival graft surgery between those receiving PBM with 660-nm diode laser and those receiving placebo treatment. That study showed no significant differences in VAS scores or analgesic consumption on days one through 12. The PBM group actually reported significantly greater discomfort from the donor site than the placebo group during the first three hours post-operatively. Dias et al[117] compared the effects of 660-nm diode laser on the palatal donor site after harvesting of CTGs. No statistically significant differences were found between the test and control groups when comparing post-operative analgesic usage or discomfort to a blast of air. In a similar study, da Silva Neves et al[118] evaluated the effect of PBM on CTG donor sites with a diode laser at two different power settings (30 J/cm^2 and 60 J/cm^2). VAS scores between the two test groups and a control group showed no significant differences in discomfort to air blast or in consumption of painkillers.

The inconsistent findings regarding the efficacy of laser treatment for pain relief are likely a result of the difficulty with objectively quantifying discomfort. Pain is experienced uniquely by different individuals. Not only is pain affected by many different factors, how these factors are perceived and tolerated by different people can vary widely. Pradhan et al[119] evaluated the impact of several surgical and patient factors on the perception of pain following different periodontal surgeries. The quantity of local anesthetic and the sex of the patient were reported to be the only significant factors. Duration of the surgery, age of the patient, site of the procedure, and use of periodontal dressing had no significant impact. While Curtis et al[120] found post-operative pain to only be significantly associated with the type and duration of the surgery. Neither sex of the patient nor use of periodontal dressing showed to be a statistically significant

factor. Comparing different gingival augmentation procedures, Griffin et al[121] found the duration of the surgery to be the most consistently significant factor associated with post-operative pain while age and sex of the patient had no significant impact. The effect of other aspects, such as the site of the procedure and smoking habit, varied depending on the type of surgery.

It is also important to note that the emotional and psychological state of the patient have a pronounced influence on pain perception. Beaudette et al[16] evaluated the relationship between pre-surgical anxiety and post-surgical pain following implant and mucogingival surgeries. Patients consistently anticipated more pain than they actually experienced and those who anticipated higher amounts of pain were more likely to experience more pain than those who anticipated low levels of pain. In addition, they also found that patient age was significantly associated with pain, but that the type of surgery performed and the sex of the patient were not. A study by Eli et al[14] demonstrated a significant correlation between patients' anxiety levels and their reported pain levels associated with implant surgery. Further illustrating the complexity of pain, this study also found a significant relationship between anxiety and gender but no significant association between pain and gender. Fardal et al[122] reported similar observations, with those patients who had the highest pre-treatment anxiety also having significantly higher levels of pain than those with the lowest levels of anxiety.

It is easy to see why thus far it has been difficult to get consistent findings regarding PBM, a relatively new field of study that is still not well understood, and its potential role in pain management. Another important factor to consider that could contribute to the lack of significant differences in reported pain between the Test and Control groups in this study is that

CTG surgeries are generally well-tolerated by patients. Palatal donor sites do not typically produce severe discomfort, especially with a minimally invasive approach such as the SI technique. Del Pizzo et al[102] compared the healing of palatal tissue following three procedures: harvesting of a CTG with the SI or the TD techniques and harvesting of an FGG. CTG-harvesting procedures showed significantly less post-operative discomfort in the first week compared to FGGs, with only 50% of both the SI and TD groups reporting discomfort while 100% of the FGG group reported discomfort. Fickl et al[113] also assessed healing and patient morbidity of the SI and TD techniques. Both procedures reported low mean VAS scores following surgery. 2.16±1.18 and 2.67±2.28 for the SI and TD groups, respectively, with no significant difference between the two. Also, the SI group consumed significantly less painkillers than the TD group. Findings from Wessel and Tatakis[123] reaffirm the minimal patient morbidity associated with harvesting of a CTG. At three days post-operatively, 50% of CTG patients reported pain from the palate compared to 90% of FGG patients. To accurately assess pain relief at CTG donor sites, it may require methods that are more precise than a VAS questionnaire or a significant increase in sample size to increase the power of that test.

To the knowledge of this author, this is the first study to expand the investigation of patient-centred outcomes immediately following periodontal surgery to include not just post-operative pain but also how the procedure affected the patient's OHQoL during this recovery period. As such, there are no studies with which to directly compare the results of the OIDP. The short-term post-operative OHQoL was studied by McGrath et al[124] following extraction of third molars. The questionnaire they used for their assessment included the Oral Health Impact Profile (OHIP-14) and the UK Oral Health-Related Quality of Life measure (OHQoL-UK). Both

these surveys indicated a significant decrease in OHQoL at one week post-operatively, compared to pre-operative ratings. By one month, the subjects' self-rated OHQoL had surpassed their pre-operative scores and continued to improve throughout six months. A similar study by Shugars et al[125] utilized the OHIP-14 and the Health-Related Quality of Life instrument (HRQOL) to evaluate the effect of third molar extractions on patients' OHQoL. Results indicated that patients were most affected on the first post-surgical day, followed by continued improvement until most categories reached pre-operative levels by day 14. For the day after surgery, more than 70% of subjects reported a lot of trouble opening their mouths, eating their regular diet, and chewing while more than 50% said it caused a lot of trouble to their social life and recreation. These studies help provide some context regarding how oral surgery in general affects patients' well-being, beyond the scope of just pain, during the short-term post-operative period.

The findings from our study allow a more in-depth exploration of how patients' day-to-day functioning is affected during the post-operative period following periodontal surgery. The results of this study also provide insight into whether the OHQoL during this period is improved by PBM therapy. The lack of any significant differences between the groups regarding the severity of disturbances likely reflects the fact that the palatal wound is not generally reported to cause a great deal of post-operative discomfort, as has already been discussed. The significantly higher frequency of disturbances during certain activities (light work, going out, sleeping, smiling, and interacting) for the Test group is unclear. It could be postulated that if PBM does provide any level of analgesia, it is not enough to be perceived by the patient. However, if it is a patient's natural tendency to try to protect an area of injury, such as the donor site, a negligible improvement in comfort level may be enough to reduce this protective reflex to the point that the

patient notices the wound more often but not with any appreciable difference in intensity. This theory could also help explain why the activities that had the greatest difference in severity of disturbances were almost mutually exclusive of those that demonstrated a significantly greater frequency of disturbances. Of the four categories (Eating, Speaking, Going Out, and Emotional State) that had the most significantly greater severity in the Test group, three of them (Eating, Speaking, and Emotional State) were separate from those that had a significantly higher frequency. While this could help clarify the relationship between the scores of frequency and severity on the OIDP, the reason for the consistently higher severity scores in the Test group remains uncertain.

Neither group in this study demonstrated a statistically significant change in MEHI or HI scores from the one-week to the two-week post-op appointment, nor were there any statistically significant differences between the groups at any time point. This is in agreement with Almeida et al[126] who evaluated the effect of 780-nm and 660-nm diode lasers on the healing of FGGs. This split-mouth study compared post-operative photographs of test and control sites. It too found no significant differences in healing between the two groups. Damante et al[127] similarly conducted a split-mouth study to evaluate the effects of PBM with a 670-nm diode laser on soft tissue healing following gingivoplasty. Histological analysis compared the width and composition of epithelium and connective tissue, as well as the density of inflammatory cells present. This comparison found no significant difference between test and control sites.

While no statistically significant differences were found in the healing, the Test group did demonstrate a trend of greater improvement between the two appointments compared to the

Control group (Figs. 2 and 3). This trend is in accordance with the findings of a split-mouth pilot study by Ozcelik et al[88] which compared the healing of gingivectomies and gingivoplasties, half the sites were treated with a 588-nm diode laser and half acted as controls. Patients were followed for 15 days post-operatively and surgical sites that were treated with PBM demonstrated faster epithelization at all time points.

Other human clinical trials have shown mixed results regarding the influence of PBM on the healing of periodontal soft tissues. While they provide some initial insight and basic understanding into the concept of PBM and its potential applications, their results cannot be directly compared to this study as they have primarily used diode lasers and our study implemented the use of an Er:YAG laser.

A major challenge for a pilot study such as this is the lack of similar studies with which to build off of and design the protocol. As this is the first human clinical trial attempting to use PBM with an Er:YAG laser to improve the healing of intraoral soft tissues, the rationale for this investigation was based on findings from *in vitro* studies. Pourzarandian et al[79] and Ogita et al[80] exposed cultured human gingival fibroblasts to PBM treatment with an Er:YAG laser. Three days after irradiation, both studies found significantly increased cellular proliferation of the laser-treated fibroblasts compared to that of the control group. Additionally, Ogita et al[80] tested for a potential cytotoxic effect of PBM by evaluating any changes in extracellular levels of lactate dehydrogenase (LDH), which is released from damaged cells. The test group showed no significant change in LDH, indicating minimal damage to exposed cells. Kong et al[77] confirmed

the findings of these two previous studies and expanded on them by demonstrating that ATP production was significantly elevated 30 minutes following laser treatment.

Certain limitations are common to a clinical pilot study of this nature. Foremost among these limitations is the small sample size, as was the case for this study. This limits the statistical power of the research, makes it more difficult to detect significance in our outcomes, and hinders the external validity of the findings. Despite randomization of the participants, there was an imbalance in the distribution of males and females between the two groups. It is plausible that this could influence the results, as males and females have shown to experience and report pain differently.[128,129] Another limitation lies in the inherent variability of the protocol, with regards to differences in the dimensions of the harvested tissue and the thickness of the remaining mucosal flap, which was dictated by the requirements of each individual surgery. While the clinical measurements of the donor sites presented no significant differences between the groups, one must consider how this lack of standardization could confound the relationship between the variables being investigated. Furthermore, the procedures were performed by four different clinicians, introducing another element of variability into the surgical protocol. Thus, the results of this study should be interpreted with caution.

The merit of a pilot study can be primarily accredited to its role as a preliminary step in a developing field of research. It provides information on the viability of conducting larger studies and imparts guidance for the methodology of future research. It is recommended that larger clinical studies be conducted on the applications of PBM, specifically with the use of Er:YAG lasers. Histological evaluations are planned for future investigations and will be beneficial to

gain a better understanding of the mechanism of action of PBM and the effects it has on different cell types. With a larger sample size, it would also be advisable to have multiple treatment groups with which to compare different parameters of the laser therapy (power, pulse frequency, exposure time, etc) so as to assess how these different factors influence treatment outcomes. Lastly, as this study aimed at evaluating the analgesic effects of PBM therapy on the palatal wound following CTG harvesting, which has not typically shown to cause significant post-operative pain, investigating a procedure that causes more discomfort would make it less complex to accurately measure the treatment effect.

Conclusion

Within its limitations, the findings of this pilot study did not demonstrate that any significant analgesic effect or clinically visible improvement in healing was provided by PBM therapy with an Er:YAG laser. Harvesting of CTGs from the palate remains a predictable procedure with a relatively low risk of complications and minimal patient morbidity. The PBM-treated group did report more frequent disruptions of certain day-to-day activities, specifically doing light work, going out, sleeping, smiling, and interacting with others. The Test group also exhibited a trend of more appreciable improvement in healing between the first and second post-operative weeks. However, this finding did not reach the level of significance and cannot be extrapolated to the general population. Further research on this topic is recommended.

Literature Cited

1. Amler MH. The time sequence of tissue regeneration in human extraction wounds. *Oral Surgery, Oral Med Oral Pathol*. 1969;27(3):309-318. doi:10.1016/0030-4220(69)90357-0
2. Engler WO, Ramfjord SP, Hiniker JJ. Healing Following Simple Gingivectomy. A Tritiated Thymidine Radioautographic Study. I. Epithelialization. *J Periodontol*. 1966;37(4):298-308. doi:10.1902/jop.1966.37.4.298
3. Nobuto T, Imai H, Yamaoka A. Microvascularization of the Free Gingival Autograft. *J Periodontol*. 1988;59(10):639-646. doi:10.1902/jop.1988.59.10.639
4. Wilderman MN, Pennel BM, King K, Barron JM. Histogenesis of Repair Following Osseous Surgery. *J Periodontol*. 1970;41(10):551-565. doi:10.1902/jop.1970.41.10.551
5. Wilderman MN, Wentz FM. Repair of a Dentogingival Defect with a Pedicle Flap. *J Periodontol*. 1965;36:218-231. doi:10.1902/jop.1965.36.3.218
6. Berglundh T, Abrahamsson I, Lang NP, Lindhe J, Tord Berglundh OD. *De Novo Alveolar Bone Formation Adjacent to Endosseous Implants A Model Study in the Dog*.; 2003.
7. Caffesse RG, Ramfjord SP, Nasjleti CE. Reverse Bevel Periodontal Flaps in Monkeys. *J Periodontol*. 1968;39(4):219-235. doi:10.1902/jop.1968.39.4.219
8. Gordon HP, Sullivan HC, Atkins JH. Free autogenous gingival grafts. II. Supplemental findings--histology of the graft site. *Periodontics*. 1968;6(3):130-133. http://www.ncbi.nlm.nih.gov/pubmed/5241945.
9. Wikesjö UME, Crigger M, Nilvéus R, Selvig KA. Early Healing Events at the Dentin-Connective Tissue Interface. Light and Transmission Electron Microscopy Observations. *J Periodontol*. 1991;62(1):5-14. doi:10.1902/jop.1991.62.1.5
10. Susin C, Fiorini T, Lee J, De Stefano JA, Dickinson DP, Wikesjö UME. Wound healing

following surgical and regenerative periodontal therapy. *Periodontol 2000*. 2015;68(1):83-98. doi:10.1111/prd.12057

11. Wikesjö UME, Nilvéus RE, Selvig KA. Significance of Early Healing Events on Periodontal Repair: A Review. *J Periodontol*. 1992;63(3):158-165. doi:10.1902/jop.1992.63.3.158

12. Lindhe J, Lang NP. *Clinical Periodontology and Implant Dentistry*. 6th ed. John Wiley & Sons, Ltd; 2015.

13. Mei CC, Lee FY, Yeh HC. Assessment of pain perception following periodontal and implant surgeries. *J Clin Periodontol*. 2016;43(12):1151-1159. doi:10.1111/jcpe.12618

14. Eli I, Schwartz-Arad D, Baht R, Ben-Tuvim H. Effect of anxiety on the experience of pain in implant insertion. *Clin Oral Impl Res*. 2003;14:115-118.

15. Burkhardt R, Hämmerle CHF, Lang NP. Self-reported pain perception of patients after mucosal graft harvesting in the palatal area. *J Clin Periodontol*. 2015;42(3):281-287. doi:10.1111/jcpe.12357

16. Beaudette JR, Fritz PC, Sullivan PJ, Piccini A, Ward WE. Investigation of factors that influence pain experienced and the use of pain medication following periodontal surgery. *J Clin Periodontol*. 2018;45(5):578-585. doi:10.1111/jcpe.12885

17. Mendell LM, Albers KM, Davis BM. *Neurotrophins, Nociceptors, and Pain*. Vol 45.; 1999.

18. Waldmann R, Champigny G, Bassilana F, Heurteaux C, Lazdunski M. A proton-gated cation channel involved in acid-sensing. *Nature*. 1997;386(6621):173-177.

19. Waldmann R, Champigny G, Lingueglia E, De Weille JR, Heurteaux C, Lazdunski M. H+-Gated Cation Channels. *Ann N Y Acad Sci*. 1999;868:67-76.

20. Burkhardt R, Lang NP. Influence of suturing on wound healing. *Periodontol 2000*. 2015;68(1):270-281. doi:10.1111/prd.12078

21. Dragovic M, Pejovic M, Stepic J, et al. Comparison of four different suture materials in respect to oral wound healing, microbial colonization, tissue reaction and clinical features—randomized clinical study. *Clin Oral Investig*. 2019. doi:10.1007/s00784-019-03034-4

22. Selvig KA, Biagiotti GR, Leknes KN, Wikesjö UM. Oral tissue reactions to suture materials. *Int J Periodontics Restorative Dent*. 1998;18(5):474-487. doi:10.11607/prd.00.0285

23. Leknes KN, Røynstrand IT, Selvig KA. Human Gingival Tissue Reactions to Silk and Expanded Polytetrafluoroethylene Sutures. *J Periodontol*. 2005;76(1):34-42. doi:10.1902/jop.2005.76.1.34

24. Vastani A, Maria A. Healing of intraoral wounds closed using silk sutures and isoamyl 2-cyanoacrylate glue: A comparative clinical and histologic study. *J Oral Maxillofac Surg*. 2013;71(2):241-248. doi:10.1016/j.joms.2012.08.032

25. Soheilifar S, Bidgoli M, Faradmal J, Soheilifar S, Bidgoli M. *Effect of Periodontal Dressing on Wound Healing and Patient Satisfaction Following Periodontal Flap Surgery*. Vol 12.; 2015. www.jdt.tums.ac.ir.

26. Jones TM, Cassingham RJ. Comparison of Healing Following Periodontal Surgery With and Without Dressings in Humans. *J Periodontol*. 1979;50(8):387-393. doi:10.1902/jop.1979.50.8.387

27. Allen DR, Caffesse RG. Comparison of Results Following Modified Widman Flap Surgery With and Without Surgical Dressing. *J Periodontol*. 1983;54(8):470-475.

doi:10.1902/jop.1983.54.8.470

28. Checchi L, Trombelli L. Postoperative Pain and Discomfort With and Without Periodontal Dressing in Conjunction With 0.2% Chlorhexidine Mouthwash After Apically Positioned Flap Procedure. *J Periodontol*. 1993;64(12):1238-1242. doi:10.1902/jop.1993.64.12.1238
29. Sachs HA, Famoush A, Checchi L, Joseph CE. Current Status of Periodontal Dressings. *J Periodontol*. 1984;55(12):689-696. doi:10.1902/jop.1984.55.12.689
30. Edel A. Clinical Evaluation of Free Connective Tissue Grafts Used to Increase the Width of Keratinized Gingiva. *J Clin Periodontol*. 1974;1:185-196.
31. Harris RJ. The Connective Tissue and Partial Thickness Double Pedicle Graft: A Predictable Method of Obtaining Root Coverage. *J Periodontol*. 1992;63(5):477-486. doi:10.1902/jop.1992.63.5.477
32. Harris RJ. A Comparison of Two Techniques for Obtaining a Connective Tissue Graft From the Palate. *Int J Periodont Rest Dent*. 1997;17(3):261-271.
33. Hurzeler MB, Weng D. A Single-Incision Technique to Harvest Subepithelial Connective Tissue Grafts from the Palate. *Int J Periodontics Restorative Dent*. 1999;19(3):279-287.
34. Lorenzana ER, Allen EP. The single-incision palatal harvest technique: a strategy for esthetics and patient comfort. *Int J Periodontics Restorative Dent*. 2000;20(3):297-305. doi:10.1017/CBO9781107415324.004
35. Zuhr O, Akakpo DL, Hürzeler M. Wound closure and wound healing. Suture techniques in contemporary periodontal and implant surgery: Interactions, requirements, and practical considerations. *Quintessence Int (Berl)*. 2017;48(8):647-660. doi:10.3290/j.qi.a38706
36. Takei HH, Han TJ, Carranza FAJ, Kenney EB, Lekovic V. Flap technique for periodontal bone implants. Papilla preservation technique. *J Periodontol*. 1985;56(4):204-210.

doi:10.1902/jop.1985.56.4.204

37. Cortellini P, Prato GP, Tonetti MS. The Modified Papilla Preservation Technique. A New Surgical Approach for Interproximal Regenerative Procedures. *J Periodontol*. 1995;66(4):261-266. doi:10.1902/jop.1995.66.4.261

38. Cortellini P, Prato GP, Tonetti MS. The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. *Int J Periodontics Restorative Dent*. 1999;19(6):589-599.

39. Murphy KG, Gunsolley JC. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Ann Periodontol*. 2003;8(1):266-302. doi:10.1902/annals.2003.8.1.266

40. Graziani F, Gennai S, Cei S, et al. Clinical performance of access flap surgery in the treatment of the intrabony defect. A systematic review and meta-analysis of randomized clinical trials. *J Clin Periodontol*. 2012;39(2):145-156. doi:10.1111/j.1600-051X.2011.01815.x

41. Harrel SK, Rees TD. Granulation tissue removal in routine and minimally invasive procedures. *Compend Contin Educ Dent*. 1995;16(9):960, 962, 964 passim.

42. Cortellini P, Tonetti MS, Lang NP, et al. The Simplified Papilla Preservation Flap in the Regenerative Treatment of Deep Intrabony Defects: Clinical Outcomes and Postoperative Morbidity. *J Periodontol*. 2001;72(12):1702-1712. doi:10.1902/jop.2001.72.12.1702

43. Cortellini P, Nieri M, Pini Prato G, Tonetti MS. Single minimally invasive surgical technique with an enamel matrix derivative to treat multiple adjacent intra-bony defects: Clinical outcomes and patient morbidity. *J Clin Periodontol*. 2008;35(7):605-613. doi:10.1111/j.1600-051X.2008.01242.x

44. Cortellini P, Pini-Prato G, Nieri M, Tonetti MS. Minimally invasive surgical technique and enamel matrix derivative in intrabony defects: 2. Factors associated with healing outcomes. *Int J Periodontics Restorative Dent*. 2009;29(3):257-265. doi:10.11607/prd.00.0861

45. Cortellini P, Tonetti MS. Improved wound stability with a modified minimally invasive surgical technique in the regenerative treatment of isolated interdental intrabony defects. *J Clin Periodontol*. 2009;36(2):157-163. doi:10.1111/j.1600-051X.2008.01352.x

46. Cortellini P, Tonetti MS. Microsurgical Approach to Periodontal Regeneration. Initial Evaluation in a Case Cohort. *J Periodontol*. 2001;72(4):559-569. doi:10.1902/jop.2001.72.4.559

47. Harrel SK, Abraham CM, Rivera-Hidalgo F, Shulman JD, Nunn ME. Videoscope-assisted minimally invasive periodontal surgery (V-MIS). *J Clin Periodontol*. 2014;41(9):900-907. doi:10.1111/jcpe.12294

48. Harrel SK. Videoscope-Assisted Minimally Invasive Periodontal Surgery: One-Year Outcome and Patient Morbidity. *Int J Periodontics Restorative Dent*. 2016;36(3). http://www.quintpub.com.uml.idm.oclc.org/journals/prd/fulltext.php?article_id=16225. Accessed November 12, 2017.

49. Harrel SK, Nunn ME, Abraham CM, Rivera-Hidalgo F, Shulman JD, Tunnell JC. Videoscope Assisted Minimally Invasive Surgery (VMIS): 36-Month Results. *J Periodontol*. 2017;88(6):528-535. doi:10.1902/jop.2017.160705

50. Ghezzi C, Ferrantino L, Bernardini L, Lencioni M, Masiero S. Minimally Invasive Surgical Technique in Periodontal Regeneration: A Randomized Controlled Clinical Trial Pilot Study. *Int J Periodontics Restorative Dent*. 2016;36(4):475-482.

doi:10.11607/prd.2550

51. Mishra A, Avula H, Pathakota KR, Avula J. Efficacy of modified minimally invasive surgical technique in the treatment of human intrabony defects with or without use of rhPDGF-BB gel - A randomized controlled trial. *J Clin Periodontol*. 2013;40(2):172-179. doi:10.1111/jcpe.12030

52. De Bruyckere T, Eghbali A, Younes F, et al. A 5-year prospective study on regenerative periodontal therapy of infrabony defects using minimally invasive surgery and a collagen-enriched bovine-derived xenograft. *Clin Oral Investig*. 2018;22(3):1235-1242. doi:10.1007/s00784-017-2208-x

53. Liu S, Hu B, Zhang Y, Li W, Song J. Minimally invasive surgery combined with regenerative biomaterials in treating intra- bony defects: A meta-analysis. *PLoS One*. 2016;11(1). doi:10.1371/journal.pone.0147001

54. Cortellini P, Tonetti MS. Minimally invasive surgical technique and enamel matrix derivative in intra-bony defects. I: Clinical outcomes and morbidity. *J Clin Periodontol*. 2007;34(12):1082-1088. doi:10.1111/j.1600-051X.2007.01144.x

55. Clementini M, Ambrosi A, Cicciarelli V, De Risi V, de Sanctis M. Clinical performance of minimally invasive periodontal surgery in the treatment of infrabony defects: Systematic review and meta-analysis. *J Clin Periodontol*. 2019;46:1236-1253. doi:10.1111/jcpe.13201

56. Goldman L, Gray JA, Goldman J, Goldman B, Meyer R. EFFECT OF LASER BEAM IMPACTS ON TEETH. *J Am Dent Assoc*. 1965;70:601-606. doi:10.14219/jada.archive.1965.0260

57. Myers TD, Myers WD, Stone RM. First soft tissue study utilizing a pulsed Nd:YAG

dental laser. *Northwest Dent*. 1989;68(2):14-17.

58. Green J, Weiss A, Stern A. Lasers and Radiofrequency Devices in Dentistry. *Dent Clin N Am*. 2011:585-597. doi:10.1016/j.cden.2011.02.017

59. Aoki A, Mizutani K, Schwarz F, et al. Periodontal and peri-implant wound healing following laser therapy. *Periodontol 2000*. 2015;68(1):217-269. doi:10.1111/prd.12080

60. Coluzzi DJ. Fundamentals of dental lasers: science and instruments. *Dent Clin North Am*. 2004;48(4):751-770. doi:10.1016/j.cden.2004.05.003

61. Goyal M, Makkar S, Pasricha S. Low Level Laser Therapy in Dentistry. *Int J Laser Dent*. 2013;3(3):82-88. doi:10.1097/00006534-199005000-00040

62. Pang P, Andreana S, Aoki A, et al. Laser energy in oral soft tissue applications. *J Laser Dent*. 2010;18(3):123-131. http://www.dcinter.com/PDF/Laser_energy_inoral_softtissue.pdf.

63. Weber JBB, Camilotti RS, Ponte ME. Efficacy of laser therapy in the management of bisphosphonate-related osteonecrosis of the jaw (BRONJ): a systematic review. *Lasers Med Sci*. 2016;31(6):1261-1272. doi:10.1007/s10103-016-1929-4

64. Lomke MA. Clinical applications of dental lasers. *Gen Dent*. 2009;57(1):47-59. www.agd.org.

65. Anders JJ, Lanzafame RJ, Arany PR. Low-Level Light/Laser Therapy Versus Photobiomodulation Therapy. *Photomed Laser Surg*. 2015;33(4):183-184. doi:10.1089/pho.2015.9848

66. Gáspár L. Professor Endre Mester, the Father of Photobiomodulation. *J Laser Dent*. 2009;17(3):146-148.

67. Huang YY, Chen ACH, Carroll JD, Hamblin MR. Biphasic dose response in low level

light therapy. *Dose-Response*. 2009;7:358-383. doi:10.2203/dose-response.09-027.Hamblin

68. Serrage H, Heiskanen V, Palin WM, et al. Under the spotlight: Mechanisms of photobiomodulation concentrating on blue and green light. *Photochem Photobiol Sci*. 2019;18:1877-1909. doi:10.1039/c9pp00089e

69. Pandeshwar P, Roa MD, Das R, Shastry SP, Kaul R, Srinivasreddy MB. Photobiomodulation in oral medicine: a review. *J Investig Clin Dent*. 2015;0:1-12. doi:10.1111/jicd.12148

70. Popović DM. Current advances in research of cytochrome c oxidase. *Amino Acids*. 2013;45:1073-1087. doi:10.1007/s00726-013-1585-y

71. Darbar A, Darbar R. Let's talk lasers part 3: Photobiomodulation (PBM). *Aesthetic Dent Today*. 2011;5(4):20-31.

72. Desmet KD, Paz DA, Corry JJ, et al. Clinical and Experimental Applications of NIR-LED Photobiomodulation. *Photomed Laser Surg*. 2006;24(2):121-128. doi:10.1089/pho.2006.24.121

73. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J Photochem Photobiol B Biol*. 1999;49:1-17.

74. Karu TI, Kolyakov SF. Exact Action Spectra for Cellular Responses Relevant to Phototherapy. *Photomed Laser Surg*. 2005;23(4):355-361. https://www.isan.troitsk.ru/dls/publ/Karu.pho.2005.23.355.pdf. Accessed July 1, 2018.

75. 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. *Photomed Laser Surg*. 2016;34(11):1-8. doi:10.1089/pho.2015.4035

76. Quirk BJ, Sannagowdara K, Buchmann E V, 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. *J Clin Orthop trauma*. 2016;7(4):234-241. doi:10.1016/j.jcot.2016.02.009

77. Kong S, Aoki A, Iwasaki K, et al. Biological effects of Er:YAG laser irradiation on the proliferation of primary human gingival fibroblasts. *J Biophotonics*. 2018;11:1-11. doi:10.1002/jbio.201700157

78. de Medeiros ML, Araújo-Filho I, da Silva EMN, 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. doi:10.1007/s10103-016-2080-y

79. Pourzarandian A, Watanabe H, Ruwanpura SMPM, Aoki A, Ishikawa I. Effect of Low-Level Er:YAG Laser Irradiation on Cultured Human Gingival Fibroblasts. *J Periodontol*. 2005;76(2):187-193. doi:10.1902/jop.2005.76.2.187

80. Ogita M, Tsuchida S, Aoki A, et al. Increased cell proliferation and differential protein expression induced by low-level Er:YAG laser irradiation in human gingival fibroblasts: proteomic analysis. *Lasers Med Sci*. 2015;30:1855-1866. doi:10.1007/s10103-014-1691-4

81. Aleksic V, Aoki A, Iwasaki K, et al. Low-level Er: YAG laser irradiation enhances osteoblast proliferation through activation of MAPK/ERK. *Lasers Med Sci*. 2010;25(4):559-569. doi:10.1007/s10103-010-0761-5

82. Chung H, Dai T, Sharma SK, Huang Y-Y, Carroll JD, Hamblin MR. The Nuts and Bolts of Low-level Laser (Light) Therapy. *Ann Biomed Eng*. 2012;40(2):516-533. doi:10.1007/s10439-011-0454-7

83. Gholami L, Asefi S, Hooshyarfard A, et al. Photobiomodulation in Periodontology and

Implant Dentistry: Part 2. *Photobiomodulation, Photomedicine, Laser Surg*. December 2019:1-20. doi:10.1089/photob.2019.4731

84. Bjordal JM, Johnson MI, Iversen V, Aimbire F, Lopes-Martins RAB. 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):158-168. doi:10.1089/pho.2006.24.158

85. Meller ST, Gebhart GF. A critical review of the afferent pathways and the potential chemical mediators involved in cardiac pain. *Neuroscience*. 1992;48(3):501-524. doi:10.1016/0306-4522(92)90398-L

86. Heidari M, Paknejad M, Jamali R, Nokhbatolfoghahaei H, Fekrazad R, Moslemi N. Effect of laser photobiomodulation on wound healing and postoperative pain following free gingival graft: A split-mouth triple-blind randomized controlled clinical trial. *J Photochem Photobiol B Biol*. 2017;172:109-114. doi:10.1016/j.jphotobiol.2017.05.022

87. Amorim JCF, De Sousa GR, Silveira LDB, Prates RA, Pinotti M, Ribeiro MS. Clinical Study of the Gingiva Healing after Gingivectomy and Low-Level Laser Therapy. *Photomed Laser Surg*. 2006;24(5):588-594. doi:10.1089/pho.2006.24.588

88. 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(3):250-254. doi:10.1111/j.1600-051X.2007.01194.x

89. Cauwels RGEC, Martens LC. Low level laser therapy in oral mucositis: a pilot study. *Eur Arch Paediatr Dent*. 2011;12(2):118-123. doi:10.1007/BF03262791

90. Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Sanford LE, Woodruff LD. The Efficacy of Low-Power Lasers in Tissue Repair and Pain Control: A Meta-Analysis

Study. *Photomed Laser Surg*. 2004;22(4):323-329. doi:10.1089/pho.2004.22.323

91. Halon A, Donizy P, Dziegala M, Dobrakowski R, Simon K. Tissue laser biostimulation promotes post-extraction neoangiogenesis in HIV-infected patients. *Lasers Med Sci*. 2015;30:701-706. doi:10.1007/s10103-013-1411-5

92. Mayer L, Gomes F, Carlsson L, Gerhardt-Oliveira M. Histologic and Resonance Frequency Analysis of Peri-Implant Bone Healing After Low-Level Laser Therapy: An In Vivo Study. *Int J Oral Maxillofac Implants*. 2015;30(5):1028-1035. doi:10.11607/jomi.3882

93. Sanz-Moliner JD, Nart J, Cohen RE, Ciancio SG. The Effect of an 810-nm Diode Laser on Postoperative Pain and Tissue Response After Modified Widman Flap Surgery: A Pilot Study in Humans. *J Periodontol*. 2013;84:152-158. doi:10.1902/jop.2012.110660

94. Parker S, Cronshaw M, Anagnostaki E, Bordin-Aykroyd SR, Lynch E. Systematic Review of Delivery Parameters Used in Dental Photobiomodulation Therapy. *Photobiomodulation, Photomedicine, Laser Surg*. December 2019:1-14. doi:10.1089/photob.2019.4694

95. Ravera S, Ferrando S, Agas D, et al. 1064 nm Nd:YAG laser light affects transmembrane mitochondria respiratory chain complexes. *J Biophotonics*. 2019;12:1-7. doi:10.1002/jbio.201900101

96. Rocca JP, Zhao M, Fornaini C, Tan L, Zhao Z, Merigo E. Effect of laser irradiation on aphthae pain management: A four different wavelengths comparison. *J Photochem Photobiol B Biol*. 2018;189:1-4. doi:10.1016/j.jphotobiol.2018.09.016

97. Khan I, Arany P. Biophysical Approaches for Oral Wound Healing: Emphasis on Photobiomodulation. *Adv Wound Care*. 2015;4(12):724-737.

doi:10.1089/wound.2014.0623

98. Zand N, Ataie-Fashtami L, Djavid GE, et al. Relieving pain in minor aphthous stomatitis by a single session of non-thermal carbon dioxide laser irradiation. *Lasers Med Sci*. 2009;24:515-520. doi:10.1007/s10103-008-0555-1

99. Suter VGA, Sjölund S, Bornstein MM. Effect of laser on pain relief and wound healing of recurrent aphthous stomatitis: a systematic review. *Lasers Med Sci*. 2017;32:953-963. doi:10.1007/s10103-017-2184-z

100. Imrigha NAA, Bidin N, Lau PS, Musa N, Zakaria N, Krishnan G. Photobiomodulation therapy on wound treatment subsequent to Q-switched Nd: YAG laser tattoo removal in rat model. *J Biophotonics*. 2017;10:1287-1291. doi:10.1002/jbio.201600295

101. Pourzarandian A, Watanabe H, Ruwanpura SMPM, Aoki A, Noguchi K, Ishikawa I. Er:YAG laser irradiation increases prostaglandin E2 production via the induction of cyclooxygenase-2 mRNA in human gingival fibroblasts. *J Periodontal Res*. 2005;40(2):182-186. doi:10.1111/j.1600-0765.2005.00789.x

102. Del Pizzo M, Modica F, Bethaz N, Priotto P, Romagnoli R. The connective tissue graft: a comparative clinical evaluation of wound healing at the palatal donor site. A preliminary study. *J Clin Periodontol*. 2002;29:848-854. doi:10.1034/j.1600-051X.2002.290910.x

103. Fernandes-Dias SB, De Marco AC, Santamaria M, Kerbauy WD, Jardini MAN, Santamaria MP. Connective tissue graft associated or not with low laser therapy to treat gingival recession: Randomized clinical trial. *J Clin Periodontol*. 2015;42(1):54-61. doi:10.1111/jcpe.12328

104. Stavropoulou C, Atout RN, Brownlee M, Schroth RJ, Kelekis-Cholakis A. A randomized clinical trial of cyanoacrylate tissue adhesives in donor site of connective tissue grafts. *J*

Periodontol. 2019;90(6):608-615. doi:10.1002/JPER.18-0475

105. Santamaria MP, Fernandes-Dias SB, Araújo CF, et al. 2-Year Assessment of Tissue Biostimulation With Low-Level Laser on the Outcomes of Connective Tissue Graft in the Treatment of Single Gingival Recession: A Randomized Clinical Trial. *J Periodontol*. 2017;88(4):320-328. doi:10.1902/jop.2016.160391

106. Tsakos G, Marcenes W, Sheiham A. Evaluation of a modified version of the index of Oral Impacts On Daily Performances (OIDP) in elderly populations in two European countries. *Gerodontology*. 2001;18(2):121-130. doi:10.1111/j.1741-2358.2001.00121.x

107. Astrom A, Haugejorden O, Skaret E, Trovik T, Klock K. Oral Impacts on Daily Performance in Norwegian adults: the influence of age, number of missing teeth, and socio-demographic factors. *Eur J Oral Sci*. 2006;114:115-121.

108. Jung SH, Ryu JI, Tsakos G, Sheiham A. A Korean version of the Oral Impacts on Daily Performances (OIDP) scale in elderly populations: Validity, reliability and prevalence. *Health Qual Life Outcomes*. 2008;6(17). doi:10.1186/1477-7525-6-17

109. Costa FO, Miranda Cota LO, Pereira Lages EJ, et al. Oral Impact on Daily Performance, Personality Traits, and Compliance in Periodontal Maintenance Therapy. *J Periodontol*. 2011;82(8):1146-1154. doi:10.1902/jop.2011.100515

110. Melas F, Marcenes W, Wright PS. Oral health impact on daily performance in patients with implant-stabilized overdentures and patients with conventional complete dentures. *Int J Oral Maxillofac Implants*. 2001;16(5):700-712. http://www.ncbi.nlm.nih.gov/pubmed/11669253.

111. Erić J, Stančić I, Šojić LT, Popovac AJ, Tsakos G. Validity and reliability of the Oral Impacts on Daily Performance (OIDP) scale in the elderly population of Bosnia and

Herzegovina. *Gerodontology*. 2012;29(2):e902-e908. doi:10.1111/j.1741-2358.2011.00584.x

112. Purohit B, Singh A, Acharya S, Bhat M, Priya H. Assessment and validation of the oral impact on daily performance (OIDP) instrument among adults in Karnataka, South India. *Community Dent Health*. 2012;29(3):203-208. doi:10.1922/CDH

113. Fickl S, Fischer KR, Jockel-Schneider Y, Stappert CFJ, Schlagenhauf U, Kebschull M. Early wound healing and patient morbidity after single-incision vs. trap-door graft harvesting from the palate—a clinical study. *Clin Oral Investig*. 2014;18:2213-2219. doi:10.1007/s00784-014-1204-7

114. Wachtel H, Schenk G, Böhm S, Weng D, Zuhr O, Hürzeler MB. Microsurgical access flap and enamel matrix derivative for the treatment of periodontal intrabony defects: A controlled clinical study. *J Clin Periodontol*. 2003;30(6):496-504. doi:10.1034/j.1600-051X.2003.00013.x

115. Landry RG, Turnbull RS, Howley T. Effectiveness of benzydamine HCl in the treatment of periodontal post-surgical patients. *Res Clin Forum*. 1988;10:105-118.

116. Heidari M, Fekrazad R, Sobouti F, et al. Evaluating the effect of photobiomodulation with a 940-nm diode laser on post-operative pain in periodontal flap surgery. *Lasers Med Sci*. 2018;33:1639-1645. doi:10.1007/s10103-018-2492-y

117. Dias SBF, Fonseca MVA, dos Santos NCC, 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(6):1695-1702. doi:10.1007/s10103-014-1685-2

118. da Silva Neves FL, Silveira CA, Dias SBF, et al. Comparison of two power densities on

the healing of palatal wounds after connective tissue graft removal: randomized clinical trial. *Lasers Med Sci*. 2016;31(7):1371-1378. doi:10.1007/s10103-016-1988-6

119. Pradhan S, Shrestha R, Gorkhali R. Pain Perception after Periodontal Therapies. *J Nepal Soc Perio Oral Implant*. 2018;2(2):56-60.

120. Curtis JW, Mclain JB, Hutchinson RA. The Incidence and Severity of Complications and following Periodontal Surgery. *J Periodontol*. 1985;56(10):597-601.

121. Griffin TJ, Cheung WS, Zavras AI, Damoulis PD. Postoperative Complications Following Gingival Augmentation Procedures. *J Periodontol*. 2006;77:2070-2079. doi:10.1902/jop.2006.050296

122. Fardal Ø, McCulloch CA. Impact of Anxiety on Pain Perception Associated With Periodontal and Implant Surgery in a Private Practice. *J Periodontol*. 2012;83(9):1079-1085. doi:10.1902/jop.2011.110562

123. Wessel JR, Tatakis DN. Patient Outcomes Following Subepithelial Connective Tissue Graft and Free Gingival Graft Procedures. *J Periodontol*. 2008;79(3):425-430. doi:10.1902/jop.2008.070325

124. McGrath C, Comfort MB, Lo ECM, Luo Y. Can Third Molar Surgery Improve Quality of Life? A 6-Month Cohort Study. *J Oral Maxillofac Surg*. 2003;61:759-763. doi:10.1016/S078-2391(03)00150-2

125. Shugars DA, Gentile MA, Ahmad N, et al. Assessment of Oral Health-Related Quality of Life Before and After Third Molar Surgery. *J Oral Maxillofac Surg*. 2006;64(12):1721-1730. doi:10.1016/j.joms.2006.03.052

126. Almeida ALPF, Esper L a, Sbrana MC, Ribeiro IWJ, Kaizer ROF. Utilization of Low-Intensity Laser During Healing of Free Gingival Grafts. *Photomed Laser Surg*.

2009;27(4):561-564. doi:10.1089/pho.2008.2292

127. Damante CA, Greghi SLA, Sant'Ana ACP, Passanezi E, Taga R. Histomorphometric Study of the Healing of Human Oral Mucosa After Gingivoplasty and Low-Level Laser Therapy. *Lasers Surg Med*. 2004;35:377-384. doi:10.1002/lsm.20111

128. Paller CJ, Campbell CM, Edwards RR, Dobs AS. Sex-based differences in pain perception and treatment. *Pain Med*. 2009;10(2):289-299. doi:10.1111/j.1526-4637.2008.00558.x

129. Bartley EJ, Fillingim RB. Sex differences in pain: A brief review of clinical and experimental findings. *Br J Anaesth*. 2013;111(1):52-58. doi:10.1093/bja/aet127

Appendix I

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RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Effects of Er:YAG Photobiomodulation Therapy on Wound Healing of Human Palatal Mucosa After Connective Tissue Graft Harvesting: A Pilot Study

Principal Investigators: Dr. Brian Wiens, Dr. Reem Atout
Dental Diagnostic and Surgical Sciences, Periodontics
University of Manitoba
D343-780 Bannatyne Ave

You are being asked to participate in a Clinical Trial (a human research study). Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

WHAT IS THE RESEARCH ABOUT?

This Clinical Trial is being conducted to study the effects of photobiomodulation with an Er:YAG laser on tissue healing following subepithelial connective tissue graft (SECTG) surgery. The "photobiomodulation" procedure involves using a low-level laser directed at the roof of the mouth to stimulate these cells, inducing a quicker and more comfortable healing process. You are being asked to take part in this study because you are a patient of the Graduate Periodontics Clinic, College of Dentistry, University of Manitoba and require a connective tissue graft.

The purpose of this study is to compare palatal tissue donor sites following stimulation with an Er:YAG laser with tissue donor sites that do not receive laser treatment to determine if there is a beneficial effect on the healing of the tissues as well as any potential effect on your post-operative discomfort following SECTG surgery.

This research is being done because currently, there are no clinical trials that have investigated the effects of photobiomodulation with Er:YAG lasers on healing tissues following periodontal surgery. **This study is voluntary. If you decide not to participate in the study or withdraw from the study, your normal dental care will not be affected in any way.**

AM I ELIGIBLE TO PARTICIPATE?

Patients of the Graduate Periodontics Clinic, College of Dentistry, University of Manitoba that require a connective tissue graft, will participate in this study. Patients will be limited to those who are periodontally and systemically healthy. Within the context of this study, this includes disease-free individuals and those with only minor conditions that do not cause significant functional limitations. Examples include, but are not limited to, well-controlled hypertension, mild lung disease, pregnancy, obesity, well-controlled diabetes, or a smoking habit of no more than 10 cigarettes per day. To participate, patients must also not be taking corticosteroids or anticoagulants.

WHAT WILL I HAVE TO DO?

In this study, each participant will be "randomized" into one of 2 study groups described below. "Randomized" means that you are put into a group by chance, like flipping a coin. One group will receive post-operative laser stimulation of the palate and the other group will not receive any laser treatment.

You will undergo SECTG surgery as per standard procedure by one of the periodontology residents. Measurements of the palatal tissue will be recorded during surgery. After successful completion of the procedure, the palate will either receive treatment with an Er:YAG laser, according to the parameters that have demonstrated to promote healing in human cells, or will receive "sham treatment" in which the resident will mimic the laser treatment procedure but with the unit turned off. Following surgery, you will be given post-operative instructions and provided with antibiotics, painkillers, an antibacterial oral rinse, and a questionnaire to take home.

You will be required to return to the clinic for the standard one-week and two-week post-operative appointments, as well as a six-week follow-up appointment. At the first post-operative appointment, you are to return your questionnaire and any remaining painkillers that you did not need. You will complete one additional questionnaire and a resident who did not perform the procedure will assess the healing of the surgical sites. At the two-week post-operative appointment, healing of the surgical sites will again be assessed by the same resident. At the six-week appointment, you will receive an injection of local anesthetic to numb the palate (similar to what was done during your surgery) and the resident will measure the thickness of the healed tissues at the donor site. At this appointment, a small tissue sample will also be taken from the palate which will be assessed under a microscope to further evaluate the healing that has taken place. The tissue samples will be transported and stored in containers labelled with a numerical code and no identifying information that could be linked to you. After being analyzed, they will be securely stored in a locked location for the duration of the study (approximately 2 years).

The researcher may decide to take you off this study if it becomes evident that there are any contraindications to the surgery or laser treatment, funding is stopped, new information becomes available, or you become medically compromised during the study. You can stop participating at any time by voluntarily withdrawing. However, if you decide to stop participating in the study, we encourage you to talk to the study staff and your regular doctor first.

WHAT ARE THE POSSIBLE HARMS OR BENEFITS?

The two treatment options offered in this study will both follow effective and efficient surgical protocols used in North America and worldwide for SECTG procedures. In addition to standard treatment, one of the options will include application of laser stimulation which has demonstrated to be safe for human tissue. Zero to minimal side effects are observed after both therapies. Possible side effects following any SECTG surgery include bleeding, swelling, bruising, infection, pain, failure of graft integration and healing, and allergic reaction to the sutures. Similarly, there are minimal risks associated with taking a tissue sample from the palate. These may include bleeding, infection, and pain.

IS THERE ANY PAYMENT FOR PARTICIPATING?

This is a research study, so you may not personally benefit by participating in this study. We will provide you with the required medication following surgery, as well as a $50 discount off of the standard fee charged for this procedure. Eventually, the results of this study may benefit you and future patients by helping to establish the applications of laser treatment and how this can be utilized to improve healing following periodontal surgery.

WHAT IS THE COST OF THE STUDY?

All clinic and professional fees used in this study fall under the standard clinical fee guide and will be at no additional cost to you.

IS THE STUDY CONFIDENTIAL?

Information gathered in this research study may be published, presented in public forums, or shared with academic journals. If any data is published, presented, or shared, it will be done so in an anonymized form. Your name and any other identifying information will not be used or revealed. Data shared with academic journals may be stored under an open access policy so that it can be utilized by other researchers for further analysis and research purposes. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. All study documents related to you will bear only your assigned patient code/number instead of your name. Only your file marked with your specific code will be kept securely in an office safe at the Graduate Periodontics Clinic. Data based on your clinical measurements will be entered into the computer and transmitted electronically based on your patient code. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Biomedical Research Ethics Board (BREB) at the University of Manitoba.

The University of Manitoba Biomedical Research Ethics Board may review research-related records for quality assurance purposes. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No

information revealing any personal information such as your name, address or telephone number will leave the Graduate Periodontics Clinic.

WHAT ELSE SHOULD I KNOW?

You will be responsible for the cost of the surgical fees. You have the right to withdraw from the study at any time. The Investigators reserve the right to end your participation for any reason. If someone withdraws, their samples and measurements already taken (if any) will be discarded. You are entitled to know the scientific and technical results at the end of the research project and may request that a copy of any reports be sent to you upon completion of the study.

WHO CAN I CONTACT FOR MORE INFORMATION?

Information regarding the study is available on a publicly available Registry Databank. ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on http://ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact:

Shelly Rempel-Rossum
Bannatyne Campus Research Ethics Board Coordinator
(204) 789-3389
shelly.rempel-rossum@umanitoba.ca

CONSENT FORM

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Wiens and his study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. Any relationship (such as employee, student or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by The University of Manitoba Biomedical Research Ethics Board.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study. I understand that I can end my participation at any time and for any reason and that this will not affect my care at the Graduate Periodontal Clinic. I agree to participate in the research protocol, Effects of Er:YAG Photobiomodulation Therapy on Wound Healing of Human Palatal Mucosa After Connective Tissue Graft Harvesting: A Pilot Study.

Participant signature_____ **Date** _____
(day/month/year)

Participant printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____ **Date** _____
(day/month/year)

Signature: _____