

# *A 810 nm Diode Laser following Modified Widman Flap Surgery*

Javier Daniel Sanz Moliner

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# **A 810 nm Diode Laser following Modified Widman Flap Surgery.**

**Doctoral Thesis**

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March 2012  
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**DEDICATION**

**To My Parents and Sister Nuria: You have always been my support through life and have done so much to make me who I am today. I feel so lucky and blessed to have your love and guidance. Thank You for everything.**

**Nancy Kate Pancko: Thank you for your grammatical guidance and emotional support during the creation of this project. It would not have been possible without you.**

**To the SUNY at Buffalo Faculty: I have learned so many things from all of you. You have helped me excel in my studies and I aspire that one day I will have the knowledge and achievements that you have obtained through the years.**

**To Dr Santos and Dr Nart: Thank you for your wisdom and guidance on this project. Thank you for giving me the opportunity to be a part of your program as a faculty.**

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## **INDEX OF ABBREVIATIONS**

Aa: *Agregatibacter actimecetemcomytans*  
APF: apically positioned flap  
AST: After surgical treatment  
BOP: bleeding on probing  
CAL: clinical attachment level  
CEJ: cementoenamel junction  
CO2: Carbon dioxide laser  
DL: Diode laser  
DNA: Nicotinamide adenine dinucleotide  
EMD: enamel matrix protein derivative  
Er,Cr:YSGG: Erbium, Cromium: Yttrium Scandium Gallium  
Er:YAG: Erbium Doped: Yttrium Aluminium Garnet  
GI: gingival index  
GM: gingival margin  
GTR: guided tissue regeneration  
J: jules  
KM: Keyes method  
Laser-ENAP: Laser-excisional new attachment procedure  
LLLT: low level laser therapy  
MWF: modified Widman flap  
Nd:YAG: Neodymium Doped: Yttrium Aluminium Garnet  
Nd:YAP: Neodymium Doped: yttrium aluminum perovskite  
PD: pocket depth  
PG: *Porphiromona gingivalis*  
PM: pain medication consumption  
Pn: *Prevotella nigrescens*  
FADH2: Reduced flavin adenine dinucleotide  
PI: plaque index  
Pi: *Prevotella intermedia*  
PS: Pain scale assessment  
SRP: scaling and root planing  
TC: tissue color





Td: Tannerella denticola

TE: tissue edema

VAS: visual analog scale

W: watts

## **1. JUSTIFICATION**

Chronic periodontitis, according to the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions, is defined as "inflammation of the gingiva extending into the adjacent attachment apparatus". The disease is characterized by loss of clinical attachment due to destruction of the periodontal ligament and loss of the adjacent supporting bone" (1). Although chronic periodontitis is the most common form of destructive periodontal disease in adults, it can occur over a wide range of ages. It can occur in both the primary and secondary dentitions.

It is widely accepted that the initiation and progression of periodontitis is dependent upon the presence of microorganisms capable of causing disease. Although more than three hundred species of microorganism have been isolated from periodontal pockets, it is likely that only a small percentage of these species are etiologic agents. (2). The histopathology of a chronic periodontitis lesion is characterized by a predominance of plasma cells, loss of connective tissue elements, and bone resorption. In response to specific stimuli, inflammatory cells chemotactically migrate and concentrate in localized areas where they phagocytize bacteria and bacterial components.

The primary goal of periodontal treatment is to remove bacterial deposits present on roots affected by periodontitis (3). Treatment focuses on the mechanical removal of the subgingival deposits of biofilm, which helps to establish a local environment and microflora compatible with periodontal health. Parameters that are commonly used to assess and monitor periodontal status include clinical attachment level (CAL), pocket depth (PD) measurements, and bleeding on probing (BOP). Treatment goals include reduction of probing PD, to maintain or improve CAL and to reduce the incidence of BOP.

In general, initial therapy involves non-surgical therapy by scaling. Scaling is a mechanical process, which removes plaque and calculus from both the supragingival

and subgingival aspects of a tooth. Root planing is a mechanical process to remove residual embedded calculus and portions of cementum from roots to produce a smooth, hard, clean surface (4).

Generally, surgery is indicated when non-surgical methods fail. The purpose of surgical pocket therapy is to eliminate the pathologic changes in the pocket walls in order to create a stable, easily maintainable state and, if possible, to promote periodontal regeneration. A variety of surgical techniques exist. These include subgingival curettage, gingivectomy, modified Widman flap, and full-thickness or split-thickness flap procedures with or without osseous recontouring and with or without bone grafting. The best surgical approach and material or device, such as lasers, to proceed remains controversial (5).

Periodontal disease is not necessarily painful. Conversely, periodontal treatment is experienced as painful by substantial numbers of patients (6). Studies have analyzed the level of pain after different periodontal treatments: 20-40% of patients experience some pain during the first week (7,8). Mucogingival surgery and osseous recontouring seem to be the most painful (6,7,9). Painless therapies are necessary in order to consolidate the patients in the importance of periodontal treatment.

Lasers may be classified in different ways. According to the power that they are used can be classified into high power or surgical and low power or therapeutic. Low level lasers are used due to their bioestimulatory, analgesic and antiinflammatory action. High power are used as an alternative to electric and conventional rotator instruments (10).

Surgical lasers have been used in periodontal therapy. In order to excise the epithelium of the periodontal pocket during scaling and root planing (11-14) and periodontal surgery (15-20); to eliminate calculus, granulation tissue and bone during periodontal resective and regenerative surgery (21); as an antialgic during hygienic phase(22,23); enhanced coagulation (24); bactericidal agent (22,25) as well as calculus elimination (26). Each investigation use different types of lasers, wavelengths and techniques making difficult to stablish a conclusion.

Low level lasers emit visible red and near-infrared wavelengths that are absorbed in the photoreceptors within subcellular components, targeting the electron transport/respiratory chain within the membranes of mitochondria (27). Absorption of laser energy increases ATP formation, which in turn stimulates cell function (28). This increase in cell function may help improve wound healing. In vitro studies have shown that low level laser therapy (LLLT) increases the synthesis of DNA (24), collagen (29), and procollagen (30) as well as to stimulate (31) and increase (32) the proliferation rate of fibroblasts.

Nevertheless, the beneficial effect seen in vitro is not completely observed in clinical studies. In dentistry, the analgesic effect of low level lasers has been extensively studied with a wide range of results. Studies have been performed to evaluate the effect of lasers on dentin hypersensitivity (33), temporomandibular joint disorders (34), analgesia after third molar extraction (35) and as an adjunct in orthodontic treatment (36). However, few studies have addressed the effect that lasers have on pain in periodontics. Masse et al. (37) and Almeida et al. (38) failed to show a significant analgesic effect of low level lasers when used after the placement of free gingival grafts. On the other hand, low level lasers have been found to enhance periodontal healing (39,40) when used as an adjunct of scaling and root planning. Overall, the effect that low level lasers have on periodontal treatment and the true analgesic effect that low level lasers provide is unclear.

The prevalence of periodontal disease is high. If untreated, it can lead to tooth lost. Its progression is usually nonpainful but its treatment could be. The major goal of the treatment is the elimination of bacteria, periodontal regeneration and prevention of disease progression, which will lead to the major objective which is maintaining the teeth. Lasers have been used with the objective to previously mentioned goals.

Despite the large number of publications, there is still controversy regarding the application of dental lasers to the treatment of chronic periodontitis due to:

- A comparison between clinical studies is difficult due to different laser wavelengths, variation in laser parameters and ways of application; lack of

information of the laser parameters used; experimental design; clinical parameters and sample size

- There is a considerable conflict in results for both laboratory studies and clinical trials, even when using the same laser wavelength.
- Although some studies shown a statistically significant differences between non-laser use and laser, the differences may be clinically irrelevant
- The ratio of economical cost/benefit of certain type of lasers questions the need for the use of lasers.
- The time needed and number of visits for certain antialgic laser therapies, such as LLLT, question the viability of these therapies in private practice.

The study hypothesized that a unique application of a 810 nm diode laser following a modified Widman flap (MWF) would have an additional benefit in clinical parameters; such as pocket depth reduction, clinical attachment gain, reduction of bleeding on probing, tissue response and reduction of postoperative pain, when compared to conventional periodontal surgery alone.

A diode laser was used once to deepithelialize the inner part of the periodontal flap and photobiostimulate the surgical area. Nowadays there are not studies evaluating the use of a diode laser as an adjunct in periodontal surgery. None of the studies evaluate the application of the laser to eliminate the inner epithelial part of a periodontal pocket, or a unique application of the laser as LLLT.

## **2.FUNDAMENTALS**

The goals of periodontal therapy are to alter or eliminate the microbial etiology and contributing risk factors for periodontitis, thereby arresting the progression of disease and preserving the dentition in a state of health, comfort, and function with appropriate esthetics; and to prevent the recurrence of periodontitis. In addition, regeneration of the periodontal attachment apparatus, where indicated, may be attempted (41).

Periodontal treatment involves initial therapy. Surgical therapy might be needed if non-surgical/initial therapy methods fail.

### **2.1 Initial therapy**

Initial therapy usually involves instruction, reinforcement, and evaluation of the patient's plaque control as well as supra and subgingival scaling and root planing to remove microbial plaque and calculus. (41)

An analysis of studies performed over the past 25 years on conventional scaling and root planing (SRP) showed that, on average, pockets of 4 to 6 mm associated with moderate periodontitis could be reduced by 1.29 mm, with gains in attachment of 0.55 mm. In addition, pockets  $\geq 7$  mm associated with more severe periodontitis could be reduced, on average, by 2.16 mm, with gains in attachment of 1.19 mm (42). Adjuncts to SRP such as local and systemic antibiotic therapy, host-modulation, and laser therapy have been used with a wide range of results (43,44,45).

### **2.2 Periodontal Surgical Treatment**

The ideal time to reevaluate periodontal therapy is 4 to 8 weeks following treatment (46). Surgical approach may be considered to enhance effective root debridement, to possibly enhance regenerative therapy, to reduce gingival recession, etc. on patients who demonstrate effective plaque control and favorable compliance in their prior dental care (41).

A variety of surgical techniques exist. These include subgingival curettage, gingivectomy, modified Widman flap, and full-thickness or split-thickness flap procedures with or without osseous recontouring and with or without bone grafting. The best surgical approach remains controversial (5).

### **2.2.1 The modified Widman flap**

Ramfjord and Nissle (47) described the modified Widman flap and emphasized the need of a sharp dissection, minimal flap separation from the alveolar process, and close interproximal flap adaptation. The following is an outline of this technique:

1. The initial incision is an internal bevel incision to the alveolar crest starting 0.5 mm to 1 mm away from the gingival margin. Scalloping follows the gingival margin.
2. The gingiva is reflected with a periosteal elevator.
3. A crevicular incision is made from the bottom of the pocket to the bone.
4. After the flap is reflected, a third incision is made in the interdental spaces coronal to the bone and the gingival collar is removed
5. Tissue tags and granulation tissue are removed, root surfaces are scaled and planed
6. Bone architecture is not corrected except if it prevents good tissue adaptation to the necks of the teeth.
7. Primary closure, without exposition of the interproximal bone, is achieved with interrupted direct sutures placed in each interdental space.

This technique is designed to maximize healing in areas of previous periodontal pockets with a minimal loss of periodontal tissues during and after surgery. This technique offers the possibility of establishing an intimate postoperative adaptation of healthy collagenous connective tissue to tooth surfaces, provides access for adequate instrumentation of the root surfaces, and allows for immediate closure of the area

(48). During healing, some crestal bone resorption and osseous repair can be expected with the establishment of a long junctional epithelium between the bone and the root surface (49).

Ramfjord et al. (50) evaluated the results of four different periodontal treatment techniques: pocket elimination or reduction surgery, MWF, subgingival curettage, and SRP on PD and AL. The authors concluded that the treatment of choice for periodontal pockets of  $\leq 6$  mm was SRP and for pockets  $\geq 7$  mm the results were similar for all the 4 methods.

## **2.3 Comparative studies of Periodontal Treatments**

### **2.3.1 Longitudinal studies comparing non-surgical and surgical treatments**

Ramfjord et al. (50) split mouth studies were separated based on the probing depth at initial examination: 1 to 3 mm, 4 to 6 mm, and 7 mm or greater. Results also were compared based on tooth type. Curettage, SRP, and MWF produced slightly better attachment level results, while pocket elimination procedures gave the greatest probing depth reduction. There were no differences found between tooth types but molars showed slightly less favorable results. Teeth with initially shallow probing depths tended to lose a small amount of attachment due to treatment, while teeth with initially deep probing depths showed the greatest AL gain and PD reduction (50-55). Although the results of different surgical methods yielded similar results, the Ramfjord study found the greatest surgical benefit to teeth with initially larger probing depths.

Lindhe, Nyman and Olsen tested various surgical techniques including: gingivectomy, MWF with and without osseous recontouring, and an apically positioned flap (APF) with and without osseous recontouring. All techniques halted attachment loss, but the greatest gain of attachment was achieved when osseous resection was avoided and soft tissue was sutured to completely cover alveolar bone (56-59). Additional studies by Lindhe et al. (60-62) utilized a split-mouth design and compared only SRP and MWF. These studies reported AL changes and PD reductions similar to that found by Ramfjord et al. (50). Critical PD for SRP and MWF were identified (60)



and were found to be 2.9 mm for SRP and 4.2 mm for MWF. Values above the critical PD responded with gain of attachment while values below tended to lose attachment. Therefore, Lindhe's studies support the results obtained by Ramfjord's in which teeth with deeper probing depths responded the best to periodontal surgical techniques where teeth with shallower probing techniques benefited less. These findings suggest that only deeper probing depths should have surgical treatment, since it would yield the greatest benefit to the patient.

Pihlstrom's studies (63-65) also compared SRP with the MWF. There was little difference found in AL results for the two treatments. The Isidor studies (66-68) compared APF, MWF and SRP and found little difference between treatments. Becker et al. (69,70) and Kerry et al. (71) found minimal differences when comparing SRP to the MWF. These studies suggest that the outcomes of APF, MWF and SRP yield similar results. Therefore, teeth requiring surgical treatment would benefit equally from APF, MWF, and SRP.

Most recent studies (72-74) evaluated the results of surgical and non surgical therapy. The studies differ from the above in several aspects. First, a coronal scaling group was included. Second, the furcation response was analyzed using horizontal AL data. Third, sites were stratified by initial and post-surgical PD. Fourthly, the annual incidence of sites breaking down was determined. The results found that the recurrence rate was greatest for sites treated with SRP, which was similar to MWF, and least for sites that received osseous surgery. It was also concluded that breakdown incidences were greater with increasing post-surgical PD. Finally, the effect of smoking on treatment outcome was assessed and found to profoundly influence disease recurrence. Smokers responded to treatment but the response was less pronounced, and the recurrence rate was higher than in nonsmokers. These studies suggested that SRP and MWF gave similar recurrence rates and that teeth would benefit equally from each surgical treatment. It was also suggested that disease severity and smoking habits may have negative implications on surgical outcomes.

### **2.3.2 Modified Widman flap verses Keyes method**

Keyes et al. (75) reported a method of periodontal therapy employing oral hygiene, SRP with the adjunctive application of a thick paste containing sodium bicarbonate, salt, hydrogen peroxide, and water, and the use of a pulsating water irrigation device. Diagnosis and reevaluation of therapy was assessed by subgingival microorganism dark field microscopy and the prevalence of white blood cells. Whitehead and Watts (76) developed a study which compared the Keyes method (KM) with the MWF. In areas with  $\geq 7$  mm PD, the KM PD reduction was 4.2 mm and the AL gain was 3.5mm. The MWF had a PD reduction of 4.8mm and an AL gain of 3.3 mm. Overall, no differences between treatments were found. This study suggests that MWF and KM provide similar benefits in terms of PD reduction and AL gain.

### **2.3.3 MWF verses APF**

Hill et al. (55) compared APF procedures to the MWF approach with respect to final PD and changes in AL. They found that MWF resulted in less attachment loss and no differences in APF in maintaining PD reduction after two years. In a long term longitudinal comparison of MWF and APF procedures, Knowles et al. (54) concluded that both modalities showed no differences in regards to sustained PD reduction and gain in clinical AL. In contrast, Zamet (77) compared MWF and APF surgery and found APF to be the most effective method in reducing PD but no difference in changes in AL. Matchtei and Ben-Yehouda (78) compared the effect of MWF and APF on PD and AL in a 2 year longitudinal study. A positive correlation was found between immediate (day of surgical procedure) postoperative PD, also known as a sound depth, and PD. Conversely, AL changes over the 2 year period showed only weak inverse correlation. Sites with SD of  $\leq 3$ mm had a mean PD of 2.52 mm. This was significantly smaller when compared to sites with SD of  $\geq 4$  mm which had a mean PD of 3.58 mm. Some studies show that there may be some advantage of APF over MWF with regard to PD reduction. However, the majority of the studies support the belief that APF and MWF yield similar surgical results in terms of PD reduction and AL gain.

### **2.3.4 MWF verses sulcular incision**

Smith et al. (79) evaluated the need for elimination of the pocket epithelium during mucoperiosteal flap surgery aimed at reattachment or readaptation. A split mouth design involving 13 patients compared MWF with removal of pocket epithelium where the contralateral side received a crevicular flap without removing the pocket epithelium. After 3 months, MWF resulted in a statistically significant greater interproximal PD reduction (PD 1.72mm versus 1.53mm). It was also found that there was less recession in the MWF approach than that found with the crevicular approach. The differences, although statistically significant, were found to be by the author clinically insignificant. This study suggests that MWF may be slightly superior to a crevicular flap in terms of PD reduction.

## **2.4 Laser Therapy**

### **2.4.1 Laser fundamentals**

LASER is an acronym for light amplification by stimulated emission of radiation. Laser light is an energy that behaves both as a particle and as a wave. The basic unit of light is called a photon. A wave of photons has three basic properties: velocity, amplitude, and wavelength (27).

Amplification occurs inside the laser. The type of laser, such as argon and CO<sub>2</sub>, is named by the type of active medium and the emission wavelength of the laser. The pumping mechanism excites the active medium and atoms are released and absorbed by the process of light emission. Laser light produced by the stimulated active medium is bounced back and forth through the axis of the laser cavity, using two mirrors placed at either end.

The term "stimulated emission" is based on the quantum theory of physics. A quantum, the smallest unit of energy, is absorbed by the electrons of an atom or molecule, causing a brief excitation. After this, a quantum is released in a process called spontaneous emission. The mirrors at each end of the active medium reflect these photons back and forth to allow further stimulated emission, and successive



passes through the active medium increase the power of the photon beam. This is the process of amplification (80).

Radiation refers to the light waves produced by the laser. The electromagnetic spectrum is the entire collection of wave energy that ranges from gamma rays ( $10^{-12}$  m) to radio waves ( $10^3$ m). The very short wavelengths can deeply penetrate biologic tissue and produce charged atoms and molecules. Wavelengths larger than 300 nm have less photon energy and cause excitation and heating of the tissue in which they interact. Dental lasers (500 nm to 10,600 nm) are within the visible or the invisible infrared non-ionizing portion of the electromagnetic spectrum and emit thermal radiation (27).

The energy from the laser can be absorbed, reflected, or scattered by the tissue. It will only exhibit scattering in cases of deep tissue penetration. The absorbed light energy is converted to heat and constitutes a photothermal event. Variable parameters affecting energy absorption include: Optical properties of the tissue (pigmentation, water content, heat capacity), emission wavelength, primary variable, power (watts (W)), waveform (continuous or pulsed), pulse duration, energy/pulse, energy density, duration of exposure, peak power of pulse, and the angulation of the energy delivery tip to the target surface (45).

Dental lasers have two basic emission modes; continuous wave and free-running pulse. Continuous wave lasers emit laser energy during laser activation. As a result, constant tissue interaction is produced. One type of continuous wave laser is a gated-pulse laser. Gating is produced by mechanically closing the opening of the mirror within the laser chamber (81). The second emission mode is free-running pulse or true pulsed. In this mode, large peak energies of laser light are emitted for a short time span, usually in microseconds, followed by a relatively long time in which the laser is off (81).

Chromophores are compounds of tissues that absorb specific wavelengths. Hemoglobin reflects red wavelengths and is strongly absorbed by the blue and green

wavelengths. Venous blood, containing less oxygen, absorbs more red wavelengths and appears darker. The pigment melanin is strongly absorbed by short wavelengths. Water, however, has varying degrees of absorption by different wavelengths.

Shorter wavelengths (500nm-1000nm) are absorbed in pigmented tissue and blood elements. Argon is highly attenuated by hemoglobin. Diode and Neodymium Doped: Yttrium Aluminium Garnet (Nd:YAG) have a high affinity for melanin but have less interaction with hemoglobin. The longer wavelengths are more interactive with water and hydroxyapatite. The largest absorption peak for water is just below 3000 nm, which is at the Erbium Doped: Yttrium Aluminium Garnet (Er:YAG) wavelength. Erbium is also well absorbed by hydroxyapatite. Carbon dioxide laser (CO<sub>2</sub>) at 10,600nm is well absorbed by water and has the greatest affinity for tooth structure (27).

#### **2.4.2 Types of lasers**

##### *Argon*

Argon is a laser with an active medium of argon gas that is energized by a high-current electrical discharge. There are two emission wavelengths used in dentistry. The wavelengths 488 nm and 514 nm are in the visible spectrum and are seen as blue and green, respectively. The wavelengths are delivered in a continuous wave or in a gated pulsed wave (27). The 488 nm laser is used for resin polymerization in composite restorative materials and for activating whitening gels and impression materials. The 514 nm laser has hemostatic capabilities that permit inflammatory and vascular healing. Both wavelengths can be used as an aid in caries detection (27).

##### *Diode*

Diode is a solid active medium laser. It is manufactured from a semiconductor crystal using a combination of aluminium or indium, gallium, and arsenic. The wavelengths for the dental use of a diode laser range from 800 nm for an aluminium medium, 980 nm for an indium medium, to a near infrared portion of the invisible non-ionizing spectrum. These wavelengths are delivered in a continuous wave or gated pulsed (27). The wavelengths are highly absorbed by pigmented tissue and are deeply

penetrating (27). The diode laser has been shown to have a similar tissue effect as the Neodymium Doped: Yttrium Aluminium Garnet (Nd:YAG) laser in comparative studies but with less thermal effects on the deeper tissues (82). On the other hand, the laser's wavelengths are poorly absorbed by tooth structures.

The diode laser is used for removal of soft tissue. The laser must contact the soft tissue in order to ablate the tissue. If deeper coagulation is desired, the laser should not contact the tissue. The diode laser has the same clinical applications as the Nd:YAG laser. It can be used for such procedures as a gingivectomy, subgingival curettage, frenectomy, and for the excision of soft tissue pathology (82).

*Neodymium Doped: Yttrium Aluminium Garnet (Nd:YAG), Neodymium Doped: yttrium aluminum perovskite (Nd:YAP)*

The Nd:YAG laser has a solid active medium, which is a garnet crystal combined with the rare earth elements yttrium and aluminium, doped with neodymium ions. The Nd:YAG laser operates at the wavelength of 1064 nm and the Nd:YAP at the wavelength of 1340 nm which is in the invisible near-infrared portion of the electromagnetic spectrum. It operates only in a free running pulse mode with short pulse durations. It is highly absorbed by melanin but is less absorbed by hemoglobin than the argon laser. The Nd:YAG laser is not readily absorbed by water, so that most of the energy is scattered in soft tissue rather than being absorbed by the tissue surface. It is, however, slightly absorbed by dental hard tissue. It can be used in contact or non-contact mode (27). The Nd:YAG laser is used for the same procedures as the diode laser (82).

*Erbium Doped: Yttrium Aluminium Garnet (Er:YAG), Erbium, Chromium: Yttrium Scandium Gallium (Er,Cr:YSGG)*

Er:YAG laser has an active medium of a solid crystal of yttrium aluminum garnet that is doped with erbium. The Er:YAG laser operates at a wavelength of 2940 nm and the Er,Cr:YSGG operates at a wavelength of 2780nm and both are emitted in a free-running pulsed mode. Those two wavelengths have the highest absorption in

water of any dental laser and the highest affinity for hydroxyapatite. The Er:YAG laser vaporizes the water within hydroxyapatite causing a massive volume expansion. This expansion causes the surrounding material to explode away (27). The Er:YAG laser is also used for cavity preparation of incipient caries and for calculus removal (82).

### *Carbon dioxide laser (CO<sub>2</sub>)*

The CO<sub>2</sub> is a gas-active medium laser that incorporates a sealed tube containing a gaseous mixture with CO<sub>2</sub> molecules pumped via electrical discharge current. The light energy, 10600 nm, is placed at the end of the mid infrared invisible nonionizing portion of the spectrum (27). The CO<sub>2</sub> laser wavelength is readily absorbed by water. As soft tissue is 75% to 90% water, about 98% of the energy is converted to heat and absorbed at tissue surface with very little scatter or penetration (82). It has the highest absorption in hydroxyapatite of any dental laser. Teeth should be protected from irradiation. Non contact mode is used. Higher energy is used to vaporize and remove tissue. Lower energy levels are used for hemostasis and photocoagulation. Carbonization and crazing of tooth structure can occur due to long pulse duration and low peak powers which limits its use for hard tissue. The CO<sub>2</sub> laser removes soft tissue by ablation. It is used for gingivectomy, frenectomy, excision of soft tissue pathology and laser deepithelialization of flaps during and after surgery (Rossman 2002).

## **2.5 Use of lasers in periodontal therapy**

Surgical lasers have many uses in periodontal therapy. Lasers can assist with the removal of diseased pocket lining epithelium and coagulation. Low level laser therapy has been found to enhance wound healing and to reduce pain. Lasers have a bactericidal effect on periodontal pocket organisms and can be used for removal of calculus deposits and root surface detoxification (24).

### **2.5.1 Removal of diseased pocket epithelium and coagulation**

*As an adjunct in the initial therapy*

Case reports have recommended the diode laser at a wavelength of 819nm and the Nd:YAG laser for treatment of periodontal pockets using laser subgingival curettage or excisional new attachment procedure. Root surface damage following laser-assisted subgingival curettage has been reported (82). Kreisler et al. (83) stated that parameters, such as power and wavelength, may be important in order to prevent root surface damage. Irradiation of dry or moist specimens did not result in any surface alterations. However, irradiation caused damage if the teeth were covered by a thin blood film and if the laser was at a power of 1.5W, 2W and 2.5 W. On the other hand, Lopez Castro et al. (84) found no tooth surface alterations histologically. This study used a 2W diode laser as an adjunct to scaling and root planing in areas with a thin blood film covering the root surfaces. Therefore, studies have shown that diode lasers applied at higher energies of 1.5 W may cause root surface damage.

*In vivo* studies using the diode laser have shown large amounts of calculus remaining after treatment, along with significant structural damage to root surfaces (85). Schwarz et al. (86) histologically determined the *in vivo* and *in vitro* effect of a 1.8W diode laser. In this study, teeth were lased *in vivo*, extracted, and irradiated *in vitro*. It was found that the diode laser was unsuitable for calculus removal since it caused severe damage to the root surfaces *in vivo* but not *in vitro*. No thermal side effects, however, were seen. Since diode lasers have been found to be ineffective at removing calculus and there is the possibility of root surface damage, lasers should not be used for calculus removal (85).

Moritz et al. (87), in a pilot study on humans, determined that the presence of *A. actinomycetemcomitans* was reduced when a diode laser at 805 nm was used as an adjunct to SRP. Moritz et al. (88) performed another study on 50 patients that were randomly subdivided in 2 groups. Patients were treated either with a laser or subgingival irrigation of H<sub>2</sub>O<sub>2</sub>. Subgingival bacteria samples were collected in all patients. After 6 months, the sides that received subgingival laser treatment exhibited



a much lower bacterial count. There was also a 96.9 % reduction of BOP values in the laser group compared to 66.7 % in the control group. It is important to mention, though, that the test patients received laser therapy at 1 week, 2 months and 4 months, while control patients received no further treatment except for subgingival rinse with H<sub>2</sub>O<sub>2</sub>. This study suggests that a single episode of subgingival irrigation with H<sub>2</sub>O<sub>2</sub> may have little beneficial effect on scaling. This results agreed with the review perform by Greenstein (1) where little benefit can be expected from subgingival irrigation as and adjunct of scaling.

Kreisler et al. (13) evaluated the use of an 809 nm, 1 W diode laser as an adjunct to SRP. The study was a split mouth randomized clinical trial on 25 patients comparing SRP alone verses SRP plus a diode laser. After 3 months, treatment evaluation showed that mobility (Periotest®) reduction, PD reduction and CAL gain differences between groups were 0.3mm (p=0.019), 0.2mm (p<0.001) and 0.3mm (p<0.001) respectively, favoring the treatment of SRP plus a diode laser. No differences were seen in BOP, PI, GI and sulcus fluid flow rate between groups. Borrajo et al. (12), in a similar study used an 980 nm, 2W diode laser on 30 patients with moderate periodontal disease. No significant differences were found between groups for CAL but there was a significant reduction in BOP for the SRP plus a diode laser group (p<0.0001). Euzebio et al. (89) in another split mouth clinical trial with 36 chronic periodontitis patients, applied a 808 nm, 1.5W continuous diode laser as an adjunct to scaling and root planing. No statistically significant differences were seen for PD,CAL, BOP, PI between the study groups at 6 weeks and 6 months after treatment. De Micheli et al. (90) and Lin et al. (91) found similar results with no benefits of diode laser application after scaling and root planing. The Kreisler study suggests that the use of a diode laser with SRP may be more beneficial than SRP alone in mobility reduction, PD reduction and CAL gain. However, the Borrajo study found that the diode laser with SRP may be more beneficial that SRP alone only in terms of BOP reduction and the Euzebio, De Micheli and Lin studies found no benefits. In summary, studies yield conflicting results and it is unclear whether lasers provide an important adjunct to SRP.

*Laser-excisional new attachment procedure (Laser-ENAP)*

Laser-ENAP is a non-flap procedure to reduce pocket depths which uses a

Nd:YAG laser at a wavelength of 1,064nm. The laser probe or fiber is placed 1-2 mm coronal to the pocket base and is angled toward the soft tissue wall. SRP is performed prior to laser application. Power values sufficient to ablate the epithelial lining are approximately 0.8 W for a diode laser, 2.0 W for a Nd:YAG and Er:YAG/YSGG laser, and 1.0 W for a CO<sub>2</sub> laser, were used. Ablation starts near the base of the pocket and proceed upwards.

The laser-ENAP policy statement of the American Academy of Periodontology (92), states that there are no beneficial effects of this procedure in comparison to traditional SRP. Clinical case reports of laser-ENAP have demonstrated improved clinical measurements and some radiographic evidence of bone regeneration in treated areas (11). Yukna (14) also found evidence that laser-ENAP application resulted in new cementum and bone growth, and periodontal ligament regeneration. Therefore, results of studies using the laser-ENAP have conflicting findings and it is unclear if this laser yields additional benefits to periodontal surgical outcomes.

Slot et al. (93) in a review paper, analyzed the effect of pulsed Nd:YAG laser in nonsurgical periodontal therapy. Only 8 clinical trials were available for review. Two studies compared the Nd:YAG laser alone with SRP; 1 compared lasers with ultrasonic instrumentation and 1 with sham therapy; 4 compared laser therapy plus SRP with SRP alone. Laser therapy was not more effective than traditional instrumentation with ultrasonic or hand instruments in terms of plaque reduction, pocket reduction, decreased bleeding or gain in clinical attachment levels.

### *Surgical de-epithelialization*

During healing, the apical epithelial proliferation along the root surface has been shown to interfere with the establishment of new connective tissue attachment and the development of its associated alveolar bone (94). Apical epithelial migration also develops during the presence of inflammation. Reduced epithelial downgrowth has been the objective of multiple methods.

Lasers have been used in periodontal therapy in order to exclude epithelial migration. Rossman (17) stated that the reason for the epithelial migration delay was that the laser wound margins showed thermal necrosis, forming a firm scar which blocked epithelial migration. Secondly, Rossman found a decrease in wound contraction due to a reduction in myofibroblasts which leaves a greater surface area remaining to be epithelialized. Thirdly, he found a thin layer of denatured collagen on the laser wound surface which acts as an impermeable dressing in the immediate postoperative period, causing a reduction in the degree of tissue irritation from oral cavity contents. Finally, a reduced inflammation in the laser-induced wound may provide less stimulation for epithelial migration. Therefore, lasers may help prevent the apical epithelial proliferation along the root surface which may aid in the establishment of new connective tissue attachment and the development of its associated alveolar bone.

Animal and human studies have used the CO<sub>2</sub> laser to de-epithelize periodontal flaps, in order to exclude the epithelium downgrowth. Rossman et al. (15) showed that the 8W pulsed mode CO<sub>2</sub> laser removed gingival epithelium without damaging the connective tissue. The underlying connective tissue 1mm below the wound was biopsied and was found to have no heat generated damage, along with complete tissue viability. A histologic evaluation of the test sites also showed a delay in epithelial migration for 14 days. Wound healing studies have shown that the epithelium should be excluded for at least 30 days after periodontal surgery for guided tissue regeneration (GTR) to be effective. This exclusion allows the preferential organization of the blood clot with periodontal ligament and alveolar bone cells.

Rossman et al. (16) determined the rate of epithelial migration after laser application of the outer portion of the keratinized gingiva. It was found that laser application every 10 days prevented epithelial cells from reaching the root surface. Abundant formation of new cementum coronal to the reference notch was seen only on the laser treated sides. Those studies concluded that laser de-epithelialization could enhance regeneration of periodontal defects.

Several studies human studies (18,19,20) have shown that the CO<sub>2</sub> laser can

completely de-epithelize the inner and outer aspects of a mucoperiosteal flap while leaving the underlying connective tissue undisturbed. The laser, when compared to the scalpel, appears to eliminate the sulcular epithelium significantly better than a reverse bevel incision with a conventional blade. The authors believed that this technique demonstrated significantly better results than those obtained through conventional osseous grafting alone, and appeared to be comparable to the results reported for GTR procedures with barrier membranes. Centty et al. (18) showed that laser-treated sites had significantly better gain of CAL when compared with control sites; 1.94mm versus 0.5mm. Re-entry showed that laser treated sites had 1.7mm of bone fill in comparison with control sites, which had no bone fill. The Centty study suggests that laser treated sites could improve CAL gain and bone fill over conventional methods but it consisted of a small sample size.

*Laser treatment as an adjunct of periodontal surgery*

Crespi et al. (95) tested the effect of CO<sub>2</sub> lasers in class III periodontal furcations and compared that with the regeneration obtained with either GTR with non-resorbable membrane or SRP. After 6 months, the laser group showed new attachment averaging 1.9 mm whereas GTR and SRP showed 0.2 mm. Mizunami et al. (96) compared periodontal tissue healing following flap surgery using an Er:YAG laser with that of conventional surgery. Degranulation and root debridement of class III furcations were performed using the Er:YAG laser. After 3 months, histological analysis revealed a higher amount of newly formed bone but similar amounts of cementum formation and connective tissue attachment.

Sculean et al. (97) compared the healing of intrabony defects treated surgically with and without debridement using an Er:YAG laser. No statistically significant differences were found between control and test sides. Gaspirc et al. (21), in a single-blinded, split-mouth, randomized controlled trial of a 60 month duration compared the clinical outcomes of Er:YAG laser assisted periodontal flap surgery versus conventional treatment with the MWF procedure. A total of 146 single-rooted periodontally involved teeth from 25 patients with advanced chronic periodontitis were involved. Er:YAG was used to debride the intrabony pockets, scale the root surface and trim the periodontal flap. The use of Er:YAG laser compared to conventional MWF

surgery resulted in greater reduction in PD and greater CAL compared to MWF surgery alone. The reductions were significantly greater in the laser group from month 6 to 36. Differences in CAL gain were in the range of 0.3-0.4 mm, favoring the laser group.

Schwarz et al. (98) evaluated the use of the Er:YAG laser in combination with enamel matrix protein derivative (EMD) for the treatment of intrabony defects. Twenty-two patients with chronic periodontitis, each of whom displayed one intrabony defect, were randomly treated with access flap surgery and defect debridement with an Er:YAG (160 mJ/pulse, 10 Hz) plus EMD (test) or with access flap surgery followed by SRP with hand instruments plus EDTA and EMD (control). No statistically significant differences in CAL, PD, and BOP were observed between the test and control groups. Dilsiz et al. (99) in a split-mouth study on 21 patients evaluated the healing of intrabony defects after treatment with an EMD with or without Nd:YAG laser application for root surface conditioning. The root conditioning of Nd:YAG (test) or EDTA (control) led to statistically significant clinical improvements but the control group showed a greater reduction in PD and gain in CAL compared to the test group ( $P < 0.05$ ). Overall, studies have conflicting outcomes when evaluating the effect of using an Er:YAG laser as an adjunct to periodontal surgery.

### **2.5.2 Enhance wound healing and reduce pain**

Most of the studies that analyze the effect of lasers on wound healing and postoperative pain are based on the concept of low level laser therapy. It will be discussed in another section of the manuscript.

However, there are few randomized human clinical trials that have analyzed the pain on surgical lasers. Several authors (22,23,100,101) have analyzed the use of Er:YAG and Nd:YAG laser alone or in conjunction with scaling and root planning with mechanical instruments during non-surgical periodontal therapy. While Braun et al. (101) and Tomasi et al. (23) have found less pain with the use of lasers, Rotundo et al. (100) and Ambrossini et al. (22) have not found differences. Overall, the true analgesic effect that low level lasers provide is unclear.

### **2.5.3 Bacterial decontamination**

Soft tissue lasers are a choice for bacterial reduction and coagulation. The soft tissue lasers such as a Argon operating at 488 nm and 514 nm, diode operating at 800-830 nm and 980 nm, and Nd:YAG operating at 1064 nm, are well absorbed by melanin and hemoglobin and other chromophores present in periodontally diseased tissues. The laser energy is transmitted through water and are poorly absorbed in hydroxyapatite. Therefore, these soft tissue lasers' properties are excellent to interact with inflamed tissue and pigmented bacteria (27).

Fontana et al. (25) in a study on rats with induced periodontal disease showed that and application of a 810 nm diode laser operating at a range of 200-1200 mW reduced the bacteria count. Microbial samples were collected before and immediately after laser irradiation. Samples were cultured in a non-selective medium, black pigmented anaerobic bacilli, *Candida sp*, *Streptococcus betahemolyico*, and *Enterobacterium*. A control group where an optical fiber was used but no laser irradiation was applied was also analyzed. No bacterial reduction was observed in the control group. Bacterial elimination was seen, especially for *Prevotella sp*, *Streptococcus betahemolitico*, *Fusobacterium sp*, and *Pseudomonas sp* in the test group. More common periodontal bacteria such as *Agregatibacter actimecetmcomytans (Aa)*, *Porphiromona gingivalis (Pg)*, *Tannerella denticola (Td)* were not found in the control and test rats. According to this study, use of an 810nm diode laser lead to a greater amount of bacterial reduction then areas without laser treatment. On the other hand, in a previously mentioned human clinical trial study by Euzebio et al. (89), the high-intensity diode laser failed to show additional benefits to the conventional periodontal treatment in terms of colony forming units and black-pigmented bacteria at 6 weeks and 6 months after treatment. De Micheli et al. (90) found similar results in another randomized controlled human trial with no benefits of the application of diode laser on microbiological parameters. It is not clear if the differences seen in the animal study by Fontana et al. have any beneficial effect clinically or if they are maintainable over time as can be seen in the Euzebio and De Micheli human studies.

#### **2.5.4 Calculus removal**

A systematic review (102) of human studies that applied Er:YAG laser in non-surgical periodontal therapy in patients with chronic periodontitis showed equal clinical outcomes compared to mechanical debridement. The authors mentioned as well the diversity of clinical studies designs.

As mentioned earlier the Er:YAG laser possesses a wavelength capable of removing calculus without damaging dental structures. Schwarz et al. (103) compared the use of an Er:YAG laser at an energy level of 160 mJ/pulse and 10 Hz to SRP. A split-mouth design was used for treatment of 20 patients with moderate to advanced periodontal disease. The amount of time needed in the SRP group was, on average, 9 minutes for single rooted and 15 minutes for multirrooted. For the laser group, on average, 5 minutes for single rooted and 10 minutes for multirrooted. At follow-up visits of one and two years, they observed significant differences (0.9mm and 0.7mm) for the CAL ( $P < 0.001$ ) between the two treatment groups favoring laser group. The study concluded that the CAL gain obtained following non-surgical periodontal treatment with the Er:YAG laser or scaling and root planing could be maintained over a 2-year period. In terms of CAL gain over prolonged periods of time, the Er:YAG laser yields greater improvements than SRP alone.

Sculean et al. (26) treated 20 patients with moderate to advanced periodontal disease. A split-mouth design was used. Subgingival debridement using an Er:Yag laser at 160 mJ/pulse, 10 Hz or an ultrasonic instrument was applied in pockets exhibiting a probing depth of greater than 4 mm. There were no statistically significant differences found between the two groups in any of the measured clinical parameters at baseline. The sites treated with Er:YAG laser demonstrated a mean CAL gain of  $1.48 \pm 0.73$ mm, ( $P < 0.001$ ) and  $1.11 \pm 0.59$ mm, ( $P < 0.001$ ) at 3 and 6 months, respectively. The sites treated by the ultrasonic device demonstrated a mean CAL gain of  $1.53 \pm 0.67$  mm ( $P < 0.001$ ) and  $1.11 \pm 0.46$  mm ( $P < 0.001$ ) at 3 and 6 months, respectively. According to this study, ultrasonic instruments when compared to Er:Yag laser for subgingival debridement were similar in treatment outcomes.

Lopes et al. (104), in a randomized controlled clinical trial (Lopes et al. 2008),

compared conventional scaling and root planing (SRP), SRP+Er:YAG laser, Er:YAG laser alone and no treatment. Clinical parameters and microbiologic analysis were performed at baseline, 12 days, and 1, 3, 6, and 12 months after treatment and were analyzed for detection of *Aa*, *Pg*, *Td*, *Prevotella intermedia* (*Pi*) and *Prevotella nigrescens* (*Pn*) using PCR. All treatment therapies showed a benefit in clinical parameters ( $P < 0.001$ ) at 1, 3, 6 and 12 months. No significant differences were observed among SRP and Er:YAG laser, Er:YAG laser alone, SRP. Microbiologically, SRP and Er:YAG and Er:YAG presented a significant reduction in the percentage of sites with bacteria 6 and 12 months after treatment ( $P < 0.05$ ). On the other hand, Rotundo et al. (100) in a similar randomized split-mouth clinical study that evaluated the adjunctive use of Er:YAG laser to conventional SRP, did not reveal a more effective result on clinical parameters than SRP alone. Furthermore, the sites treated with Er:YAG laser showed similar results of the sites treated with supragingival scaling. Therefore, Er:YAG laser may prove to be a viable but not superior alternative to mechanical debridement.

## **2.6 Low level laser therapy ( LLLT )**

Low level laser therapy has been extensively studied using different wavelength exposure times and frequencies (87). LLLT is also known as soft, cold, low intensity laser therapy and as photobiostimulation (105). The use of a low-level laser as a therapeutic agent started was first described by Mester (106) in 1967, who studied its effect on the acceleration of wound healing in rats.

The literature regarding laser therapy is inconclusive, either showing beneficial effects (28,106,107) or no effect at all (108,109). Investigations failed to demonstrate effects possibly because of the large choices of parameters. The existence of various types of lasers showing different abilities of interaction with tissues should be taken into account, as well as failures in dosimetry, the mode of application, and the animal model used. The use of a laser to accelerate the wound healing process has been extensively studied; however, most investigations have been performed on animals (108, 109) or *in vitro* (29,110). Most human studies refer to treatment after extraction of the third molars (111,112) with only few reports in the area of periodontics being



available (37,113-115). With such a variation in study design and laser applications, it is difficult to ascertain the exact benefit and method to using lasers in periodontal applications.

### **2.6.1 Indications**

A number of applications of low level laser light have emerged. The most significant uses are photobiostimulation, composite resin curing, caries detection, photo-activated disinfection, and laser scanning in restorative dentistry and orthodontics (24).

### **2.6.2 Mechanism of action**

The mechanisms of low level laser therapy are complex. They rely upon the absorption of particular visible red and near-infrared wavelengths in photoreceptors within subcellular components, particularly the electron transport/respiratory chain within the membranes of mitochondria (116). Mitochondria catabolism energy sources using metabolic pathways including the Krebs cycle, fatty acid oxidation, and amino acid oxidation. The end result of these pathways is the production of two kinds of energy-rich electron donors, Nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH<sub>2</sub>). Electrons from these donors are passed through an electron transport chain to oxygen, which is reduced to water. This is a multi-step redox process that occurs on the mitochondrial inner membrane. The enzymes that catalyze these reactions simultaneously create a proton gradient across the membrane, producing a thermodynamically unlikely high-energy state with the potential to do work. The absorption of light by the respiratory chain components causes a short-term activation of the respiratory chain, and oxidation of the NADH pool. This stimulation of oxidative phosphorylation leads to changes in the redox status of both the mitochondria and the cytoplasm of the cell. The electron transport chain is able to provide energy to the cell, through an increased supply of ATP, as well as an increase in the electrical potential of the mitochondria membrane, alkalization of the cytoplasm, and activation of nucleic acid synthesis (28). Since ATP is the "energy currency" for a cell, LLLT has a potent action that results in stimulation of normal functions of the cell

(24).

### **2.6.3 Cellular effects**

LLLT has cellular effects during wound healing. Mester et al. (106) showed evidence of accumulated collagen fibrils and electrondense vesicles intracytoplasmatically within the laser-stimulated fibroblasts as compared with untreated areas. Also, the measurement from the incorporation of H<sup>3</sup>-thymidine showed accelerated cell reproduction and increased prostaglandin levels after irradiation (105). It has been shown to cause vasodilation which brings in oxygen and also allows for greater traffic of immune cells into the tissue. LLLT can also exert vasoactive effects by its action on mast cell degranulation. Mast cells in skin, oral mucosa, and dental pulp contain pro-inflammatory cytokines which promotes leukocyte infiltration of tissues by enhancing expression of endothelial-leukocyte adhesion molecules (116).

Laboratory studies have demonstrated a range of biostimulation effects that are shown in the table. Wound healing consists of several distinct phases, all of which can be affected at the cellular level by LLLT.

Conflicting results for the analgesic effect of LLLT may be due to the placebo potential and patient's subjective findings (105).

A recent meta-analysis of the literature (117) concerning the potential of low power lasers in tissue repair and pain relief showed positive results. Thirty-four peer papers on tissue repair and nine papers in pain relief were analyzed. All studies were *in vivo* animal or human studies. Meta-analysis revealed a positive effect on tissue repair ( $d=+1.81, n= 46$ ) and pain control ( $d=+ 1.11, n= 9$ ). The positive effect of treatment on specific indices of tissue repair was evident in the treatment effect sizes determined as follows: collagen formation ( $d= + 2.78$ ), rate of healing ( $d=+1.57$ ), tensile strength ( $d=+ 2.13$ ), time needed for wound closure ( $d= +0.76$ ), tensile stress ( $d=+2.65$ ),

number and rate of degranulation of mast cells (d= +1.87), and flap survival (d=+1.95). Those studies suggest that LLLT is beneficial and may aid in tissue repair and pain relief.

**Table 1:** Biostimulation effects of lasers (116)

Fibroblast	<ul style="list-style-type: none"> <li>Proliferation</li> <li>Maturation</li> <li>Locomotion</li> <li>Transformation into myofibroblast</li> <li>Reduced secretion of PGE2 and IL-1</li> <li>Enhanced secretion of bFGF</li> </ul>
Macrophages	<ul style="list-style-type: none"> <li>Phagocytosis</li> <li>Secretion of fibroblast growth factors</li> <li>Fibrin resorption</li> </ul>
Lymphocytes	<ul style="list-style-type: none"> <li>Activation</li> <li>Enhanced proliferation</li> </ul>
Epithelial cells	<ul style="list-style-type: none"> <li>Motility</li> </ul>
Endothelium	<ul style="list-style-type: none"> <li>Increased granulation tissue</li> <li>Relaxation of vascular smooth muscle</li> </ul>
Neural tissue	<ul style="list-style-type: none"> <li>Reduces synthesis of inflammatory mediators</li> <li>Maturation and regeneration</li> <li>Axonal growth</li> </ul>

**Dental applications**

Reported applications in clinical dentistry include the following:

- 1 Dentin hypersensitivity (33)
- 2 Post-extraction socket/post-trauma sites (118)
- 3 Viral infections: herpes labialis, herpes simplex (119)
- 4 Neuropathy: trigeminal neuralgia, paraesthesia (34).
- 5 Aphthous ulceration (120)
- 6 Temporomandibular joint disorders (34)
- 7 Post-oncology: mucositis, dermatitis, post-surgery healing (121)
- 8 Accelerated human teeth movement in orthodontic patients (36)

#### **2.6.4 Dosage**

To calculate the dose (energy density) of a laser, power (mW) is multiplied by time (seconds) (eg 100 mW x 10 seconds = 1000 mJules = 1 Jule (J)). The dose is calculated by dividing the energy by the irradiated area. If this area is 1 cm<sup>2</sup>, the calculations is 1/1 = 1 J/ cm<sup>2</sup>. If the irradiated area is 0.25 cm<sup>2</sup>, the calculation is 1/0.25 = 4 J/cm<sup>2</sup> (105). Contact mode is needed for all applications with the exception of open wounds which requires a 2 to 4 mm separation distance between the laser and the target tissue (105).

Previous studies indicated that laser irradiation at energy densities up to 4 J/cm<sup>2</sup> had stimulating effects whereas higher energy densities had rather inhibitory characteristics on fibroblast growth (116,122). This phenomenon, not observed by the Kreisler (110) investigation where laser induced cellular effects, were comparable within the range of 2-8 J/cm<sup>2</sup>. The laser literature shows that biomodulatory effects are also dose dependent (123). In addition, other factors such as cell growth phase (124) and the frequency and number of sessions are important (125). The use of inappropriate wavelengths may result in negative results (126). Higher wavelengths are more resistant to dispersion than lower ones and penetrate more deeply into skin. Previous studies reviewed by Basford (122) mentioned that 632.8 nm laser light penetrates 0.5-1mm before losing 37 % of its intensity.

Woodruff et al. (127) in a review study revealed that energy density is the only treatment parameter with predictable dose dependent treatment effects. Five studies with energy densities ranging from 19 to 24 J/cm<sup>2</sup> had the largest average effect size. It was suggested that this range of energy densities has more positive effect than other dose levels but there was a variability in the experimental models used and definitive conclusions could not be made.

### **2.6.5 LLLT and Oral/Periodontal Treatment**

#### *In vitro and animal studies*

*In vitro* studies studying the use of LLLT in oral cells have been mainly focusing in skin and embryonal fibroblasts. Published data indicates that low-power irradiation can enhance DNA synthesis (29), collagen (30), and procollagen production (128), increase proliferation rates (31) and alter locomotory characteristics in connective tissue cells. Little is known about the effects on human gingival and oral fibroblasts. Kreisler (110) investigated the effect on the proliferation of human gingival fibroblasts using an 809 nm diode laser at energy fluences 1.96-7.84 J/cm<sup>2</sup>. In order to investigate a possible effect of successive laser treatments (10 mW, 75 seconds), irradiation was carried out two and three times, respectively, at 24 hours interval. The differences between test and controls were highly significant ( $p < 0.001$ ) 24 hours after irradiation. Fortyeight and 72 hours after irradiation the differences decreased in an energy-dependent manner. Pourzarandian (128) studied as well the effect of low level laser, Er:YAG, on human gingival fibroblasts using fluences 1.68-5.0 J/cm<sup>2</sup>. Er:YAG significantly stimulated proliferation. Transmission electron microscopy showed that enhanced proliferation of gingival fibroblasts were accompanied by an earlier mitosis and increased collagen fibril strand expression. The optimal stimulative energy density was found to be 3.37 J/cm<sup>2</sup>.

Stein (129) examined the effect of LLLT on proliferation and differentiation of human osteoblast *in vitro* using markers such as alkaline phosphatase. Cultured cells were irradiated using a He-Ne laser irradiation at 632 nm, 10 Mw. Different laser energy densities were applied (0.14, 0.43, and 1.43 j/cm<sup>2</sup>). A significant 31-58 % increase in cell survival and a higher cell count was seen with the 0.14 j/cm<sup>2</sup> density

irradiation as compared to the non-irradiated. Expression of alkaline phosphatase was manifested in 40-60 % of the laser-irradiated cells, whereas only 20 % of the non-irradiated cells showed positive staining of this enzyme. Expression of osteopontin and sialoprotein was much higher in the irradiated osteoblast.

Arisu (130) found that He-Ne and 20 mJ, 10 Hz, 10 s Nd:YAG laser irradiation had a stimulatory effect on the cell viability and proliferation of human osteoblast-like cell culture. The author concluded that an increase in the pulse energy, pulse repetition rate, and power output had an inhibitory effect on the cell viability and proliferation.

Garavello (131) studied the effect of LLLT on angiogenesis on rat tibia. The author surgically created a 1.6 mm diameter hole in rat tibia and irradiated the area with He-Ne laser with energies 31.5 or 94.5 J/cm<sup>2</sup> for 7 to 14 days after surgeries. The He-Ne laser significantly increased the number of blood vessels after 7 days irradiation at an energy density of 94.5 j/cm<sup>2</sup>, but significantly decreased the number of vessels in the 14 day irradiated tibiae, independent of the dosage.

Jackse (132) evaluated the effect of a 680 nm, 75 mW diode laser in the bone regeneration and osseointegration of dental implants in a sinus graft of 12 sheep. Irradiation was given intraoperatively, and repeated on the first, third, and seventh day postoperatively. Histomorphometric analysis showed no differences in bone regeneration but osseointegration measurements resulted in a significantly higher bone/implant contact on the test side ( $p= 0.045$ ).

#### *Human studies*

Ryden (113) studied the effect of low level energy infra-red laser irradiation on gingival inflammation. Gingivitis was induced in ten female dental students by discontinuing from all oral hygiene measures for 28 days. On days 21 and 24 the marginal gingiva, buccal to one of the lateral mandibular incisors, was exposed to 4 minutes of laser irradiation (total dose = 1J). The gingiva associated with the

contralateral incisors was exposed to ordinary light and served as control. There was no statistical difference between the laser-exposed sites and the control sites with regard to either plaque formation or gingival bleeding. Gingivitis was evaluated with the aid of a stereophotographic method by calculating changes in the number of gingival vessels. It was found that the number of vessels increased over time for both laser exposed and control sites. The difference between sites at day 28 was not statistically significant ( $t = 0.82, p > 0.05$ ). These results suggest that low energy laser irradiation did not influence the inflammatory reaction of the gingiva.

Yilmaz (114) studied the effect of using a GaAr diode laser ( $1.6 \text{ j/cm}^2$ ) applied 3 times per week. After scaling and root planing, single rooted teeth having an interproximal site with a PD of 4mm were irradiated on 10 patients. Each dental quadrant was randomly designated to receive SRP, laser, combination SRP-laser, or oral hygiene instructions. Plaque index, gingival index, bleeding on probing, probing depths and microbiology were evaluated after 32 days. Sterile paper points were inserted into the depth of the pocket, left for 10 seconds, and cultured. All the microbiologic data were transformed into colony forming units/milliliter. Obligate anaerobic bacteria was calculated as the total counts of anaerobically cultivable bacteria minus the total counts of facultatively anaerobic bacteria and expressed as a percentage of anaerobically cultivable bacteria. The use of laser did not have any additive effect on SRP and did not significantly reduce the parameters when it was used as monotherapy. On the other hand, Qadri (133) in a human double blind split-mouth design using a combination of lasers indium-gallium-aluminium-phosphide (635 nm) and gallium-aluminium-arsenide (820 nm) applied 6 weeks after SRP found the following: probing depths, plaque and gingival indices were reduced more in the laser gingiva than in the control side ( $p < 0.01$ ). The decrease in gingival crevicular fluid volume was greater on the laser side than on the placebo side ( $p = 0.01$ ). The total amount of matrix metalloproteinase 8 increased on the placebo side but was slightly lower on the laser side ( $p = 0.052$ ).

Aykol et al. (39) evaluate the effect of low-level laser therapy as an adjunct to non-surgical periodontal therapy of 36 patients with moderate to advanced chronic periodontitis. Periodontal treatment consisted of full-mouth subgingival scaling and root planing using hand instruments and ultrasonic devices. For the test group A diode

laser with a wavelength of 808 nm and an energy density of 4 J/cm<sup>2</sup> was applied to the gingival surface after periodontal treatment on the first, second, and seventh days. Plaque index, sulcus bleeding index, PD, CAL and gingival crevicular fluid were analyzed at baseline, 1, 3, and 6 months after the treatment. At all time points, the LLLT group showed significantly more improvement in sulcus bleeding index, clinical attachment level, and probing depth levels compared to the control group ( $P < 0.001$ ), although differences were minimal (0.2 mm). No marker level of the gingival crevicular fluid change showed significant differences between the groups ( $P < 0.05$ ).

Damante (115) analyzed histomorphometrically the effects of LLLT application (diode laser 15 mW, 670 nm every 48h for 1 week) on the healing of human oral mucosa. Incisional biopsies were taken 7, 14, 21, 60 days after surgery. The study concluded that LLLT did not accelerate the healing with respect to placebo. Masse (37) evaluated the effect that soft laser had on donor and recipient sites of 28 bilateral pairs of free gingival grafts. The laser applied was a Ga-As laser but no energy densities were mentioned. Laser sides did not improve the analgesic, anti-inflammatory, and healing effects over placebo side. To the contrary, Ozcelik (134) in a split-mouth controlled clinical trial assessing the effects of LLLT on gingivectomy/gingivoplasty and found a positive effect on epithelization. Twenty patients received gingivectomy/gingivoplasty and diode laser (588 nm, 4 j/cm<sup>2</sup> daily for 7 days) to one side of the operated area. LLLT was found to enhance surface epithelization with respect to controls post-operatively on the third, 7<sup>th</sup>, and 15<sup>th</sup> day. Almeida et al. (38) evaluated the effect of low-intensity diode laser (660-780 nm, 10J/cm<sup>2</sup>, day of the surgery and 48 h) on the healing process and analgesia in 10 individuals undergoing bilateral free gingival grafts. No statistically significant difference was found at any postoperative period between control and test sides, even though greater clinical improvement associated with treatment was observed at 15 d postoperative.

Aboelsaad et al. (135) investigated the influence of low-power 830 nm diode laser (continuous wave 40 mW and fluence 4 J/cm<sup>2</sup>, with total energy density of 16 J/cm<sup>2</sup>) on the healing of human infrabony defects treated with bioactive glass graft material. Twenty patients with chronic periodontitis and bilateral infrabony defects were included. Using a split mouth design, 20 defects with bioactive glass plus laser



irradiation during surgical procedures and on days 3, 5, 7 postoperatively; 20 contralateral defects were treated with bioactive glass only. Clinical parameters (CAL,PD) and standardized periapical radiographs were recorded at baseline and at 3 months and 6 months postoperatively. At 3 months there was a statistically significant difference between the laser and non-laser sites in the parameters investigated. However, at 6 months, no difference was observed. The authors concluded that LLLT accelerated the wound healing. It can be concluded that LLLT enhances periodontal healing during the initial period of healing.

Few studies have addressed the effect that lasers have on pain in periodontics. Masse et al. (37) and Almeida et al. (38) failed to show a significant analgesic effect of low level lasers when used after the placement of free gingival grafts.

## **2.7 Complications and pain following periodontal therapy**

Periodontal disease is not necessarily painful and may consequently have negative effects on oral health due to late recognition. Conversely, periodontal treatment is experienced as painful by substantial numbers of patients (136). Curtis et al. (77) examined the incidence and severity of postoperative pain in 304 periodontal surgical cases involving plastic soft tissue surgery, osseous surgery, and mucogingival procedures. The patient postoperative pain was assessed 1 week after the periodontal procedure by subjective reporting using a verbal rating scale and objectively by recording the number and type of analgesics. Postoperative complications (bleeding, infection, swelling or adverse tissue changes) account for only 5.5%. Osseous surgery was three times more likely to cause complications than mucogingival surgery. Minimal or no postoperative pain was reported by 51.3% of the patients. Pure mucogingival surgery was significantly related to pain and was 3.5 times more likely to cause pain than osseous surgery and 6 times more likely than plastic soft tissue surgery. The duration of the surgery was statistically significant for both complications and pain.

Mathews and McCulloch (6) used a visual analog scale to investigate differences

between patients in their assessment of surgical and non-surgical periodontal treatment. The questionnaire tested for variables of pain, sensitivity, swelling, function, altered appearance, comparison of postoperative discomfort to restorative treatment (crown or filling), discomfort experienced during the procedure, and resultant disability days. They reported that surgery, particularly soft tissue grafts and osseous surgery, produced significantly more discomfort than non-surgical treatment. Philstrom et al. (137) used a hybrid scale, which incorporated visual analog scale (VAS) to study pain-associated treatment. Almost all (90%) patients treated in a university clinic reported some discomfort following scaling and root planing under local anesthesia performed by dental students, and a considerable proportion (44 %) reported mild to severe pain.

Fardal (8) used a VAS to study the patient perceptions during and after different periodontal therapies in a private practice setting. VAS was given to all the patients after 3 weeks of completion of MWF surgical procedures. Patients quantified the discomfort they had experienced during the periodontal examination, recording of X-rays, SRP, administration of local anesthesia, and periodontal surgery. Postoperative discomfort and posttreatment sensitivity was also quantified. The average VAS score for postoperative discomfort was 5.5 and was significantly less than that registered for postoperative sensitivity. The duration of the discomfort ranged from a few hours (3%), 1 day (10%), 2 to 3 days (7%) 5 days (1%) a more than 7 days (2%). Fifteen percent had to take pain medication. The levels of discomfort were found to decrease with age. The last three studies mentioned that the patients found periodontal therapy less uncomfortable the second time around. It is unclear why patients experience less pain after a second surgical procedure but may be related to psychological expectations.

Jorklend and Skoglund (138) used VAS to evaluate the influence of various local anesthetic agents and periodontal dressings. They concluded that the choice of local anesthetic and periodontal dressing could influence the postoperative pain reported by patients following gingivectomy. They reported low mean discomfort (10 to 25 out of maximum score of 100) regardless of the periodontal dressing, type of local anesthetic, or the concentration used.

Canakci (9) assessed the levels of postoperative pain, postoperative dentin

hypersensitivity, and the discomfort patients experience during various periodontal treatments by using a VAS. No statistically significant differences were found between the patients' discomfort levels associated with the four therapy types during periodontal treatment. However, postoperative pain was significantly higher for flap with osseous resection ( $p < 0.01$ ) and gingivectomy ( $p < 0.05$ ) procedures than for SRP and MWF procedures. All surgical procedures produced significantly more dentin hypersensitivity than did nonsurgical therapy. The analysis showed no statistically significant differences between male and female patients' discomfort during periodontal treatments. For all periodontal treatments, VAS scores decreased with increasing age

### **3.HYPOTHESIS/HIPÓTESIS**

#### **3.1 General Null Hypothesis/Hipótesis General Nula**

- The application of a 810 nm diode laser following modified Widman flap does not improve healing and reduce postoperative pain.
- El uso de un laser de diodo de 810nm después de un colgajo de Widman modificado no mejora la cicatrización ni reduce el dolor postoperatorio.

#### **3.2 General Alternative Hypothesis/Hipótesis General Alternativa**

- The application of a 810 nm diode laser following modified Widman flap improves healing and reduces postoperative pain.
- El uso de un laser de diodo de 810 nm después de un colgajo de Widman modificado mejora la cicatrización y reduce el dolor postoperatorio

#### **3.3 Specific Alternative Hypothesis/Hipótesis Específicas Alternativas**

1. The application of a 810 nm diode laser following modified Widman flap
  - a. Improves clinical periodontal parameters (CAL, PD, REC, BOP, GI, PI)
  - b. Improves tissue response (edema and erythema)
  - c. Reduces postoperative pain (pain and medication consumption)
2. Smoking does not negatively impact the healing after periodontal treatment when a 810 nm diode laser is used following modified Widman flap.

1. El uso del laser de diodo de 810 nm después de haber realizado un colgajo de Widman modificado:
  - a. Mejora los parámetros periodontales clínicos (nivel de inserción,

profundidad de bolsa, recesión, sangrado al sondaje, índice gingival e índice de placa)

- b. Optimiza la respuesta tisular (edema y eritema)
  - c. Reduce el dolor postoperatorio (dolor y cantidad de medicación ingerida).
2. El hábito tabáquico no perjudica la cicatrización cuando se usa un laser de diodo de 810 nm después de un colgajo de Widman modificado

## **4.OBJECTIVES/OBJETIVOS**

### **4.1 General Objective/Objetivo General**

- Evaluate the effect of a 810nm diode laser following modified Widman flap on periodontal healing and postoperative pain.
- Evaluar el efecto del uso de un laser de diodo de 810 nm después de un colgajo de Widman modificado en la cicatrización y el dolor postoperatorio

### **4.2 Specific Objectives/Objetivos Específicos**

1. The specific objectives of the study about the application of a 810 nm diode laser following a modified Widman flap were:
    - a. Assess the clinical periodontal parameters.
    - b. Prove enhanced periodontal tissue response
    - c. Reduce postoperative pain
  2. Stablish the effect of smoking on the periodontal healing when a 810 nm diode laser is used following modified Widman flap.
- 
1. Los objetivos específicos del estudio sobre el uso de laser de diodo de 810 nm después de un colgajo modificado de Widman fueron:
    - a. Evaluar el efecto en los parámetros clínicos periodontales.
    - b. Demostrar una mejora en la respuesta tisular
    - c. Reducir el dolor postoperatorio
  2. Establecer el efecto del tabaco en la cicatrización periodontal cuando un laser de diodo de 810 nm es usado después de un colgajo de Widman.



## **5.MATERIAL AND METHODS**

### **5.1 Population**

Subjects from the dental clinic at the University of Buffalo, School of Dental Medicine in New York were recruited and treated in the periodontal clinic. A total of fifteen patients (9 males and 6 females) with a mean age of 48 years (Range 31) were included in the study. Each patient had two contra-lateral periodontal pockets (same type of tooth in opposite quadrants of the mouth treated). Two patients dropped out of the study after the first surgical procedure since they could not keep subsequent appointments, leading to 26 periodontal sites in 13 patients with a mean age of 52 years (Range 31) for study analysis.

### **5.2 Exclusion and Inclusion Criteria**

Health conditions that excluded patients to participate in to the study were:

- Candidates who reported long-term steroidal or antibiotic therapy,
- Systemic diseases likely to affect wound healing
- Current pregnancy.

Criteria that included patients in to the study were:

- Age Range: 18-75 years
- Sex: Male and Female.
- Bilateral pair of maxillary or mandibular single or multi-rooted qualifying teeth. A qualifying tooth must have the following:
  - PD of  $\geq 7$ mm.
  - CAL of  $\geq 7$  mm
  - BOP and a GI  $\geq 1$  (139).



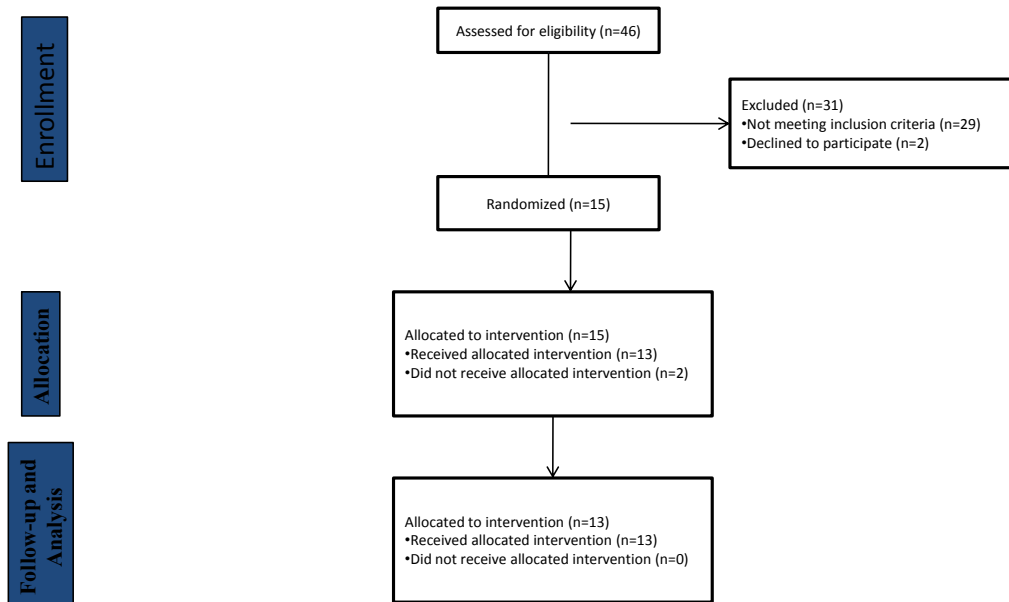
### 5.3 Study Design

This study was a randomized single masked split mouth clinical study. The patient did not know which site received laser (810nm) therapy. One operator (JS) enrolled, evaluated, and treated all patients. The primary outcome with respect to the effect of laser (810nm) use during periodontal surgery were clinical parameters (CAL,PD,BOP,PI,GI).

The outline of the study is described in a flowchart (Fig. 1; Consolidated Standards of Reporting Trials [CONSORT] statement flowchart).

### 5.4 Duration of the study

Overall treatment time averaged 5.5 months. This allowed 4 weeks for the patient to complete the screening and qualification process, and 4.5-5 months to complete the study.



**Figure 1.** CONSORT statement flowchart.



## **5.5 Clinical Procedures**

### **5.5.1 Screening examination**

Patients were evaluated by the investigator (JS). Patients underwent a screening examination to determine whether they qualified for entry to the study. At the screening visit patients completed a health history questionnaire to insure that they were medically qualified for participation in the study. The investigator reviewed with the patient the information they provided and verified the contents of the Medication History Forms. All information was recorded.

If patients still qualified up to this point, they were advised of their role in this study. An informed consent (Appendix 1) was read by the patient. Any questions relative to the consent form and the study were answered prior to the patient signing the informed consent. An informed consent form was signed by each patient. The study and informed consent form was approved by the University at Buffalo Health Sciences Institutional Review Board (P/E0510307E) (Appendix 2).

After giving consent, patients underwent an oral tissue examination. After completion of the oral tissue exam patients underwent a full mouth manual probing (using a UNC-15 probe) to determine their periodontal status.

At this visit, full mouth manual probing measurements were recorded. The PD, CAL and recession (REC) measurements were recorded to the nearest millimeter for six sites about each tooth. The sites were mesial buccal contact, mid-buccal, distal buccal contact, mesial lingual contact, mid-lingual and distal lingual contact. PD was measured from the free gingival margin with the manual probe. REC was measured from the cemento-enamel junction reference point. When the gingival margin (GM) was located coronal to the CEJ the measure (GM-CEJ) was recorded as a positive value. When the gingival margin was located apical to the CEJ the measure was recorded as a negative value. CAL was calculated as the sum of PPD and REC.

Bleeding on probing (BOP) (140) was assessed at six sites per tooth through gentle probing of the orifice of the gingival crevice. If bleeding occurred within 10 seconds a positive finding was recorded and the number of positive sites was recorded and then expressed as a percentage of the number of sites examined.

The modified gingival index (139) is a non-invasive (no probing) gingival index rating inflammation form 0 to 4. The following criteria are adopted:

- 0 = absence of inflammation;
- 1 = mild inflammation or with slight changes in color and texture but not in all portions of gingival marginal or papillary;
- 2 = mild inflammation, such as the preceding criteria, in all portions of gingival marginal or papillary;
- 3 = moderate, bright surface inflammation, erythema, edema and/or hypertrophy of gingival marginal or papillary;
- 4 = severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration.

The scores of the four areas of the tooth can be summed and divided by four to give the GI for the tooth. The mGI of the individual can be obtained by adding the values of each tooth and dividing by the number of teeth examined. The Gingival Index may be scored for all surfaces of all or selected teeth or for selected areas of all or selected teeth..

The Turesky plaque index (141) was evaluated after PD and REC measurements. It was assessed at six sites per tooth as follows:

- 0 = No Plaque
- 1 = Flecks of plaque at gingival margin
- 2 = Line of plaque at gingival margin
- 3 = Plaque covering gingival third of surface
- 4 = Plaque covering two-thirds of surface
- 5 = Plaque covering more than two-thirds of surface

If after the initial full mouth probing the patient had at least one site per quadrant that qualified for entry into the study, a repeat probing of that site, in the same order of examination, was conducted. These repeat measurements were being conducted in order to establish the intra-examiner analysis. For CAL, strength of agreement in two measurements was very good (weighted Kappa=0.85,  $p<0.0001$ ) (142)

Periapical radiographs and bitewings of the selected sites were obtained using a parallel cone technique.

### **5.5.2 Baseline examination**

Patients who qualified for the study at the screening examination returned for the baseline visit within thirty (30) days after the screening examination.

At this visit, clinical measurements were recorded as described in the screening examination section.

At the baseline visit, the contra-lateral surgical sites were randomly assigned to control or to test using a coin flip.

Following the examination, the investigator (JS) provided a full-mouth supragingival prophylaxis and subgingival scaling and root planing of each tooth selected plus all teeth in the quadrants in which that tooth was located. The time spent for each quadrant was a minimum of 45 minutes. If other non-study quadrants needed subgingival scaling and root planing, this procedure was done in another non-study visit.

Before ending the visit, patients were given oral hygiene instructions. It was recommended to the participants to use a sulcular brushing technique with a soft

brush, to floss, and to use interproximal brushes after every meal.

### **5.5.3 Week 6**

The patients returned six weeks after their baseline visit for another examination. At this visit, clinical measurements were recorded as described in screening examination.

The same oral hygiene instructions at the baseline visit were again given at this visit.

### **5.5.4 Week 7**

At this visit, patients received a Modified Widman flap at one of the study sites selected plus the quadrant in which that tooth was located.

At the baseline visit, the contra-lateral surgical sites were randomly assigned to control or to test using a coin flip. Contra-lateral meant same type of tooth in opposite quadrants of the mouth. The study tooth/site was treated plus any additional teeth in the quadrant in which the site was located, if needed. One of the study quadrants was selected at random to receive deepithelialization of the flap using the diode laser. The control sites received a MWF (47) and sham application of a diode laser (810nm). The test sites received a MWF with the application of an active diode laser (DL) (810nm) to the inside of the modified Widman flap. The time between the two surgeries was 3 weeks. The second surgery was scheduled for week 10.

#### **5.5.4.1 Surgical procedures**

##### *Anesthesia*

Topical anesthesia with 20 % benzocaine in a water soluble polyethylene glycol

base was applied to the surgical site. Local infiltration of 2 % lidocaine with 1:50000 epinephrine was then administered buccally and lingually in the maxilla and mandible. For mandibular procedures, an additional inferior alveolar nerve block was given using 2% lidocaine with 1: 50000 epinephrine.

*Flap design*

1. The MWF was used:
  - a. The initial incision was an internal bevel incision to the alveolar crest starting 1 mm away from the gingival margin. If the amount of attached gingiva present was less than 3mm, a sulcular incision was performed. Scalloping followed the gingival margin. The flap involved the study teeth plus the periodontally involved teeth with a pocket depth  $\geq 5$  mm in the same study quadrant. No vertical incisions were performed.
  - b. A crevicular incision was made from the apical aspect of the pocket to the bone.
  - c. A mucoperiosteal flap was reflected with a periosteal elevator.
  - d. A third incision was made in the interdental spaces coronal to the bone with an interproximal knife, and the gingival collar was removed.
  - e. Tissue tags and granulation tissue were removed with hand, ultrasonic and rotary instruments. Rotary instrumentation with a high-speed handpiece using multifluted finishing burs or fine diamonds with copious irrigation facilitated efficient debridement of the lesion with minimal trauma. The root surfaces were scaled and root planed using curettes and scalers.
  - f. In one control quadrant and in one test quadrant in two different patients, minor osteoplasty was performed in order to improve adaptation of periodontal flaps.
2. Control sites: laser (810nm) application was simulated without pushing the start button. The MFW was sutured with an interrupted suture using 4-0 black silk suture in all patients

3. Test sites: an aluminium, gallium and arsenide diode laser (Odyssey 2.5 G, Ivoclar Vivadent, Amherst, NY, USA) with a wavelength 810 +/- 20 nm and a power of 1 watt in a continuous mode was applied to the MWF. A 400 microns diameter tip was used to remove all visible epithelium in the inner side of the flap from the free gingival margin to the bottom of the apical aspect of the flap (both labial and lingual/palatal). The tip was initiated with the use of a blue articulating paper. The treatment was performed from the coronal to the apical aspect in parallel paths and the laser (810nm) emission was interrupted for 30 seconds after irradiation exceeded 10 seconds in time. The resultant char layer was totally removed with moist gauze prior to replacing the flaps. Care was taken to avoid any laser (810nm) contact to the root surface or the alveolar bone by placing a periosteal retractor between the hard and soft tissue and aiming the laser (810nm) beam at a 45° angle to the soft tissue flap. A second laser (810nm) application with the same laser (810nm) in continuous mode at 0.1 watts was made. All surfaces of the flap, inner and outer, exposed bone and exposed root structures involved in the surgery were irradiated, leading to a total dosage of 4 J/cm<sup>2</sup> per surface. The MFW was sutured with an interrupted suture using 4-0 black silk suture in all patients.

All surgeries were performed by a third year periodontics resident (JS). Surgeries performed on all patients were virtually identical. In each patient and all procedures were completed within a one hour time frame. The power output of the laser (810nm) was assessed throughout the duration of the study using a hand held meter provided with the unit.

#### *Postoperative Instructions*

Postoperative instructions were given to the patients (Appendix 3). A soft toothbrush was to be used during the first two weeks after the surgical procedure. Patients were requested to avoid brushing with their pre-operative daily tooth brush, flossing and chewing in the treated area for a period of 2 weeks. Patients resumed full oral hygiene and function after 2 weeks. Ibuprofen 200 (up to 3 tablets) every 8 hours

was allowed as the pain medication. Patients evaluated postoperative pain or discomfort daily at night time for one week using a modified visual analogue scale graduated from 0 to 10. Patients reported the number of 200 mg ibuprofen tablets they use and the daily amount of pain medication used was recorded. Self-reported postoperative pain as measured daily at night using a modified visual analogue scale and daily consumption of pain medication. Tissue response with regard to levels of tissue edema and color was based upon visual analysis.

### **5.5.5 Week 8**

The daily modified visual linear scale and the self-reported consumption of pain medication was returned to the investigator one week later. Sutures were also removed and tissue response was evaluated at the study site. The examiner did not know which site received which treatment.

The tissue edema was scored as follows: 1= absent, 2=slight, 3= moderate, 4= severe. The color of the gingiva was recorded as follows: 1= pink, 2= mixed, 3= red. Five patients were used to calibrate the examiner. The patients were evaluated on two occasions, 1 hour apart. The reliability coefficient of the examiner for erythema was substantial (weighted Kappa=0.71) and substantial (weighted kappa 0.75) for edema.

### **5.5.6 Week 10**

Two to three weeks after the patient received a Modified Widman flap, the second Modified Widman flap at the other paired site and the quadrant in which it was located was performed. Postoperative instructions, the daily modified visual linear scale and the self-reported consumption of pain medication were given as described in week 7.



### 5.5.7 Week 11

Patients returned to the clinic one week after the second Modified Widman flap was performed. The same procedures were performed as in week 8.

### 5.5.8 Month 5

At this visit, 6-8 weeks after the last surgical procedure, the same clinical measurements were recorded as described in screening examination.

Patient also received a supragingival prophylaxis, subgingival scaling and root planing and oral hygiene instructions reinforcement. The 5 month visit was based on a 30 day month. Each visit occurred within the designated time frame plus or minus 10 days.

### 5.5.9 Times and events schedule

Event	Screen	Baseline	6 wks	7 wks	8 wks	10 wks	11 wks	5 mon
<b>Medical history</b>	X							
<b>Informed consent</b>	X							
<b>Full mouth probing</b>	X	X	X					X
<b>Oral tissue exam</b>	X							X
<b>BOP/GI/PI</b>	X	X	X					X
<b>Radiographs</b>	X							
<b>Supragin prophylaxis</b>		X						X
<b>Scaling and root planing</b>		Test and Control sites						X
<b>MWF</b>				Control sites				
<b>MWF+DL</b>						Test sites		
<b>Suture removal, Tissue response evaluation</b>					X		X	

## **5.6 Statistical Analysis**

A power calculation before the initiation of this study revealed that a sample size of 15 was necessary to detect a difference of 2 mm in CAL, assuming a maximal mean standard deviation of 2.5 mm using a paired test with 80% power and a 0.05 significance level.

The results of this study were analyzed by presenting descriptive statistics and making comparisons between treatment sites in each patient. Descriptive statistics by treatment group for continuous variables consisted of sample sizes, means, standard deviations, standard errors, and range. For categorical variables, frequencies and percentages were displayed. Hypothesis testing of the difference between treatment groups were examined on both categorical and continuous variables.

Due to the design of this study, both test and control treatments were on different teeth and quadrants of the same patient. In hypothesis testing, for categorical variables, a McNemar's test was used to examine the relationship of treatment effect on dental variables. Study hypothesis of treatment differences were analyzed using paired t tests or Wilcoxon Signed Rank Test where appropriate. A Shapiro-Wilk test was used to assess the normality of the data. A paired-t test was used if distribution of the data was normal. If data was non-normal, a Wilcoxon Signed Rank Test was used instead. To control the possible effects of certain factors such smoking and plaque on clinical measurements after treatment, an unpaired-t test was used if distribution of the data was close to normal. If normality was in question, a Wilcoxon Signed Rank Test was used instead.

Clinical parameters were analyzed per study site and per quadrant site separately due to differences on number of teeth treated per study quadrant.

Study hypothesis for pain scale, medication consumption, edema and erythema were analyzed using paired t tests, Wilcoxon signed rank test and repeated-measures

analysis of variance (ANOVA) where appropriate. The effect of smoking on edema and erythema was analyzed using a nested ANOVA. A Shapiro-Wilk test was used to assess the normality of the data. A paired-t test was used if distribution of the data was normal. A Wilcoxon signed rank test was used if distribution of the data was non-normal.

All statistical tests utilized two-sided p-values and a statistical software (SAS version 9.01, Cary, NC, USA) was used in the analysis of the study data. Differences associated with p-values  $\leq 0.05$  were declared statistically significant.

Reliability of measurement of clinical (CAL) and tissue response (edema and erythema) variables by examiner was assessed. Results of the repeated measurements were assessed by means of percent of agreement and weighted Kappa statistics. Landis and Koch (142) proposed that Kappa coefficient of 0 is a poor agreement, 0.01-0.20 is slight, 0.21-0.40 is fair, 0.41-0.60 is moderate, 0.61-0.80 is substantial, and 0.81 to 1 is almost perfect.

## **6.RESULTS**

### **6.1 Demographics**

The number of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome are shown in Figure 1. Two patients dropped out of the study after the first surgical procedure since they could not keep subsequent appointments, leading to 26 periodontal sites in 13 patients for study analysis. Demographic and clinical characteristics of the 13 patients for study analysis are shown in table 2.

Clinical parameters were analyzed per study site and per quadrant site separately due to differences on number of teeth treated per study quadrant. Same results were seen. Results shown are per study sites.

There was no statistically significant difference between test and control sites concerning clinical criteria (PI, GI, BOP, PD, CAL, REC) at baseline.

**Table 2** Demographics of study participants.

	Participants
Number	13
Age (years)	52 ± 8.5
Gender (female)	5 (38.5%)
Smoking status	7 (53.8%)
African	2 (15.4%)
Caucasian	11 (84.6%)

## **6.2 Clinical data**

### **6.2.1 Clinical Attachment Level (Table 3,4)**

Mean CAL was initially  $7.87 \pm 1.19$ mm for the control sites and  $7.53 \pm 1.60$ mm for the test sites. After SRP, the mean CAL was  $6.80 \pm 2.04$ mm for control sides and  $6.80 \pm 1.32$ mm for test site. Both treatments modified the CAL ( $p \leq 0.05$ ) but differences between groups (test and control) were not statistical significantly different ( $p = 0.7305$ ). After surgical treatment, the mean CAL was  $5.92 \pm 1.66$ mm at control sites and  $6.15 \pm 1.41$ mm at the test sides. Both treatments modified the CAL ( $p \leq 0.05$ ). CAL gain for control sites was  $1.38 \pm 1.45$ mm and  $1.85 \pm 1.63$ mm for test sites. Differences between groups were not statistical significantly different ( $p = 0.5488$ ).

### **6.2.2 Pocket Depth (Table 5,6)**

The baseline PD mean was  $7.20 \pm 0.86$ mm in the control sites and  $7.13 \pm 0.52$ mm in the test sites. After SRP, PD changed to  $5.93 \pm 1.3$ mm in the control sites and  $5.80 \pm 0.94$ mm for the test sites. Both treatments demonstrated a decreased PD ( $p < 0.001$ ). Differences between sites control and test were not statistically different ( $p = 0.7813$ ). After surgical treatment, PD changed to  $3.15 \pm 0.9$ mm in the control sites and  $3.23 \pm 0.93$ mm for the test sites. Both treatments demonstrated a decreased PD ( $p < 0.001$ ). PD reduction for control sites was  $3.92 \pm 0.95$ mm and  $3.85 \pm 1.21$ mm for test sites. Differences between sites treated MWF or MWF + laser were not statistically different ( $p > 0.9999$ ).

### **6.2.3 Recession (Table 7,8)**

The baseline REC mean was  $0.77 \pm 1.30$ mm in the control sites and  $0.43 \pm 0.97$ mm in the test sides. After SRP, REC changed to  $0.92 \pm 1.32$ mm at the control sites and  $1.15 \pm 1.573$ mm at the test sites. Both treatments demonstrated an increased REC that reach statistical significance at test sites ( $p < 0.05$ ). Differences between sites control and test were not statistically different ( $p = 0.7026$ ). After surgical treatment, REC changed to  $2.61 \pm 1.26$ mm in the control sites and  $2.69 \pm 1.65$ mm for the test sites. REC increase for control sites was  $1.84 \pm 1.54$ mm and  $2.23 \pm 1.71$ mm for test sites. Both treatments demonstrated a REC increase ( $p < 0.001$ ). Differences

between sites treated MWF or MWF + laser were not statistically different ( $p=0.1902$ ).

#### **6.2.4 Bleeding on probing (Table 9,10)**

At baseline the percentage of sites with BOP was 93% in both test and control sites. After SRP the percentage of sites with BOP was 73% for control sites and 66% for test. No statistical differences between groups ( $p=0.9547$ ). After surgical treatment the percentage of sites with BOP was 69% for control sites and 92% for test sites. No statistical differences were found between groups ( $p=0.0833$ ).

#### **6.2.5 Gingival Index (Table 11,12)**

Mean GI was initially  $1.60\pm 0.63$  for control and  $1.4\pm 0.74$  for test. GI values after SRP were reduced to  $0.80\pm 0.77$  at control and  $0.80\pm 0.68$  at test sites. Both group demonstrated a decrease in GI that reach statistical significance ( $p<0.05$ ). There were not statistical differences between groups ( $p=0.6250$ ).

GI values after surgical treatment were reduced to  $1.15\pm 0.99$  at control and  $1\pm 0.82$  at test sites, but did not reach statistical differences with respect to baseline values ( $p\geq 0.05$ ). GI reduction for control sites was  $0.46\pm 1.20$  and  $0.38\pm 1.04$  for test sites. There were not statistical differences between groups ( $p>0.9999$ ).

#### **6.2.6 Plaque Index (Table 13, 14)**

At baseline, mean PI was  $1.4\pm 0.63$  for control sites and  $1.6\pm 0.91$  for test sides. PI values after surgical treatment were reduced to  $0.73\pm 0.70$  at control sites and  $0.80\pm 0.77$  Statistical differences with respect to baseline values reached statistically significance ( $p<0.05$ ). There were not statistical significant differences between control and test sites ( $p>0.9999$ ).

PI values after surgical treatment were reduced to  $1.09\pm 1.09$  and  $0.91\pm 0.83$  but did not reach statistical differences with respect to baseline values ( $p\geq 0.05$ ). PI reduction for control sites was  $0.27\pm 0.79$  and  $0.73\pm 1.10$  for test sites. There were not statistical significant differences between control and test sites ( $p=0.625$ )

### **6.2.7 Oral hygiene and clinical parameters (table 15)**

The data was divided depending on the patient's mean PI ( $< 1$  or  $\geq 1$ ) after surgical treatment. No statistical differences were seen between sites (control and test) with respect to PI ( $p \geq 0.05$ ).

### **6.2.8 Smoking and clinical parameters (table 16)**

Pocket depth reduction after surgical treatment was statistically different ( $p=0.012$ ) at the test sites of smokers ( $4.57 \pm 0.53$ ) compared to nonsmokers ( $3.71 \pm 2.25$ ). GI reduction after surgical treatment was statistically different ( $p=0.002$ ) at the test sites of smokers ( $1.14 \pm 0.69$ ) compared to nonsmokers ( $0.28 \pm 0.75$ ).

## **6.3 Tissue Response**

No patient reported any adverse effect from treatment. Tissue color and tissue edema at surgical sites were evaluated one week after surgery. The average tissue color was  $1.9 \pm 0.4$  at the control sites and  $1.8 \pm 0.4$  at the test sites. The color of the tissue was not statistically different ( $p=0.9766$ ) between the study sites. With respect to tissue edema, the average of control sites was  $2.1 \pm 0.8$  where the average of test sites was  $1.7 \pm 0.7$ . Statistically significant less edema was seen at the test sites ( $p=0.0410$ ).

The effect of smoking on tissue response was analyzed. Tissue edema ( $p=0.2155$ ) and tissue color ( $p=0.2346$ ) were not influenced by the smoking habit of the patient.

## **6.4 Postoperative discomfort (figure 2,3)**

The pain scale mean value for control sites was  $3.6 \pm 2.7$  and for the test site was  $2.4 \pm 1.9$ . As seen in figure 2, statistically significant differences ( $p < 0.0001$ ) between study sites were seen, favoring test sites. The pain medication consumption taken after surgical procedures was  $2.1 \pm 2$  for control sites and  $0.9 \pm 1$  for test sites. As seen in figure 3, a statistically significant greater consumption in pain medication was observed in the control group ( $p < 0.0001$ ).

#### **6.4.1 Effect of the surgery order on postoperative discomfort (table 17)**

Overall, the surgical treatment (test and control) performed first was significantly more painful than the second. As shown in table 17, this difference was seen in the pain scale scores ( $p=0.0005$ ) and in the amount of pain medication consumed ( $p=0.002$ ).



**Table 3 Comparison of CAL between Test and Control Sites**

Baseline			SRP			After surgical treatment (AST)		
Test	Control	P*	Test	Control	P*	Test	Control	P*
7.53±1.19	7.87±1.60	0.5625	6.80±1.32	6.80±2.04	0.7305	6.15±1.41	5.92±1.66	0.5488

\*Wilcoxon Signed Rank Test

**Table 4 Comparison of CAL between Time Point in Test and Control Sites**

	Baseline-SRP	p* <sup>&amp;</sup>	Baseline- AST	p* <sup>¶</sup>
Test	0.73±0.70	0.0059	1.38±1.45	0.0078
Control	1.07±0.96	0.0024	1.85±1.63	0.0046
p* <sup>^</sup>	0.4219		0.4053	

\*Wilcoxon Signed Rank Test.

<sup>&</sup>Differences between baseline and SRP.

<sup>¶</sup> Differences between baseline and AST.

<sup>^</sup>Differences between test and control

**Table 5 Comparison of PD between Test and Control Sites**

Baseline			SRP			AST		
Test	Control	P*	Test	Control	P*	Test	Control	P*
7.13±0.52	7.20±0.86	>0.9999	5.80±0.94	5.93±1.39	0.8281	3.23±0.93	3.15±0.90	>0.9999

\*Wilcoxon Signed Rank Test



**Table 6 Comparison of PD between Time Point in Test and Control Sites**

	Baseline-SRP	p* <sup>&amp;</sup>	Baseline- AST	p* <sup>¶</sup>
Test	1.33±0.98	0.0005	3.85±1.21	0.0002
Control	1.27±0.88	0.0005	3.92±0.95	0.0002
p* <sup>^</sup>	0.7813		>0.9999	

\*Wilcoxon Signed Rank Test.

<sup>&</sup>Differences between baseline and SRP.

<sup>¶</sup> Differences between baseline and AST. <sup>^</sup>Differences between test and control.

**Table 7 Comparison of REC Between Test and Control Sites**

Baseline			SRP			AST		
Test	Control	P*	Test	Control	P*	Test	Control	P*
0.43±0.97	0.77±1.30	0.1263	1.15±1.57	0.92±1.32	0.7026	2.69±1.65	2.61±1.26	0.1902

\*Wilcoxon Signed Rank Test

**Table 8 Comparison of REC between Time Point in Test and Control Sites**

	Baseline-SRP	p* <sup>&amp;</sup>	Baseline- AST	p* <sup>¶</sup>
Test	0.69±1.30	0.0128	2.23±1.71	0.0006
Control	0.15±1.26	0.1654	1.84±1.54	0.0009
p* <sup>^</sup>	0.1902		0.8443	

\*Wilcoxon Signed Rank Test.

<sup>&</sup>Differences between baseline and SRP.

<sup>¶</sup> Differences between baseline and AST.

<sup>^</sup>Differences between test and control st

**Table 9 Comparison of GI between Test and Control Sites**

Baseline			SRP			AST		
Test	Control	P*	Test	Control	P*	Test	Control	P*
1.40±0.74	1.60±0.63	0.5313	0.80±0.68	0.80±0.77	>0.9999	1.00±0.82	1.15±0.99	0.625

\*Wilcoxon Signed Rank Test

**Table 10 Comparison of GI between Time Point in Test and Control Sites**

	Baseline-SRP	P* <sup>&amp;</sup>	Baseline- AST	p* <sup>¶</sup>
Test	0.60±0.83	0.0313	0.38±1.04	0.2578
Control	0.80±0.77	0.0054	0.46±1.20	0.1855
p* <sup>^</sup>	0.5625		0.8828	

\*Wilcoxon Signed Rank Test.

<sup>&</sup>Differences between baseline and SRP.

<sup>¶</sup> Differences between baseline and AST. <sup>^</sup>Differences between test and control

**Table 11 Comparison of PI between Test and Control Sites**

Baseline			SRP			AST		
Test	Control	P*	Test	Control	P*	Test	Control	P*
1.60±0.91	1.40±0.63	0.5	0.80±0.77	0.73±0.70	>0.9999	0.91±0.83	1.09±0.83	0.625

\*Wilcoxon Signed Rank Test



**Table 12 Comparison of PI between Time Point in Test and Control Sites**

	Baseline-SRP	p* <sup>&amp;</sup>	Baseline- AST	p* <sup>¶</sup>
Test	0.80±0.56	0.001	0.73±1.10	0.0859
Control	0.67±0.49	0.002	0.27±0.79	0.5
p* <sup>^</sup>	0.6875		0.1875	

\*Wilcoxon Signed Rank Test.

<sup>&</sup>Differences between baseline and SRP.

<sup>¶</sup> Differences between baseline and AST.

<sup>^</sup>Differences between test and control

**Table 13 BOP**

	Test	Control	P*
Baseline 0	1	1	*
1	14	14	
Total	15	15	
SRP 0	5	4	0.6547
1	10	11	
Total	15	15	
AST 0	1	4	0.0833
1	12	9	
Total	13	13	

\*McNemar's Test

\*\*No statistical test because there are no discordant pairs.



**Table 14 BOP in test group and control sites**

Test	Baseline (n)	SRP (n)			P*	AST (n)			P*
		0	1	Total		0	1	Total	
	0	1	0	1		1	0	1	
	1	4	10	14		0	12	12	
	Total	5	10	15	0.0455	1	12	13	*
Control	Baseline (n)	0	1	Total		0	1	Total	
	0	1	0	1		1	0	1	
	1	3	11	14		3	9	12	
	Total	4	11	15	0.0833	4	9	13	0.0833

\*McNemar's Test

\*\*No statistical test because there are no discordant pairs.



**Table 15 Treatment effect in patients with PI ≤ 1 or PI > 1**

		Baseline-AST						
		n	CAL	p <sup>¶*</sup>	PD	p <sup>¶*</sup>	GI	p <sup>¶*</sup>
Test	PI ≤ 1	8	1±1	0.089	4.2±0.84	0.448	0.375±1.06	0.477
	PI > 1	5	1.5±1.52	0.026	3.625±1.41	0.008	0.375±1.06	0.351
	p <sup>^</sup>		0.529		0.062		1	
Control	PI ≤ 1	8	1.8±1.17	0.037	4.2±0.4	0.063	1±1.09	0.142
	PI > 1	5	1.75±1.72	0.031	3.75±1.09	0.008	0.125±1.05	0.763
	p <sup>*^</sup>		0.724		0.724		0.214	
PI ≤ 1	Test	8	1±1	0.089	4.2±0.84	0.448	0.375±1.06	0.477
	Control	8	1.8±1.17	0.037	4.2±0.4	0.063	1±1.09	0.142
	p <sup>*^^</sup>		0.308		1		0.446	
PI > 1	Test	5	1.5±1.52	0.026	3.625±1.41	0.008	0.375±1.06	0.351
	Control	5	1.75±1.72	0.031	3.75±1.09	0.008	0.125±1.05	0.763
	p <sup>*^^</sup>		0.770		0.959		0.655	

\*Wilcoxon Signed Rank Test.

¶ Differences between baseline and AST.

^Differences between PI ≤ 1 and PI > 1

^^Differences between Test and Control

**Table 16 Treatment effect in patients smokers and non smokers.**

		Baseline-AST								
		n	CAL	p* <sup>¶</sup>	PD	p* <sup>¶</sup>	GI	p* <sup>¶</sup>	PI	p* <sup>¶</sup>
Test	Smoker	7	1.86±1.36	0.011	4.57±0.53	0.016	1.14±0.69	0.005	0.42±0.97	0.289
	Non Smoker	6	1.71±2.92	0.5	3.71±2.25	0.002	0.28±0.75	0.076	0.86±1.07	0.141
	p* <sup>^</sup>		0.138		0.012		0.002		0.510	
Control	Smoker	7	2±1.15	0.004	4.42±0.53	0.016	0.86±1.22	0.111	0.28±0.76	1
	Non Smoker	6	2.14±2.55	0.137	3.71±1.38	0.003	0.14 ± 1.06	1	0.14±0.90	1
	p* <sup>^</sup>		0.594		0.073		0.234		0.731	
Smoker	Test	7	1.86±1.36	0.011	4.57±0.53	0.016	1.14±0.69	0.005	0.42±0.97	0.289
	Control	7	2±1.15	0.004	4.42±0.53	0.016	0.86±1.22	0.111	0.28±0.76	1
	p* <sup>^^</sup>		0.835		0.710		0.598		0.7	
Non Smoker	Test	6	1.71±2.92	0.5	3.71±2.25	0.002	0.28±0.75	0.076	0.86±1.07	0.141
	Control	6	2.14±2.55	0.137	3.71±1.38	0.003	0.14±1.06	1	0.14±0.90	1
	p* <sup>^^</sup>		0.310		0.699		0.485		0.196	

\*Wilcoxon Signed Rank Test.

<sup>¶</sup> Differences between baseline and AST.

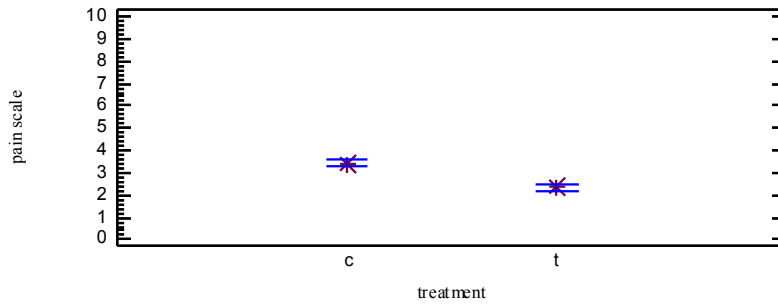
<sup>^</sup>Differences between smoker and non smoker

<sup>^^</sup>Differences between Test and Control

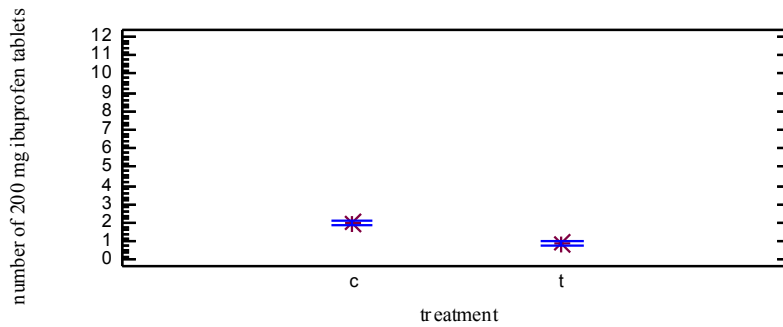
**Table 17** Comparison pain values by surgery order

	First	Second	P*
Pain medication (number of tablets)	1.80±2.18	1.09±1.48	0.002
Pain scale	3.63±3.09	2.13±1.48	0.0005

\*repeated-measures ANOVA



**Figure 2.** Mean and 95 % confidence intervals of the least significance difference for pain scale at control and test sites.



**Figure 3.** Mean and 95 % confidence intervals of the least significance difference for pain medication at control and test sites.





## **7.DISCUSSION**

The results of the present study showed that both treatment modalities were effective in improving clinical parameters. Diode laser use did neither lead to postoperative complications nor to impair clinical healing, indicating that this type of laser may not have any detrimental effect when employed in conjunction with periodontal surgery. However, with the exception of tissue edema and less post operative pain, no other statistically significant and clinically important differences in any of the investigated parameters were observed between both treatment modalities. However, the reduction of post operative pain and swelling is important to patients and represents a benefit of use of the diode laser during periodontal surgery.

It should be pointed out that the sample size of this study was small, and does not allow for definitive conclusions to be established. On the other hand, it should be noted that this is the first data from a human randomized clinical trial that uses a diode laser at cutting settings in conjunction with periodontal surgery.

Results obtained in the present study with MWF surgery, such as a reduction in PD and gains in CAL, confirm those reported by other investigators. For example, Ramfjord (50) reported a PD reduction of  $3.41 \pm 1.69$ mm and a CAL gain  $1.16 \pm 1.66$ mm after open flap debridement in teeth with initial  $PD \geq 7$  mm. In the present study, PD reduction obtained for the study sites was  $3.85 \pm 1.21$ mm and CAL gain was  $1.38 \pm 1.45$ mm. However, the present study re-evaluated patients five months following periodontal treatment, while the Ramfjord study re-evaluated patients one year following treatment.

Numerous studies have shown that smoking may negatively impact the results of periodontal treatment (143-145). Seven of the 13 patients that finished treatment in the current study had a smoking habit. Data was analyzed in order to establish the effect of smoking on clinical parameters and tissue response. Overall, smoking did not affect any of the parameters. These results differ from the one previously mentioned due probably to the small sample size available in the present study. It may be advantageous for future studies utilizing lasers in conjunction with periodontal therapy to include only non-smokers or include

both smokers and non smokers and evaluate each group separately if the sample size is large enough.

Poor oral hygiene have contributed to a smaller improvement in the test and control surgical treatment outcomes (146-148) than that seen in typical periodontal surgical outcomes. Plaque index values were statistically and clinically reduced reduced after SRP treatment but increased to baseline values after surgical treatment. Data was analyzed in order to establish the effect of plaque control. As smoking, this factor did not affect the clinical parameters but sample size was also small to establish a definitive conclusion.

As stated by Cobb (45), meaningful comparisons between various clinical studies or between laser and conventional therapy is difficult because of different wavelengths, laser parameters, experimental design, lack of proper controls, differences in severity of disease and measurements at different clinical endpoints.

The laser in conjunction with conventional surgery was applied in order to remove the epithelial lining of the pocket as well as the marginal epithelium. This procedure to deepithelialize modeled previous studies (13,15,16,18-20). Although conceptually similar, they differ widely in the design and the results. In the current study, a flap was raised using the MWF in order to deepithelialize the epithelial lining and marginal epithelium once using a diode laser. Rossman et al. (15,16), however, raised a flap using a reverse bevel incision and deepithelialized the inner and outer portions of the flap using a CO<sub>2</sub> laser. In addition, Rossman et al. deepithelialized the outer portion of the flap every ten days for a month in order to prevent the migrating epithelial cells from reaching the root surface. Rossman et al. studies were either animal studies (15,16) or human case control studies (17) focused on histological outcomes. This was in contrast to the current study which was a randomized clinical control study focused on clinical parameters.

The present study, in contrast to the studies conducted by Rossman et al, used a diode laser. A diode laser has been demonstrated to effectively remove epithelium without damaging the connective tissue (149). However, more studies need to be conducted in order to evaluate the differences in treatment outcomes of the CO<sub>2</sub> verses a diode laser when laser

deepithelialization is desired. Finally, the present study did not repeatedly remove epithelium from the buccal surface of the flap since there are not many studies available demonstrating the clinical effectiveness of this additional procedure.

The Kreisler et al. study (13) used a diode laser to remove the inner epithelium, without raising a flap, as an adjunct to scaling and root planing (SRP). Kreisler et al.(13) used the diode laser in a similar way as the current study with the same power output and wavelength. Kreisler results showed statistically significant improvement in CAL and PD with respect to SRP alone, but the CAL differences ranging from 0.3 to 0.4 were so small that they may lack clinical significance.

Another laser used as an adjunct in periodontal surgery has been the Er:Yag laser. In contrast to the CO<sub>2</sub> and diode lasers, the Er:YAG laser has been investigated more often in clinical trials. These trials have used the Er:YAG laser as an adjunct in periodontal surgery for the treatment of chronic periodontitis. Er:YAG laser was used to debride intrabony pockets, scale root surfaces and trim the periodontal flap. Studies conducted by Sculean et al. (97) and Schwarz (98) have found that the use of the Er:YAG laser produced no statistically significant differences between periodontal surgical outcomes. On the other hand, Gaspirc (21) found statistical differences in the range of 0.3-0.4mm for CAL, which has questionable clinical significance. The present study was in agreement with the studies conducted by Sculean and Schwartz.

The present study did not access the microbiota in the baseline periodontal pockets and in the residual pockets after surgery. This was not accessed since according to Armitage et al. (150), a clinical indicator of inflammation, BOP, roughly reflects the level of periodontal pathogens in the pocket. The current study found that neither treatment groups had a significant reduction in the percentage of BOP and that there were no statistically significant differences between groups. A possible reason for the lack of BOP reduction may be due to the poor plaque control of the patients after surgical treatment.

There are no current studies that demonstrate the optimal values for laser power and wavelength to use during periodontal surgeries. Kreisler et al. (13) used one watt continuous

wavelength for 10 seconds followed by an interrupted period of 30 seconds. This dosage used for soft tissue cutting has been demonstrated to be safe *in vitro* and to not cause tooth damage even with direct application to the tooth surface (83). In the same study, Kreisler (83) and later Schwarz (103) found that power higher than 1.5 w can cause damage *in vitro* (83) and *in vivo* (103). Lopez Castro et al. (84) on the other hand did not find any side effects when a diode laser at two watts of power was used as an adjunct in scaling and root planing. In order to find the optimal values of laser power and wavelengths to use in periodontal surgery, more studies need to be performed.

One of the major problems in evaluating the low level laser therapy efficacy is the determination of the optimal dosage and treatment schedule. Although there is some guidance from other studies, the choices remain discouragingly broad due to the variability of clinical findings. Furthermore, there are great differences in the published literature in terms of experimental and assessment methods and irradiation conditions.

The literature shows little correlation between *in vitro* and *in vivo* studies when using low level laser therapy (LLLT). An *in vitro* study performed by Kreisler (110) has shown a positive effect of LLLT on human gingival fibroblasts. Another *in vitro* study performed by Stein (129) showed a positive effect of LLLT on osteoblasts where Khadra (151) found a positive effect on bone formation in rat calvaria. Given the problems in extrapolating irradiation parameters and findings from *in vitro* research to human practice, trials in humans are essential.

Despite the proposed benefit of LLLT, there are very few clinical studies using LLLT in gingival surgery. This makes the comparison of the current study's results with previous studies difficult. Various human randomized control clinical studies that analyzed the periodontal healing response using LLLT are available. While Amorim et al. (152) and Ozelik et al. (134) had reported that LLLT significantly promoted healing of various periodontal surgical procedures, Masse et al. (37) and Damante et al. (115) had found that LLLT with a diode laser did not improve healing. This difference in results may have occurred since various lasers, intervals of application, surgical procedures and ways to evaluate laser wound

healing were used. The finding of a statistically significant reduction in edema in the 810 nm diode treated sites found in the present study may have occurred due to the promotion of healing as shown in the studies by Amorim et al. (152) and Ozcelik et al. (134). In the present study, the difference in edema reduction in the test sites with respect to control sites was minimal. Although statistically significant, this may not be clinically significant.

It has been suggested that daily treatment with LLLT is required to achieve maximum results (129). Ozcelik et al. (153) compared the use of enamel matrix derivative in intrabony defects with or without the application of a diode laser at a wavelength of 580 nm ( $4\text{j}/\text{cm}^2$ ) during surgery and daily for 5 days after surgery. Clinical parameters such as clinical attachment and pocket depth did not differ between treatment sites. The current study found that tissue edema was improved significantly ( $p < 0.05$ ) more in the laser (810nm) treatment verses control treatment when LLLT (810nm) was applied once during the surgical procedure. The study performed by Ozcelik et al. (153) found that swelling was statistically reduced after 1 week ( $p < 0.05$ ), although the application of LLLT added up to 40 minutes more to the surgical procedure. In the current study, additional time utilizing the laser (810nm) was minimal. This could indicate that only minor application of LLLT (810nm) can provide improvement in tissue edema.

The pain scale used in this study was subjective and highly dependent upon individual experience. However, the patient served as both the control and test subject. The patient was not aware of which surgical site received laser (810nm) treatment in order to reduce the "placebo effect of laser treatment". The subjective measure of the pain using the pain scale found statistically significant differences between the control sites and the test (810nm) sites. In disagreement with our study, Masse et al. (37) found no statistically significant differences in pain scale values in control surgical sites when compared to the laser sites. In contrast, Ozcelik et al. (153) found a statistically significant improvement in pain values for the laser group during the first 2 days following surgery when compared to conventional treatment.

There was statistically significantly less pain experienced by patients following the second surgical procedure. This is in accordance with other studies. (6,8,137). It is unclear why patients experience less pain after a second surgical procedure but it may be related to

psychological expectations. We found that use of a 810 nm diode laser during surgical treatment resulted in less pain than the control treatment, with the difference reaching statistical significance. This finding suggests that laser (810nm) application may be more beneficial in procedures where postoperative pain is expected by the patient and/or the practitioner.

Two patients received minor osteoplasty during the procedure, one in the control site and the other in the test site. According the study performed by Canakçi and Canakçi (9) patients perceived more pain when osseous resection was performed with a modified Widman flap than that experienced by patients with only the modified Widman flap procedure. Since osteoplasty was performed in only two patients, the sample size was too small to analyze the effect that this factor had on pain. Therefore the data was analyzed twice, once including the patients that required osteoplasty and the second time without including these patients. No difference was found between these two data regarding the significance of pain between treatment modalities. Pain was found to be statistically significantly greater in the control groups. Since a minor amount of osteoplasty was performed, it was unlikely that it would have an effect on pain. In the current study, clinical parameters and surgical procedures were performed by a single investigator. Since this was not a double blinded experiment, bias with data collections may have occurred. Therefore, it may be beneficial for future studies to be double blinded to prevent the introduction of bias.

The current study was one of the first human clinical randomized studies using a diode laser as an adjunct in periodontal surgery. Lasers currently have a variety of uses in dentistry and some benefits have been found to occur in *in vitro* studies. However, clinical outcomes of *in vivo* application of lasers is still unclear and little is known regarding the optimal type, wavelength, power and methods of using lasers in conjunction with periodontal surgery. In order to assess if lasers will provide additional benefits to periodontal treatments, further studies in larger numbers of patients are needed.

## **8.CONCLUSION/CONCLUSIONES**

It can be concluded that:

1. A diode laser (810nm) use did not lead to postoperative complications or to impair tissue response, indicating that this type of laser (810nm) has no detrimental effects when used in conjunction with MWF surgery.
2. No statistical differences on clinical parameters (CAL gain, PD, REC, GI and PI reduction) were seen between test and control sites.
3. No statistical differences for erythema values were seen between test and control sites.
4. The tissue edema and postoperative pain were reduced with the use of the diode laser (810nm). The differences were statistically different.
5. There was statistically significantly less pain experienced by patients following the second surgical procedure.
6. Smoking habit did not affect the outcome of any of the parameters.

Se puede concluir que:

1. El laser de diodo (810 nm) no demostró complicaciones postoperatorias ni dificultó la cicatrización, permitiendo el uso de éste laser como adjunto en el tratamiento periodontal quirúrgico junto con un colgajo modificado de Widman.
2. No se observarán diferencias estadísticamente significativas entre test y control respecto a los parametros periodontales clínicos (ganancia de inserción, reducción de sondaje, recesión, índice gingival e índice de placa)
3. El edema tisular y el dolor postoperatorio fueron menores con el uso del laser de diodo (810nm). Las diferencias entre test y control fueron estadísticamente significativas
4. El segundo acto quirúrgico demostró un menor dolor postoperatorio siendo la diferencia respect al primero estadísticamente significativa
5. El hábito tabáquico no influyó negativamente en el resultado de los parametros clínicos.





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## **10.ABSTRACT**

The purpose of this study was to clinically compare the use of a 810 nm diode laser (DL) following modified Widman flap (test sites) to that of modified Widman flap (MWF) alone (control sites). Thirteen patients with periodontal pockets in two different quadrants with at least one tooth exhibiting a pocket depth of  $\geq 7$  millimeters and attachment levels of  $\geq 7$ mm were selected. One side was randomly selected to receive MWF and the other to receive a diode laser following MWF. The DL was used to de-epithelialize the inner part of the periodontal flap and photo-biostimulate the surgical area. Probing depth (PD), clinical attachment levels (CAL), plaque index (PI), and gingival index (GI) were evaluated at baseline, 6 weeks and 5 months. Pain scale assessment (PS), pain medication consumption (PM), tissue edema (TE), and tissue color (TC) were evaluated one week following surgery. Treatment differences were analyzed using McNemar's test, paired t-tests or a Wilcoxon Signed Rank Test. No statistical differences of CAL gain, PD, GI and PI reduction between laser sites and control sites were seen. Statistically significant differences were seen for TE ( $p=0.041$ ), PM ( $p<0.001$ ) and PS ( $p<0.001$ ) favoring test sites. TC did not show a statistically significant difference ( $p=0.9766$ ). Patients rated the first surgical treatment performed as more painful than the second ( $p<0.002$ ). The use of a 810 nm diode laser following modified Widman flap provided additional benefits in terms of less edema and post operative pain.

El objetivo del estudio fue evaluar la efectividad del uso de un laser de diodo de 810nm después de un colgajo de Widman modificado. Participaron en el estudio 13 pacientes con bolsas periodontales bilaterales en dos cuadrantes diferentes. Dichos cuadrantes debían presentar en la misma zona una bolsa de  $\geq 7$  milímetros y una pérdida de inserción  $\geq 7$ mm. Después de una fase higiénica periodontal, los pacientes recibieron un tratamiento quirúrgico periodontal. Un cuadrante se trató mediante un colgajo modificado de Widman (control) y el contralateral con un colgajo modificado de Widman y una posterior aplicación de un laser de diodo (test). El laser de diodo eliminó el epitelio de la cara interna del colgajo periodontal y fotobioestimuló toda la zona quirúrgica. Se evaluó el nivel de inserción, profundidad de bolsa, recesión, sangrado al sondaje, índice gingival e índice de placa al inicio, a las 6 semanas del tratamiento de fase higiénica y a los 5 meses después del inicio



tratamiento periodontal. Una semana después de las intervenciones quirúrgicas se evaluó el edema y eritema de area tratadada. Se monitorizó el dolor postoperatorio así como la cantidad de medicación analgésica ingerida durante la semana posterior a la intervención. Se evaluaron las diferencias entre tratamientos mediante los test estadísticos McNemar, t-test pareados y Wilcoxon. No se observaron diferencias estadísticamente significativas entre test y control con respecto al nivel de ganancia de inserción, reducción de profundidad de bolsa, índice gingival e índice de placa. Se observaron diferencias estadísticamente significativas entre los grupos de estudio con respecto al edema ( $p=0.041$ ), cantidad de medicación analgésica ingerida ( $p<0.001$ ) y dolor postoperatorio ( $p<0.001$ ) favoreciendo el grupo test ( $p=0.9766$ ). La primera intervención quirúrgica fue más dolorosa que la segunda ( $p<0.002$ ). Se puede concluir que el uso de un laser de diodo de 810 nm después de un colgajo de Widman modificado es beneficioso debido a una reducción del edema y el dolor postoperatorio.



# 11. Appendix

## 11.1 Appendix 1



**University at Buffalo**  
The State University of New York

Health Sciences Institutional Review Board &  
Institutional Animal Care and Use Committee

### MEMORANDUM

**TO:** Javier Sanz Moliner, DDS

**FROM:** Monica B. Spaulding, MD  
Professor of Medicine  
HSIRB Chair *MBS*

**DATE:** June 1, 2007

**RE:** **TITLE: A Clinical Evaluation of a Diode Laser as an Adjunct in Periodontal Surgery**  
**HSIRB Project #: P/E0510307E**

The Health Sciences Institutional Review Board (HSIRB) by expedited review has approved the following for the above referenced study:

- Revised protocol
- Revised flyer
- Revised consent form document with HIPAA authorization incorporated (dated 4/23/07).  
The Authorization has met the required elements of the federal regulations of HIPAA.

The minor revision(s) does not substantially change the risks of the study.

MBS/aa

Amendment expedited minor

150 Parker Hall, Buffalo, NY 14214-8004  
Tel: (716) 829-2752 Fax: (716) 829-3610  
HSIRB: [www.wings.buffalo.edu/smb/hsirb](http://www.wings.buffalo.edu/smb/hsirb) IACUC: [www.wings.buffalo.edu/smb/iacuc](http://www.wings.buffalo.edu/smb/iacuc)



11.2 Appendix 2

University at Buffalo
Consent to Participate in a Research Study

A Clinical Evaluation Of A Diode Laser As An Adjunct In Periodontal Surgery

You are being asked to participate in a research study. The purpose of this document is to provide you with information to consider in deciding whether to participate in this research study. Your consent should be made based on your understanding of the nature and risks of the treatment, device, or procedure. Please ask questions if there is anything you do not understand. Your participation is voluntary and will have no effect on the quality of your medical care if you choose not to participate.

1. INVESTIGATOR(S) CONDUCTING THE STUDY

Javier Sanz Moliner Principal Investigator 250 Squire Hall 829-3850
Dr. Sebastian Ciancio Faculty Sponsor 250 Squire Hall 829-3848

2. SOURCE OF SUPPORT

There is no outside funding for this project

3. SITES OF THE RESEARCH STUDY

University of Buffalo, School of Dental Medicine

4. PURPOSE OF THE RESEARCH STUDY

The objective of this study is to evaluate the clinical, microbiological and analgesic effect of diode laser as an adjunct to surgical periodontal therapy in patients with moderate to severe chronic periodontitis. Periodontal surgery is the usual treatment for this disease. However, some studies have suggested benefits from use of a laser in conjunction with periodontal surgery and this study will evaluate if the benefits occur. This laser is approved by the FDA for the procedure used in this study.

5. ELIGIBILITY

Inclusion Criteria: You will be eligible for this study if

- 1. You are a male or female between the ages of 18-75 years.
2. You must have 1 qualifying tooth in 2 different quadrants of your mouth.

Each

qualifying tooth must have a pocket, and must bleed when probed.

Exclusion Criteria: You will be excluded from this study if you:

- 1. Are on clinically significant concomitant drug therapy.
2. Have with systemic infection.
3. Require antibiotic coverage for routine dental therapy.
4. Have received antibiotics (excluding tetracyclines) within six weeks of baseline.
5. You have been on antibiotic therapy, or used antibiotics within 90 days of there



baseline visit

7. Have a serious medical illness such as kidney or liver disease.
8. Patients who have previously received a deep dental cleaning and or oral surgery within the past 90 days.
9. Have received a regular dental cleaning within the past 90 days.
10. You do not require surgical periodontal therapy.

## **6. PROCEDURES**

This study requires 7 visits after your original screening visit and the study will take 5 months to complete. You will be one of 26 subjects who will participate in this study.

### **Screening Visit**

At the screening visit you must complete a health history questionnaire. The dentist will review with you your medical history forms. You will also be advised of your role in this study and asked to sign a HIPAA and informed consent. After giving consent, you will undergo an examination of your mouth. The dentist will be looking at the gums and tissue around each tooth. The dentist will then probe you mouth with some dental instruments to determine if the gums around your tooth have become detached from the tooth.

If you have not had a recent x-ray of the selected teeth, new x-rays will be taken.

If you have qualified for the study at the screening examination you will return for the baseline visit within thirty (30) days after the screening examination.

### **Baseline Visit**

At your baseline visit a full mouth manual probing and pocket measurements will be taken.

Following the examination, the examiner or a dental hygienist will provide a full-mouth supragingival cleaning and subgingival scaling and root planing of each study tooth selected plus the quadrants in which that tooth is located. Subgingival scaling and root planing of other non-study quadrants will be provided, if necessary. Oral hygiene instructions will also be given to you.

### **Week 6**

At this visit a bacterial plaque sample will be collected in each study tooth. A paper tip will be placed at the bottom of the selected pockets and remain there for 30 seconds. Sample will be frozen for analysis. After the sample is taken, full mouth manual probing and measurements will be taken.

Your mouth will be re-evaluated to assess the response to the previous scaling and root planing. If the response to treatment resolves your periodontal problem, no surgery will be necessary

Oral hygiene instructions will be given to you again.

**Week 7**

At this visit, you will receive periodontal surgery for pocket reduction in a study teeth as well as any other teeth in that quadrant which require periodontal surgery. If more than the study teeth require surgery, this does not increase risks associated with treatment. The determination as to which quadrant requires laser treatment will be decided at random. During the procedure, your gum will be opened to permit better access to the roots and the eroded bone. Inflamed and infected gum tissue will be removed, and the root surfaces will be thoroughly cleaned. Bone irregularities may be reshaped, and application of a laser will be applied around the teeth. Your gum will then be sutured back into position.

You will evaluate your pain once a day for the next week using a diary that will be handed and explained to you after the surgeries.

**Week 8**

You will return 1 week after your surgical visit to have your stitches removed. This occurs in week 8 of the study. You will return the pain evaluation diary to the investigator at this time.

**Week 10**

At this visit, you will receive periodontal surgery for pocket reduction in the study teeth as well as any other teeth in that quadrant which require periodontal surgery. If more than the study teeth require surgery, this does not increase risks associated with treatment. The determination as to which quadrant requires laser treatment will have been decided at random at your visit week 7. During the procedure, your gum will be opened to permit better access to the roots and the eroded bone. Inflamed and infected gum tissue will be removed, and the root surfaces will be thoroughly cleaned. Bone irregularities may be reshaped, and application of a laser will be applied around the teeth. Your gum will then be sutured back into position.

You will evaluate your pain once a day for the next week using a diary that will be handed and explained to you after the surgeries.

**Week 11**

You will return 1 week after your surgical visit to have your stitches removed. This occurs in week 11 of the study. You will return the pain evaluation diary to the investigator at this time.

**Month 5**

Prior to this examination a bacterial plaque sample will be collected as before. You will receive an oral tissue examination. Also, full mouth manual probing measurements (attachment level and pocket depth) will be obtained as well as Bleeding on Probing, Gingival Index and Plaque Index.

You will receive a supragingival cleaning and subgingival scaling and root planing at visit 5 along with reinforcement of oral hygiene instructions.

You will have completed the study after finishing the 5 month examination



**7. RISKS**

As with any study, the possibility of an adverse side effect is possible. After scaling and root planning, and or use of the dental laser one may experience short term discomfort such as gum soreness, redness, inflammation and bleeding of the gums for up to one week. Scaling and root planing is a standard procedure to treat periodontal pockets and a laser such as that used in this study is sold in the United States to treat periodontal pockets.

A small number of patients do not respond successfully to periodontal surgery, and in such cases, the involved teeth may eventually be lost. Periodontal surgery may not be successful in preventing function or appearance. Because each patient's condition is unique, long term success may not occur.

Some complications may result from the periodontal surgery, drugs, or anesthetics. These complications include, but are not limited to post-surgical infection, bleeding, swelling and pain, facial discoloration, transient but on occasion permanent numbness of the jaw, lip, tongue, teeth, chin or gum, jaw injuries or associated muscle spasm, transient but on occasion permanent increased tooth looseness, tooth sensitivity to hot, cold, sweet or acidic foods, shrinkage of the gum upon healing resulting in elongation of some teeth and greater spaces between some teeth, restricted ability to open the mouth for several days or weeks and impact of speech.

**8. BENEFITS**

The purpose of periodontal surgery is to reduce infection and inflammation and to restore you gum and bone to the extent possible. The surgery is intended to help keep your teeth in the operated areas and to make your oral hygiene more effective.

**9. ALTERNATIVES TO PARTICIPATION IN THE RESEARCH STUDY**

Alternatives to periodontal surgery include standard periodontal surgery without the use of diode laser, non-surgical scraping or no treatment.

**10. NEW FINDINGS**

You will be notified of any significant new findings that may cause you to change your mind about participating in the research study.

**11. COST ASSOCIATED WITH THE RESEARCH STUDY**

Neither you nor your insurance provider will be charged for costs of any of the procedures performed for the purpose of this research study (e.g., screening procedures, experimental procedures, monitoring/follow-up procedures, etc.) described above.



There are costs to you associated with the non-surgical therapy ( subgingival scaling and root planing ) part of the study.

**12. REIMBURSEMENT FOR MEDICAL TREATMENT**

Routinely, the Buffalo General Hospital, Erie County Medical Center, Millard Fillmore Hospital, and/or the University at Buffalo, State University of New York, its agents, or its employees do not compensate for or provide free medical care for human subjects/participants in the event that any injury results from participation in a human research project. In the unlikely event that you become ill or injured as a direct result of participating in this study, you may receive medical care, but it will not be free of charge even if the injury is a direct result of your participation.

**13. COMPENSATION FOR SUBJECT PARTICIPATION.**

You will be compensated a total of \$25.00 for your time and travel at your 5 month recall visit The cost of the actual surgery is covered by the study.

**14. CONFIDENTIALITY**

Information related to you will be treated in strict confidence to the extent provided by law. Your identity will be coded and will not be associated with any published results. Your code number and identity will be kept in a locked file of the Principal Investigator. In order to monitor this research study, representatives from the Health Sciences Institutional Review Board and other federal agencies such as NIH (National Institutes of Health) and OHRP (Office of Human Research Protection) may inspect the research records which may reveal your identity.

**15. Authorization for the Use and Disclosure of Identifiable Health Information for Research Purposes**

You have been asked to be part of a research study under the direction of Javier Sanz Moliner, the Principal Investigator, and his or her research team. The study is called A clinical evaluation of a diode laser as an adjunct in periodontal surgery The purpose of this study is to evaluate the clinical, microbiological and analgesic effect of diode laser as an adjunct of surgical periodontal therapy in patients with moderate to severe chronic periodontitis.

This authorization form describes information about you and about your health that will be obtained by the researchers when you participate in the research study. Health information is considered "protected health information" when it may directly identify you as an individual. By signing this form you are agreeing to permit the researchers and/or other parties (described in detail below) to have access to this information. If there are any parts of this form that you do not understand, please be sure to ask us for further clarification.

**1. What protected health information will be collected about you as part of this research study?**

- new Health Information created from study related tests, procedures, visits, and/or questionnaires



as described in the attached consent form.

*General description of information:*

*General description of information: First, middle and last names; telephone number where we may contact you if necessary, a full address with zip code, date of birth and social security number so that you may receive financial reimbursement for the study. We will ask you to complete a health history form that asks questions regarding your health status, medication taken, allergies, etc.*

---

**2. Who is authorized to provide or collect this information?**

- Principal Investigator or designee
- University at Buffalo School of Dental Medicine

**3. With whom may your protected health information be shared?**

Your health information may be shared with others outside of the research group for purposes directly related to the conduct of this research study or as required by law, including but not limited to:

- clinical staff not involved in this research study who may become involved in your care if it is potentially relevant to your treatment
- the organization(s) responsible for administering this research : University of Buffalo

Your information may also be shared with individuals or entities responsible for general administration, oversight and compliance of research activities. Examples of this include the institution's Privacy and Security Officers or other internal oversight staff, Safety Monitoring Boards, an Institutional Review Board, The Research Foundation of the State University of New York, University at Buffalo Foundation Services, and accrediting bodies, or with certain government oversight agencies that have authority over the research including the Department of Health and Human Services (HHS), the Food and Drug Administration (FDA), the National Institutes of Health (NIH), and the Office of Human Research Protections (OHRP). Your information may also be shared with other entities as permitted or required by law. All reasonable efforts will be used to protect the confidentiality of your individually identifiable health information that may be shared with others as described above.

All reasonable efforts will be used to protect the confidentiality of your protected health information. There is the potential for individually identifiable information and the associated health information obtained with this authorization to be re-disclosed by the recipient(s). After such a disclosure, the information may no longer be protected by the terms of this authorization against further re-disclosure.

**4. How long will this information be kept by the Principal Investigator?**





- This authorization has no expiration date. The researchers may continue to rely on this authorization to obtain and use protected health information about you unless you revoke this authorization in writing.

**5. What are your rights after signing this authorization?**

You have the right to revoke this authorization at any time. If you withdraw your authorization, no additional efforts to collect individually identifiable health information about you will be made. You should know, however, that protected health information acquired using this authorization prior to its withdrawal may continue to be used to the extent that the investigator(s) have already relied on your permission to conduct the research. If you chose to withdraw this authorization, you must do so in writing to the following individual(s):

Javier Sanz Moliner  
250 Squire Hall  
School of Dental Medicine  
829-3850

If you send us a request to withdraw your authorization, we will forward that request to the institutions we have shared it with in order to collect your individually identifiable health information. You may also withdraw this authorization directly with those institutions by writing to the following:

Sebastian Ciancio  
250 Squire Hall  
School of Dental Medicine  
829-3848

**6. What will happen if you decide not to sign this authorization?**

Refusing to sign this authorization will not affect the present or future care you receive at this institution and will not cause any penalty or loss of benefits to which you are otherwise entitled. If you decide not to sign this authorization, you will not be able to participate in the research study.

**16. FREEDOM TO WITHDRAW**

Your participation in this study is voluntary and you may stop your participation at any time without prejudice and without affecting future health care.

**17. REMOVAL FROM STUDY**

It is possible that you may be removed from the research study by the researcher at any time during the study. I



VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I am encouraged to ask questions about any aspects of this research study. If I have questions in the future, I should contact Sebastian Ciancio at 829-3848

Any questions I have about my rights as a research participant will be answered by the staff at the Office of the Health Sciences Institutional Review Board, University at Buffalo: (716) 829-2752.

By signing this form I do not waive any of my legal rights.

By signing this form, I agree to participate in this research study.

_____	_____
(Print) _____ Name of Participant Date	Signature of Participant

I certify that the nature and purpose, the potential benefits and possible risks associated with participation in this research study have been explained to the above individual and that any questions about this information have been answered. A signed copy of this consent will be given to the participant.

_____	_____
(Print) _____ Name of Person Obtaining Consent Date (PI or Designee)	Signature of Person Obtaining Consent  (PI or Designee)

I certify that the individuals named above as “participant” and “person obtaining consent” signed this document in my presence.

I certify that the “Person Obtaining Consent” is an authorized “Designee.”

_____	_____
(Print) _____ Name of Principal Investigator Date	Signature of Principal Investigator



### **11.3 Appendix 3**

#### **POST-OPERATIVE INSTRUCTIONS FOLLOWING PERIODONTAL SURGERY**

Do not eat anything or drink hot fluids until the numbness has worn off to avoid injury from accidental biting or burning.

No vigorous rinsing, drinking through a straw, or spitting for 3 days so the blood clot will not be disturbed.

Today, rest as much as possible with your head elevated. No vigorous exercising for 3 days.

Slight bleeding is normal for several hours following surgery. If persistent bleeding occurs place a wet teabag on the involved area and apply direct pressure for 15-20 continuous minutes. Repeat as necessary.

Do not pull back the lips or cheeks to look at the area as this can disturb the surgical site and increase complications.

Do not eat by the surgical area for 1 week. Soft nutritious foods will be necessary for the first few days. Avoid acidic and crunchy foods. Drink plenty of fluids.

Do not use tobacco or drink alcohol as these will delay healing.

Swelling can be lessened by placing a cold compress on the face alternating on and off for 20 minutes during the first 72 hours.

Take all medications the doctor has prescribed for you according to directions. Ibuprofen 200 (up to 3 tablets) every 8 hours

After the periodontal treatment, proper oral hygiene must be maintained in the surgical area to aid in healing. Use the soft toothbrush provided by the doctor to carefully brush the teeth while avoiding the gums in the area of surgery. After one week, Regular brushing and flossing can be resumed after 2 weeks.

After periodontal surgery, sensitivity of the teeth may occur. This is remedied by maintaining proper oral hygiene and by the use of specially formulated sensitive toothpaste, such as, Sensodyne.

If you have any questions, please feel free to call.

## 11.4 Appendix 4



Universitat  
Internacional  
de Catalunya

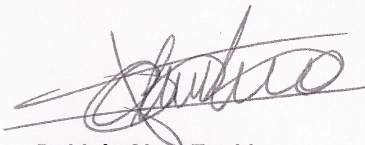
FACULTAD DE ODONTOLOGÍA  
Comisión científica

La comisión de doctorado del Departamento de Odontología de la Universitat Internacional de Catalunya, CERTIFICA que


El presente proyecto de Tesis Doctoral titulado: "**Diode laser treatment as an adjunct in periodontal surgery**", cuyo director es el Dr. José Nart Molina y cuyo investigador principal es el doctorando **Javier Sanz Moliner**,

ha sido evaluado satisfactoriamente y es apto para entrar en el programa de doctorado.

Firmado en Sant Cugat del Vallès, a 24 de noviembre del 2010. /



**Dr Lluís Giner Tarrida**  
Director de la comisión de doctorado de Odontología



Universitat Internacional  
de Catalunya  
Facultat d'Odontologia

Título:	Diode laser treatment as an adjunct in periodontal surgery
Investigador principal:	Javier Sanz Moliner
Director de la tesis:	Dr. José Nart Molina
Número de estudio:	PER-ECL-2010-02-NF



## 11.4 Appendix 4

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CC: javisanz79@hotmail.com; josenart@hotmail.com; rcohen@buffalo.edu;  
ciancio@buffalo.edu

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29-Mar-2012

Dear Dr. Sanz-Moliner,

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**11.6 Appendix 6**

**The Effect of a 810 nm Diode Laser on Postoperative Pain and Tissue Response following modified Widman Flap Surgery: A Pilot Study in Humans.**

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**Wordcount:** 3157

**TABLES:** 2

**FIGURES:** 3

**Short Running title:** Diode laser (810nm) during modified Widman flap surgery.

**Summary:** The use of a diode laser (810nm) as an adjunct to modified Widman flap provided additional benefits in terms of less edema and post operative pain.

### **Abstract**

**Background:** The purpose of this single-masked pilot clinical study (P/E0510307E) was to compare the tissue response and postoperative pain following the use of a diode laser (810nm) (DL) as an adjunct to modified Widman flap (MWF) surgery to that of MWF alone. **Methods:**

Thirteen patients with generalized severe chronic periodontitis completed the study. Control sites were randomly selected to receive a MWF and the contra-lateral test sites a MWF in conjunction with a DL. The study tooth/site was treated plus any additional teeth in the quadrant in which the site was located, if needed. Randomization was done using a coin flip.

The DL was used to de-epithelialize the inner part of the periodontal flap and photobio-stimulate the surgical area. Pain scale assessment (PS), pain medication consumption (PM), tissue edema (TE), and tissue color (TC) were evaluated one week following surgery.

**Results:** Statistically significant differences were seen for TE ( $p=0.041$ ), PM ( $p<0.001$ ) and PS ( $p<0.001$ ) favoring test sites. TC did not show a statistically significant difference ( $p=0.9766$ ). Patients rated the first surgical treatment performed as more painful than the second ( $p<0.002$ ).

**Conclusion:** The use of a 810nm diode laser provided additional benefits to modified Widman flap surgery in terms of less edema and post operative pain.

Key Words: Clinical trial(s), Laser(s), Pain, Periodontal surgery, Wound healing.

### **Introduction**

Lasers have many uses in periodontal therapy. Low level lasers have been found to enhance periodontal healing<sup>1,2</sup> and to reduce pain.<sup>3,4</sup> Surgical lasers can remove diseased pocket lining epithelium,<sup>5,6</sup> detoxify root surfaces,<sup>7-9</sup> and assist with coagulation.<sup>10</sup> The use of low level laser therapy (LLLT) as a therapeutic agent was first investigated by Mester in 1971, who

found that it improved wound healing in rats.<sup>11</sup> However, the role of LLLT in periodontal treatment is still under investigation.

Low level lasers emit visible red and near-infrared wavelengths that are absorbed in the photoreceptors within subcellular components, targeting the electron transport/respiratory chain within the membranes of mitochondria.<sup>12</sup> Absorption of laser energy increases ATP formation, which in turn stimulates cell function.<sup>13</sup> This increase in cell function may help improve wound healing. In vitro studies have shown that LLLT increases the synthesis of DNA,<sup>10</sup> collagen,<sup>14</sup> and procollagen<sup>15</sup> as well as to stimulate<sup>16</sup> and increase<sup>17</sup> the proliferation rate of fibroblasts.

Nevertheless, the beneficial effect seen in vitro is not completely observed in clinical studies. Low level lasers have not been found to reduce gingival inflammation on gingivitis patients.<sup>18</sup> Another study<sup>19</sup> showed that they have no effect on bacterial load and clinical parameters when LLLT was used as an adjunct to scaling and root planing. On the other hand, Almeida et al.<sup>20</sup> have shown improved healing of free gingival grafts during the early period of healing. Ozelik et al.<sup>21</sup> found a noticeable improvement after a gingivectomy when LLLT was applied daily over a period of one week. Also, the use of lasers as an adjunct in scaling and root planning in the Qadri et al.<sup>22</sup> study showed a significant improvement in clinical parameters. Overall, the effect that low level lasers have on periodontal treatment is unclear.

In dentistry, the analgesic effect of low level lasers has been extensively studied with a wide range of results. Studies have been performed to evaluate the effect of lasers on dentin hyperesensitivity,<sup>23</sup> temporomandibular joint disorders,<sup>24</sup> analgesia after third molar extraction<sup>25</sup> and as an adjunct in orthodontic treatment.<sup>26</sup> However, few studies have addressed the effect that lasers have on pain in periodontics. Masse et al.<sup>27</sup> failed to show a significant analgesic effect of low level lasers when used after the placement of free gingival grafts.



However, in the study performed by Tomasi et al.<sup>4</sup> low level lasers showed an analgesic effect when used during periodontal maintenance. Overall, the true analgesic effect that low level lasers provide is unclear.

Periodontal disease is not a painful disease. However, the treatment of periodontal problems is experienced as painful by many patients.<sup>28</sup> Studies have analyzed the level of pain experienced by patients following periodontal treatments. It has been reported that on average, 30 percent of patients experience some pain during the first week following periodontal surgery.<sup>29,30</sup> Mucogingival surgery and osseous recountouring have been reported to be the most painful.<sup>28,29,31</sup> Since low level laser therapy may reduce the pain experienced by patients receiving periodontal therapy which in turn may encourage them to treat their periodontal problems, further studies should be performed.

The use of low level laser therapy on periodontal healing has been studied previously but results regarding the effect that LLLT has on healing and postoperative pain are still under investigation. Also, the literature is deficient in studies that specifically use a 810 nm diode laser as an adjunct to periodontal surgery. In view of the lack of evidence for benefits of laser assisted surgery versus non laser assisted surgery, the present study evaluated if a diode laser would improve the tissue response and would reduce postoperative pain following a modified Widman flap (MWF) surgery.

#### **Materials and Methods**

Subjects from the dental clinic at the University of Buffalo, School of Dental Medicine in New York were recruited and treated in the periodontal clinic. A total of fifteen patients (9 males and 6 females) with a mean age of 48 years (Standard Deviation (SD)  $\pm 8.5$ ) were included in the study. Each patient had two contra-lateral periodontal pockets (same type of tooth in opposite quadrants of the mouth treated).



### *A 810 nm Diode Laser following modified Widman Flap Surgery*

To qualify for this study, patients had to have, in two contra-lateral quadrants, at least the same posterior or anterior tooth with one site each with a pocket depth  $\geq 7$  mm, clinical attachment levels  $\geq 7$  mm and a gingival index  $\geq 1$ .<sup>32</sup> Candidates who reported long-term steroidal or antibiotic therapy, systemic diseases likely to affect wound healing, or current pregnancy were excluded from the study.

The number of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome are shown in FIGURE 1. Two patients dropped out of the study after the first surgical procedure since they could not keep subsequent appointments, leading to 26 periodontal sites in 13 patients for study analysis. Demographic and clinical characteristics of the 13 patients for study analysis are shown in TABLE 1. An informed consent form was signed by each patient. The study and informed consent form was approved by the University at Buffalo Health Sciences Institutional Review Board (P/E0510307E).

This study was a randomized single masked split mouth clinical study. The patient did not know which site received laser (810nm) therapy. One operator (JS) enrolled, evaluated, and treated all patients. The primary outcome with respect to the effect of laser (810nm) use during periodontal surgery was the reduction of self reported post operative pain as measured by the modified visual analogue scale and daily intake of pain medication.

All patients received a hygienic treatment phase consisting of oral hygiene instructions, supragingival prophylaxis and scaling and root planing at least 6 weeks prior to the surgical phase of treatment.

At the baseline visit, the contra-lateral surgical sites were randomly assigned to control or to test using a coin flip. Contra-lateral meant same type of tooth in opposite quadrants of the mouth. The study tooth/site was treated plus any additional teeth in the quadrant in which the site was located, if needed. The control sites received a MWF<sup>33</sup> and sham application of a

diode laser (810nm). The test sites received a MWF with the application of an active diode laser (DL) (810nm) to the inside of the modified Widman flap. The time between the two surgeries was 3 weeks. In control and test sites, a MWF was performed followed by the elimination of granulation tissue using hand instruments, ultrasound, and rotary instruments. Root surfaces were scaled and planed with curets. In one control quadrant and in one test quadrant in two different patients, minor osteoplasty was performed. In the test sites, an aluminium, gallium and arsenide diode laser<sup>‡</sup> with a wavelength 810 +/- 20 nm and a power of 1 watt in a continuous mode was applied to the MWF. A 400 microns diameter tip was used to remove all visible epithelium in the inner side of the flap from the free gingival margin to the bottom of the apical aspect of the flap (both labial and lingual/palatal). The tip was initiated with the use of a blue articulating paper. The treatment was performed from the coronal to the apical aspect in parallel paths and the laser (810nm) emission was interrupted for 30 seconds after irradiation exceeded 10 seconds in time. The resultant char layer was totally removed with moist gauze prior to replacing the flaps. Care was taken to avoid any laser (810nm) contact to the root surface or the alveolar bone by placing a periosteal retractor between the hard and soft tissue and aiming the laser (810nm) beam at a 45° angle to the soft tissue flap. A second laser (810nm) application with the same laser (810nm) in continuous mode at 0.1 watts was made. All surfaces of the flap, inner and outer, exposed bone and exposed root structures involved in the surgery were irradiated, leading to a total dosage of 4 J/cm<sup>2</sup> per surface. In the control sites, laser (810nm) application was simulated without pushing the start button. The MFW was sutured with an interrupted suture using 4-0 black silk suture in all patients. All surgeries were performed by the same operator (JS). Surgeries performed on all patients were virtually identical. In each patient and all procedures were completed within a one hour time frame. The power output of the laser (810nm) was assessed throughout the duration of



the study using a hand held meter provided with the unit.

Postoperative instructions were given to the patients. A soft toothbrush was to be used during the first two weeks after the surgical procedure. Patients were requested to avoid brushing with their pre-operative daily tooth brush, flossing and chewing in the treated area for a period of 2 weeks. Patients resumed full oral hygiene and function after 2 weeks. Ibuprofen 200 (up to 3 tablets) every 8 hours was allowed as the pain medication. Patients evaluated postoperative pain or discomfort daily at night time for one week using a modified visual analogue scale graduated from 0 to 10. Patients reported the number of 200 mg ibuprofen tablets they use and the daily amount of pain medication used was recorded. The primary outcome with respect to the effect of laser (810nm) use during periodontal surgery was the reduction of self-reported postoperative pain as measured daily at night using a modified visual analogue scale and daily consumption of pain medication. The secondary outcome was tissue response with regard to levels of tissue edema and color based upon visual analysis.

The daily modified visual linear scale and the self-reported consumption of pain medication was returned to the investigator one week later. Sutures were also removed and tissue response was evaluated at the study site. The examiner did not know which site received which treatment.

The tissue edema was scored as follows: 1= absent, 2=slight, 3= moderate, 4= severe. The color of the gingiva was recorded as follows: 1= pink, 2= mixed, 3= red. Five patients were used to calibrate the examiner. The patients were evaluated on two occasions, 1 hour apart. The reliability coefficient of the examiner for erythema was substantial (weighted Kappa=0.71) and substantial (weighted kappa 0.75) for edema.

### **Statistical Analysis**

A power calculation before the initiation of this study revealed that a sample size of 15 was

necessary to detect a difference of 2 in the pain scale, assuming a maximal mean standard deviation of 2.5 using a paired test with 80% power and a 0.05 significance level. Study hypothesis of the primary and secondary outcomes were analyzed using paired t tests, Wilcoxon signed rank test and repeated-measures analysis of variance (ANOVA) where appropriate. The effect of smoking on secondary outcomes was analyzed using a nested ANOVA. A Shapiro-Wilk test was used to assess the normality of the data. A paired-t test was used if distribution of the data was normal. A Wilcoxon signed rank test was used if distribution of the data was non-normal. All statistical tests utilized two-sided p-values and a statistical software<sup>s</sup> was used in the analysis of the study data. Differences associated with p-values  $\leq 0.05$  were declared statistically significant.

## **Results**

### **Tissue Response**

No patient reported any adverse effect from treatment. Tissue color and tissue edema at surgical sites were evaluated one week after surgery. The average tissue color was  $1.9 \pm 0.4$  at the control sites and  $1.8 \pm 0.4$  at the test sites. The color of the tissue was not statistically different ( $p=0.9766$ ) between the study sites. With respect to tissue edema, the average of control sites was  $2.1 \pm 0.8$  where the average of test sites was  $1.7 \pm 0.7$ . Statistically significant less edema was seen at the test sites ( $p=0.0410$ ).

The effect of smoking on tissue response was analyzed. Tissue edema ( $p=0.2155$ ) and tissue color ( $p=0.2346$ ) were not influenced by the smoking habit of the patient.

### **Postoperative discomfort**

The pain scale mean value for control sites was  $3.6 \pm 2.7$  and for the test site was  $2.4 \pm 1.9$ . As seen in FIGURE 2, statistically significant differences ( $p < 0.0001$ ) between study sites were seen, favoring test sites. The pain medication consumption taken after surgical procedures was

2.1±2 for control sites and 0.9±1 for test sites. As seen in FIGURE 3, a statistically significant greater consumption in pain medication was observed in the control group ( $p<0.0001$ ).

**Effect of the surgery order on postoperative discomfort**

Overall, the surgical treatment (test and control) performed first was significantly more painful than the second. As shown in TABLE 2, this difference was seen in the pain scale scores ( $p=0.0005$ ) and in the amount of pain medication consumed ( $p=0.002$ ).

**Discussion**

This study found that diode laser (810nm) use did not lead to postoperative complications or to impair tissue response, indicating that this type of laser (810nm) has no detrimental effects when used in conjunction with MWF surgery. Also, this study found that the tissue edema and postoperative pain were reduced with the use of the diode laser (810nm).

Since the optimal dosage and treatment schedule has not been determined, it is difficult to evaluate the efficiency of low-level laser therapy. As a result, studies have encountered a wide range of clinical findings due to the differences in experimental and assessment methods and irradiation conditions.

The literature shows little correlation between both in vitro and in vivo animal studies and human trials when using low level laser therapy. An in vitro study performed by Stein et al.<sup>34</sup> showed a positive effect of LLLT on osteoblasts where an in vivo animal study performed by Khadra et al.<sup>35</sup> found a positive effect on bone formation in rat calvaria. Given the problems in extrapolating irradiation parameters and findings from in vitro or animal research to human practice, trials in humans are essential.

Despite the proposed benefit of LLLT, there are very few clinical studies using LLLT in gingival surgery. This makes the comparison of the current study's results with previous

studies difficult. Various human randomized control clinical studies that analyzed the periodontal healing response using LLLT are available. While Amorim et al.<sup>36</sup> and Ozcelik et al.<sup>21</sup> had reported that LLLT significantly promoted healing of various periodontal surgical procedures, Masse et al.<sup>27</sup> and Damante et al.<sup>37</sup> had found that LLLT with a diode laser did not improve healing. This difference in results may have occurred since various lasers, intervals of application, surgical procedures and ways to evaluate laser wound healing were used. The finding of a statistically significant reduction in edema in the 810 nm diode treated sites found in the present study may have occurred due to the promotion of healing as shown in the studies by Amorim et al.<sup>36</sup> and Ozcelik et al.<sup>21</sup> In the present study, the difference in edema reduction in the test sites with respect to control sites was minimal. Although statistically significant, this may not be clinically significant.

It has been suggested that daily treatment with LLLT is required to achieve maximum results.<sup>38</sup> Ozcelik et al.<sup>39</sup> compared the use of enamel matrix derivative in intrabony defects with or without the application of a diode laser at a wavelength of 580 nm ( $4\text{j}/\text{cm}^2$ ) during surgery and daily for 5 days after surgery. Clinical parameters such as clinical attachment and pocket depth did not differ between treatment sites. The current study found that tissue edema was improved significantly ( $p < 0.05$ ) more in the laser (810nm) treatment verses control treatment when LLLT (810nm) was applied once during the surgical procedure. The study performed by Ozcelik et al.<sup>39</sup> found that swelling was statistically reduced after 1 week ( $p < 0.05$ ), although the application of LLLT added up to 40 minutes more to the surgical procedure. In the current study, additional time utilizing the laser (810nm) was minimal. This could indicate that only minor application of LLLT (810nm) can provide improvement in tissue edema.

Numerous studies have shown that smoking may negatively impact the results of periodontal treatment.<sup>40-42</sup> Seven of the 13 patients that finished treatment in the current study had a smoking habit. Data was analyzed in order to establish the effect of smoking on tissue response. Overall, smoking did not affect the tissue response. These results differ from the previously mentioned studies. It may be advantageous for future studies utilizing lasers in conjunction with periodontal therapy to include only non-smokers.

The pain scale used in this study was subjective and highly dependent upon individual experience. However, the patient served as both the control and test subject. The patient was not aware of which surgical site received laser (810nm) treatment in order to reduce the “placebo effect of laser treatment”. The subjective measure of the pain using the pain scale found statistically significant differences between the control sites and the test (810nm) sites. In disagreement with our study, Masse et al.<sup>27</sup> and Ambrossini<sup>43</sup> found no statistically significant differences in pain scale values in control surgical sites when compared to the laser sites. In contrast, Ozcelik et al.<sup>39</sup> found a statistically significant improvement in pain values for the laser group during the first 2 days following surgery when compared to conventional treatment.

There was statistically significantly less pain experienced by patients following the second surgical procedure. This is in accordance with other studies.<sup>28,30,44</sup> It is unclear why patients experience less pain after a second surgical procedure but it may be related to psychological expectations. We found that use of a 810 nm diode laser during surgical treatment resulted in less pain than the control treatment, with the difference reaching statistical significance. This finding suggests that laser (810nm) application may be more beneficial in procedures where postoperative pain is expected by the patient and/or the practitioner.



Two patients received minor osteoplasty during the procedure, one in the control site and the other in the test site. According the study performed by Canakçi and Canakçi<sup>31</sup> patients perceived more pain when osseous resection was performed with a modified Widman flap than that experienced by patients with only the modified Widman flap procedure. Since osteoplasty was performed in only two patients, the sample size was too small to analyze the effect that this factor had on pain. Therefore the data was analyzed twice, once including the patients that required osteoplasty and the second time without including these patients. No difference was found between these two data regarding the significance of pain between treatment modalities. Pain was found to be statistically significantly greater in the control groups. Since a minor amount of osteoplasty was performed, it was unlikely that it would have an effect on pain.

The current study was one of the first human clinical studies that used a diode laser (810nm) as an adjunct to periodontal surgery. Lasers currently have a variety of uses in dentistry and some low level lasers have been found beneficial in in vitro studies. However, clinical outcomes of in vivo application of low level lasers is still unclear and little is known regarding the optimal type, wavelength, power, energy delivered and method of using lasers in conjunction with periodontal surgery. In order to assess if lasers will provide additional benefits to periodontal treatment, further studies in a larger number of patients are needed.

#### **Footnotes**

‡Odyssey 2.5 G, Ivoclar Vivadent, Amherst, NY, USA

§SAS version 9.01

#### **Conflict of interest**

The authors declare no conflict of interest related to this study. No funding was provided for the study.

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**Table 1** Demographics of study participants.

	Participants
Number	13
Age (years)	52 ± 8.5
Gender(female)	5 (38.5%)
Smoking status	7 (53.8%)
Ethnic origin	
African	2 (15.4%)
Caucasian	11 (84.6%)

**Table 2** Comparison pain values by surgery order

	First	Second	P*
Pain medication (number of tablets)	1.80 ± 2.18	1.09 ± 1.48	0.002
Pain scale	3.63 ± 3.09	2.13 ± 1.48	0.0005

\*repeated-measures ANOVA

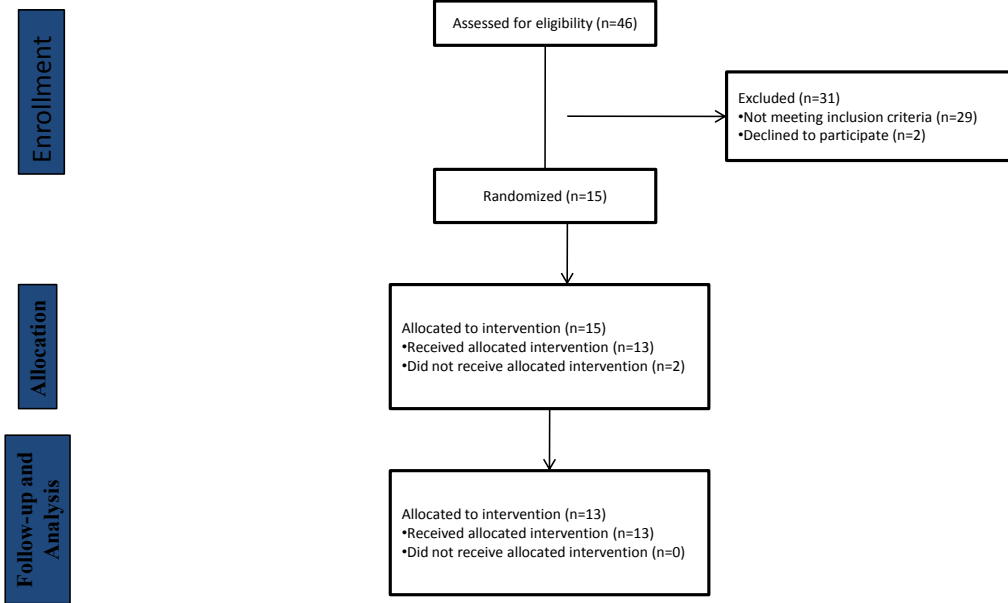


Figure1. CONSORT statement flowchart.

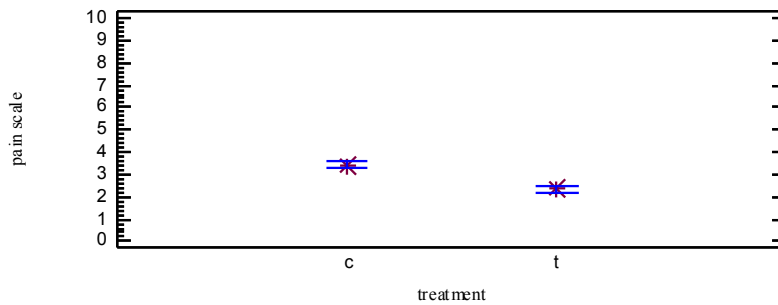
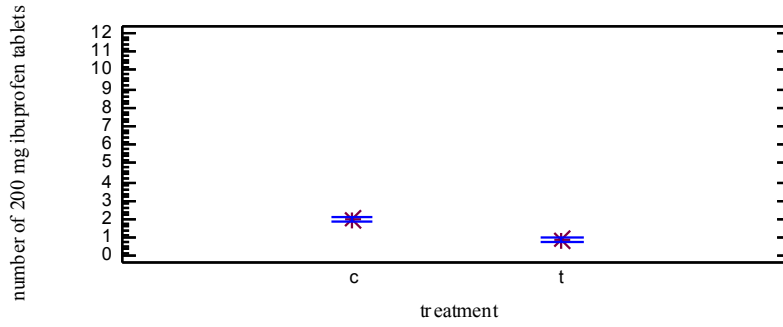


Figure 2. Mean and 95 % confidence intervals of the least significance difference for pain scale at control and test sites.





**Figure 3.** Mean and 95 % confidence intervals of the least significance difference for pain medication at control and test sites.