# The Inhibition Effect of 940 nm Diode Laser on Some Microorganisms Associated With Gingivitis

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## ABSTRACT

**Background :**Poor oral hygiene lead to gingival inflammation due to the accumulation of plaque and microorganisms. Scaling and polishing was considered as an effective treatment of gingivitis. Several systemic and local antimicrobials were found to enhance the mechanical treatment. However, these antimicrobial agents may lead to bacterial resistance and systemic side effects in addition to limitation in the accessibility to the affected areas. The aim of this study is to explore the effect of diode laser (940nm) in combination to the traditional mechanical treatment.

**Materials and methods:** twenty patients with bilateral gingivitis were selected. For each patient, one side of the mouth was treated traditionally. The other side was treated with 2 W diode laser (940nm) after scaling and polishing. Smears were taken from deepest points of the gingival sulci of both sides. The collected bacterial samples were inoculated in brain-heart infusion and inoculated into blood agar plates and MacConkey agar plates for 24 hours aerobically and 24-48 hours anaerobically (using jar and CO2 gas pack) at 37ºC. The collected data of bacterial identification were tabulated in excel tables. Means, standard deviations, ANOVA test and p value were calculated.

**RESULTS** there is highly significant decrease in the identification of aerobic bacteria after using diode laser. Anaerobic bacteria were less identified, but they were completely absent after diode laser treatment.

Conclusion: Diode laser (940nm) could be successfully used adjunctive to mechanical scaling and polishing to integrate the antimicrobial action within the gingival sulci.

**Keywords:** Diode laser, Gingivitis, bacteria .

## INTRODUCTION

Gingivitis is the most common form of oral diseases, which is often caused by poor oral hygiene. It can progress to the destruction of bone and loss of teeth.[1] If good oral hygiene is restored, gingivitis is usually eradicated and the tissues become clinically normal again. Estimates of the incidence of gingivitis are difficult to determine but probably the whole dentate population is affected by this condition at some stage. Generally, gingivitis is regarded as resulting from a non-specific proliferation of the normal gingival crevice micro-flora due to poor oral hygiene.[2] Oral cavity is colonized by over 1000 different types of microorganisms during our lifespan. The key factor for oral health is the normal balance among these microorganisms. These bacteria are most often found in oral mucosa, on dental surfaces, in saliva and gingival fluid. Dental plaque is formed by these microorganisms (which is an adherent complex structure) functions as a multicellular organism with over 100 microorganisms in one cubic millimeter.[3]

Although gingivitis is a reversible inflammatory condition, continuous accumulation of microbial biofilm due to impaired or poor oral hygiene results in the clinical manifestation of gingivitis and may precede periodontitis.[4]Pathogenic occurrences during this inflammatory process in the gingival tissue enhance the release of pro-inflammatory mediators, such as cytokines and prostaglandins, which cause damage the periodontal tissue. This biofilm is an independent entity with a strong ability to survive, and together with its bacteria and their byproducts (lipopolysaccharides), results in damage to the periodontal tissue.[5]Gingival diseases can also be modified by systemic factors. The clinical signs are exaggerated and the gingivae are more edematous and inflamed in individuals undergoing hormonal disturbances (e.g. during puberty or pregnancy). Certain drug therapies (e.g. immunosuppressive drugs) can also result in gingivitis.[2] On the other hand, there is significant effects of periodontal microorganisms on certain systemic conditions and diseases, like myocardial infarction, atherosclerosis, stroke, osteoporosis, premature birth and low-birth-weight babies; and by increasing the damage caused by diabetes mellitus and chronic respiratory diseases.[6]

Conventional treatment of periodontal disease is based on reduction of pathogenic microbiota by scaling and root planing.[7]However, conventional periodontal treatment are not equally effective in removal of all types of perio pathogens and their toxins from

infected sites. Thus adjunctive treatment plans have evolved to manage periodontal diseases[8] Under specific circumstances systemic antimicrobials may be used as adjuncts to treatment, leading to significant bacterial reduction and additional clinical benefits to the mechanical procedure. On the other hand, systemic antimicrobials may have potential for adverse reactions and development of resistant bacteria strains.[9,10]The local antimicrobial agents have wider applications, since they create higher concentrations of the effective agent within the pocket and compared to systemic antimicrobials, they cause less side effects. The main problem associated with the use of these local agents is the difficulty of maintaining therapeutic concentrations of antimicrobials in the affected sites.[8]

Because of these shortages of antimicrobials, the use of laser irradiation has become a topic of much interest and is a promising field in periodontal therapy.[11]Antimicrobial effect of laser is considered as a safe coadjutant in nonsurgical treatment of gingivitis, as it has been proved to reduce the signs of inflammation and microbial infection without any harmful effects on adjacent periodontal tissues.[12]The most common laser wavelengths used in periodontics include those of diode, neodymium:yttrium-aluminium-garnet (Nd:YAG), erbium:yttrium-aluminium-garnet(Er:YAG) lasers and carbon dioxide ($CO_2$) laser.[13]Diode lasers are solid-state semiconductor lasers (800-980 nm) poorly absorbed in water, but highly absorbed in hemoglobin. Since diodes basically do not interact with dental hard tissues, the FDA approved the use of a diode laser for soft tissue surgery in 1995 and for sulcular debridement in 1998.[14]

The goal of the current study was to evaluate the efficacy of diode laser (940 nm) as a coadjunct to conventional scaling and polishing to achieve bacterial reduction in chronic gingivitis.

## MATERIALS AND METHODS

**Study design and population:**A randomized, split mouth design double blind controlled trial was conducted at the periodontics education clinic of Tikrit University/College of dentistry. Prior to the beginning of this study, the patients were assessed for eligibility. The subjects should had presence of chronic gingivitis on both right and left sides. One side used to test and the other side used as control. The exclusion criteria were: (1) patients with systemic diseases or conditions that might interfere with oral health; (2) patients who had undergone previous periodontal treatment or had received antibiotics during the past three months. Before inclusion in this study, informed consents were obtained from all patients, according to Helsinki declaration (ethical principles for medical research involving human subjects).

Twenty patients (10 males and 10 females) with ages ranged between 25 to 35 years old were selected for this study. The patients complained of chronic gingivitis bilaterally with moderate plaque and calculus accumulation.

**Treatment procedure:** On the control side, the affected area was scaled by using manual and ultrasonic device. Polishing was performed with low speed hand-piece, rubber cup and pomuce powder. The experimental side underwent the same listed steps. In addition, 940 nm diode laser (Ezlase, Biolase, USA) were used with 2 W output power and 0.5 ms pulse duration within the gingival sulcus for 20-30 seconds. The treated area on both sides (experimental and control) were isolated with cotton roll and dried by using triple syringe. Smears were taken from both sides, bu introduction of sterile paper points into the deepest points of the gingival sulci.

**Microbiological examination:** The collected bacterial samples were inoculated in brain-heart infusion aerobically and anaerobically and incubated for 24 hours at 37ºC. After that the samples were inoculated into blood agar plates and MacConkey agar plates for 24 hours aerobically and 24-48 hours anaerobically (using jar and $CO_2$ gas pack) at 37ºC. The isolated bacterial strains were identified by Api-20Afor anaerobic bacteria and ApiEnterobacteriase, Api Staph and Apistrept for aerobic bacteria (Biomeriaex Co., France).

Statistical analysis: collected data including identification of microorganisms from both control and experimental sides were tabulated in excel tables (Microsoft office 2010). Means, standard deviations, ANOVA test and p values were calculated using IBM SPSS 19.0.0 statistical package for windows.

## RESULTS

In this study, five aerobic bacteria (*E coli, Klebsiellapneumonia,Serratia sp.,Staph aureus*and *Viridans streptococci*) as well as three anaerobic bacteria (*Villonilla sp., Fusobacterium sp.*and *Peptostreptococcusniger*) were isolated and identified. The results obtained from MacConkey and blood ager media for identification of bacterial species isolated from gingival sulci of both experimental and control samples are shown in table (1).Regardless the method of identification, all bacterial species were less identified from the experimental samples.

Table (2) shows the means and the standard deviations of the identified aerobic bacterial strains in

both control and experimental samples. The isolated aerobic bacteria showed very highly significant decrease in experimental samples ($p<0.001$).

Anaerobic bacterial strains and their means and standard deviations of identification are listed in table (3). The three strains (*Villonilla sp., Fusobacterium sp.*and *Peptostreptococcusniger*) were identified in control samples. However, they were completely absent in experimental samples.

## DISCUSSION

Several studies have shown a close relationship between the appearance of gingivitis and oral hygiene.[4] Bacterial accumulation on the tooth surface is the main cause of the gingival inflammation. These bacteria are in equilibrium with the gum tissues. Plaque related gingival disorders occur when this balance is altered.[15] Early diagnosis and treatment of the initial gingival inflammation is important to reduce the prevalence and severity of subsequent periodontitis.[4]The limited access of topical and systemic antimicrobial agents to dental plaque and the development of bacterial resistance toward antibiotics have led to search alternative strategies to control dental biofilm and to treat periodontal diseases.[16] For the past decades, many lasers have been investigated in the treatment of periodontal diseases among other oral conditions.[17]The use of modern laser technology in periodontal therapy has the advantage of reaching areas that are almost not accessible to conventional treatment. In addition, periodontaics studies show that the bactericidal effect of the lasers is the main purpose for their us as adjuncts to conventional periodontal treatment.[18]So the inhibition of the bacteria within the gingival sulcus is the deciding factor for the choice of the laser parameters. However, it has not been found, in previous literature, any standardization of these parameters.[17]

The result of current study demonstrated that 940 nm diode laser associated with conventional scaling and polishing promote additional enhancement of the conventional scaling and polishing treatment alone, as regard of microbiological inhibition. There is very compelling evidence in the dental literature that the addition of diode laser irradiation to conventional mechanical treatment has a significant bactericidal and detoxifying effect in periodontal therapy.[8] It is difficult to compare the results of our study with the previous studies because of differences in the laboratory settings and irradiation constants. Although most of these studies used diode laser, they do not mention which laser wavelength, energy density, duration, mode or frequency used. It seems that the differences in these factors led to different results. Another limitation is that most of the previous studies were performed on the effect of lasers on periodontitis. This study used laser on gingivitis which precedes periodontitis. That means that our study is more conservative ant absolutely better in preventive measures In agreement with the current study findings, Mortiz*et al*. reported significant bacterial inhibition with diode laser therapy compared to conventional treatment.[19] Several other studies have revealed that diode laser may significantly suppress microorganisms related to periodontal diseases.[20-22] In contrast, another study reported that the use of diode laser had no significant additional effects on bacterial inhibition.[23] An interesting study stated that the low power diode laser could stimulate, instead of inhibit, the viability of *Klebsiella pneumonia*.[24] However, in the present study the significant inhibition to this bacteria is less than that to other studied species. Other study stated that mechanical treatment alone has been shown to be clinically and microbially effective. The clinical benefits are derived from the removal of subgingival plaque and disruption of bacterial counts.[25] In our study , diode laser was used as an adjunctive treatment to conventional mechanical treatment, but not as a primary treatment of gingivitis. It should kept in mind that the diode laser therapy used to perform decontamination to the previously scaled and polished areas and conventional mechanical treatment should not be discarded.

The mechanism of diode laser inhibition of bacteria has not been determined. Two possible mechanisms were suggested. In the first mechanism, the laser light considered to be absorbed by the substrate onto which the bacteria adhere. The resultant heating causes high local rise in temperature and lead to death of the attached bacteria. The second mechanism suggested that the laser light is absorbed directly by the bacteria which causes damage of the bacterial cell structures.[26]

The presence of bacteria in the gingival sulcus and periodontal connective tissues is a determinant factor in the development of periodontitis. In areas of difficult access, the use of manual or ultrasonic instruments is not enough to ensure the eradication of periodontal pathogenic bacteria. Likewise, antibiotic resistant strains may also damage the efficacy of conventional treatment.[27] This gives laser therapy a great advantage over systemic antibiotic therapy, since laser radiation does not negatively affect the rest of the body and does not cause resistance. The minimal risk

of creating resistant bacterial strains is laser therapy's greatest advantage.[6] According to previous reports, there are many other advantages of diode laser gingival therapy like the positive effects on wound healing.[28]Moreover, diode laser therapy may significantly enhance patient comfort during the post-operative healing phase because it involves minimal pain.[29]

**Table 1: Isolated microorganisms from the gingival sulci.**

| Isolated microorganisms | MacConkey agar | | Blood agar | |
|---|---|---|---|---|
| | control | experimental | control | experimental |
| *E coli* | 20 | 0 | 1 | 2 |
| *Klebsiellapnemonia* | 20 | 16 | 20 | 14 |
| *Serratia sp.* | 20 | 0 | 19 | 0 |
| *Staph aureus* | 3 | 0 | 19 | 0 |
| *Viridans streptococci* | 0 | 0 | 19 | 9 |
| *Villonilla sp.* | - | - | 1 | 0 |
| *Fusobacterium sp.* | - | - | 3 | 0 |
| *Peptostreptococcusniger* | - | - | 1 | 0 |

**Tables 2: Means and standard deviation of identified aerobic bacterial strains from the experimental and control group using MacConkey and blood agar.**

| Group | E coli | | Klebsiellapnemonia | | Serratia sp. | | Staph aureus | | Viridans streptococci | |
|---|---|---|---|---|---|---|---|---|---|---|
| MacConkey agar | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD |
| Control | 1 | 0 | 1 | 0 | 1 | 0 | 0.15 | 0.134211 | 0 | 0 |
| Experimental | 0 | 0 | 0.8 | 0.1684 | 0 | 0 | 0 | 0 | 0 | 0 |
| Blood agar | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD |
| Control | 0.05 | 0.05 | 1 | 0 | 0.95 | 0.05 | 0.95 | 0.05 | 0.95 | 0.05 |
| Experimental | 0.1 | 0.0947 | 0.7 | 0.2210 | 0 | 0 | 0 | 0 | 0.45 | 0.2652 |

**($p<0.001$)**

**Tables 3: Means and standard deviation of identified anaerobic bacterial strains from the experimental and control group.**

| Group | Villonilla sp. | | Fusobacterium sp. | | Peptostreptococcusniger | |
|---|---|---|---|---|---|---|
| Blood agar | mean | SD | mean | SD | mean | SD |
| Control | 0.05 | 0.218 | 0.15 | 0.3571 | 0.05 | 0.218 |
| Experimental | 0 | 0 | 0 | 0 | 0 | 0 |

## REFERENCES


1. Azadeh M, Kermanshahi RK, Sadat naghavi N, Ghalayani P, Salamat F. The profile of pathogenic bacteria isolated from dental plaque induced gingivitis. International Journal of Molecular and Clinical Microbiology 2011;1:36-39.
2. Marsh PD, Martin MV. Oral microbiology. Fifth edition. Elsevier Limited 2009.p120.
3. Sánchez MC, Liama-Palacios A, Blanc V. Structure, viability and bacterial kinetics of an in vitro biofilm model using six bacteria from subgingival microbiota. J Periodontal Res 2011;46:252-260.
4. Igic M, Kesic L, Lekovic V, Apostolovic M, Mihalovic D, Kostadinovic L, M ilasin J. Chronic gingivitis: the prevalence of periodontopathogens and therapy efficiency. Eur J ClinMicrobiol Infect Dis 2012;31:1911-1915.
5. Hojo K, Nagaoka S, Oshima T, Maeda N. Bacterial interactions in dental biofilm development. J Dent Res 2009;88(11):982-990.
6. Atanasovska-Stojanovska A. Modern approach to the identification and elimination of periodontal infection. Macedonian Journal of Medical Sciences 2012;5(2):215-221.
7. Theodoro LH, Silva SP, Pires JR, Garcia Soares-GH,Pontes AEF, Zuzu EP, Spolidó DMP, Correa de Toledo BE, Garcia VG. Clinical and microbiological effects of photodynamic therapy associated with nonsurgical periodontal treatment. A 6-month

follow-up. Lasers Med Sci 2012;27:687-693.
8. Birang R, Yaghini J, Adibard M, Kiany S, Mohammadi Z, Birang E. The effect of diode laser (980 nm wavelength) and chlorhexidin gel in the treatment of chronic periodontitis. Journal of Lasers in Medical Sciences 2011;2(4):131-138.
9. Walker CB. Selected antimicrobial agents: mechanisms of action, side effects and drug interaction. Periodontology 2000:12-28.
10. Quirynen M, Teughels W. van Steenberge D. Microbial shifts after subgingival debridement and formation of bacterial resistance when combined with local or systemic antimicrobials. Oral Dis 2003.9(Suppl 1):30-37.
11. Sennhenn-Kirchner S, Klaue S, Wolff N, Mergeryan H, Borg von Zepelin M, Jacobs HG. Decontamination of rough titanium surfaces with diode lasers: microbiological findings on in vivo grown biofilms. Clin Oral Implants Res 2007;18:126-132.
12. Luan XL, Qin YL, Bi LJ, Hu CY, Zhang ZG, Lin J, Zhou CN. Histological evaluation of the safety of toluidine blue-mediated photosensitization to periodontal tissues in mice. Lasers Med Sci 2009.24(2):162-166.
13. Romeo U, Palaia G, Botti R, Leone V, Rocca JP, Polimeni A. Non-surgical periodontal therapy assisted by potassium-titanyl-phosphate laser: a pilot study. Lasers Med Sci 2010;25:891-899.
14. Aoki A, Saski KM, Watanabe H, Ishikawa I. Lasers in nonsurgical periodontal therapy. Periodontal 2004;36:59-97.
15. Liebana J, Castillo AM, Alvarez M. Periodontal diseases: microbiological consideration. Med Oral Cir Bucal 2004.9(Suppl):75-91.
16. Schneider M, Kirfel, Berthold M, Frentzen M, Kraus F, Braun A. The impact of antimicrobial photodynamic therapy in an artificial biofilm model. Lasers Med Sci 2012;27:615-620.
17. Alves VTE, de Andrade AKP, Toaliar JT, Conde MC, Zezell DM, Cai S, Pannuti CM, De Micheli G. Clinical and microbiological evaluation of high intensity diode laser adjutant to non-surgical periodontal treatment: a 6-month clinical trial. Clin Oral Invest 2013;17:87-95.
18. Kamma JJ, Vasdekis VGS, Romanos GE. The effect of diode laser (980 nm) treatment on the survival rate of gingival fibroblast cell cultures. Lasers Surg Med 2011;28:445-450.
19. Mortez A, Schoop U, Goharkhay K, Schauer P, Doetbudak O, Wernisch J. Treatment of periodontal pockets with a diode laser. Lasers Surg Med 1998;22:302-311.
20. Nussbaum EL, Lilge L, Mazzualli T. Effects of 810 nm laser irradiation on in vitro growth of bacteria: Comparison of continuous wave frequency modulated light. Lasers Surg Med 2002;31:343-351.
21. Wilson M. Lethal photosensitization of oral bacteria and its potential application in the photo dynamic therapy of oral infections. PhotochemPhotobiolSci 2004;3:412-418.
22. Mortez A, Gutknecht N, Doertbudak O, Goharkhay K, Schoop U, Schauer P. Bacterial reduction in periodontal pockets through irradiation with a diode laser: a pilot study. J Clin Laser Med Surg 1997;15:33-37.
23. Ryden H, Persson L, Perber H, Bergstorm J. Effect of low level energy laser irradiation on gingival inflammation. Swed Dent J 1994;18:35-41.
24. Hassan LM, Gizar R, Majeed LJ. Effect of diode laser 805 nm on the viability of some types of gram negative and gram positive pathogenic bacteria. Iraqi Journal of Science 2010;51(4):665-669.
25. Qadri T, Miranda L, Tuner J, Gustafsson A. The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation. J ClinPeriodontol 2005;32:714-719.
26. Pirnat S, Lukac M, Ihan A. Study of direct bactericidal effect of Nd:YAG and diode laser parameters used in endodontics on pigmented and nonpigmented bacteria. Lasers Med Sci 2011;26:755-761.
27. Eduardo Cd, de Freitas PM, Esteves-Oliveira M,Aranha ACC, Ramalho KM, Simões A, Bello-Silva MS, Tunér J. Laser phototherapy in the treatment of periodontal disease. A review. Lasers Med Sci 2010;25:781-792.
28. Savafi SM, Kazemi B, Esmaeili M, Fallah A, Modarresi A, Mir M. Effects of low-level He-Ne laser irradiation on the gene expression of IL-1beta, TNF-alpha, IFN-gamma, TGP-beta, Bfgf, and PDGF in rats gingiva. Lasers Med Sci 2008;23:331-335.
29. Andersen R, Loebel N, Hammond D, Wilson M. Treatment of periodontal disease by photodisinfection compared to scaling and root planning. J Clin Dent 2007;18:34-38.