

help of finite elements method (FEM), it has been possible to show the penetration of Nd:YAG laser inside the root canal and surrounding tissues [15]. We can suggest that the same investigation be performed on periodontal pockets to let the investigators talk more clearly about the energy density in three dimensional way.

It is recommended that the other pulse lengths of Nd:YAG laser like 300 microsecond pulses, be investigated in other studies in order to compare the results with the present study to demonstrate the positive effects on fibroblast activity [8].

Conclusion

The laser group of teeth pockets demonstrated a significant reduction in *Porphyromonas gingivalis* (PG)

and *Prevotella intermedia* (PI) bacteria compared to the control group.

100 us Nd:YAG laser is effective to decrease the depth of pockets and controlling the bleeding after treatment, because there was significant difference Probing Depth (PD) and Bleeding Index (BI) between lased and non lased groups. There was no significant difference in Clinical Attachment Level (CAL), Gingival Index (GI) and Recession (Rec.) between the groups at the condition of this study.

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Table-2- Comparison of clinical indexes before, immediately after and 1 and 2 month later are reported (P value numbers) between non lased and lased groups. Significant cells are underlined. The other indexes show no significant difference (n= 26 pockets in 6 patients).

Time Clinical Index	Before treatments	Immediately after treatments	1 month later	2 month later
Clinical Attachment Level (CAL)	0.848	0.978	<u>0.031<0.05</u>	<u>0.047<0.05</u>
Bleeding Index (BI)	0.552	0.999	<u>0.002<0.01</u>	<u>0.006<0.01</u>
Gingival Index(GI)	0.223	0.003	0.439	0.032
Recession (Rec.)	0.273	0.852	-----	-----
Probing Depth (PD)	0.798	0.845	<u>0.004<0.01</u>	<u>0.001≤0.001</u>

amount of PG micro-organisms which are the most responsible pathogenic micro-organisms in periodontal diseases, were much less in laser treated pockets significantly.

The PI specimen showed same reduction later but more profound. After two month the bacteria counts in laser treated sulcuses were significantly less than the non-laser treated ones (P< 0.0005).

In the laser group the PI count after one month was equal to the 0 in all the sulcuses. After two months this specimen only could be counted in one socket. It is necessary to note that because of the statistical analysis, less than 5% bacteria present was coded as 0. 5-30% was called as positive (+), 30-50% (++) and more than 50% (+++).

The same statement could be applied to PG counts reported one month after the treatment. However after 2 months only from 2 sockets this bacteria could be colonised.

Discussion

According to the most recent published literatures, the bactericidal effects of Nd:YAG laser in periodontal pockets is clear which the results of this study also supports it [6].

By evaluating laser therapy through clinical signs in patients, like the study of Liu et al. [7] in 1999 which completely replaced the conventional methods by laser therapy with the wavelenght of 1064 nm, no improvement was observed. But the study of Gutknecht's [1] research group published on 2002, described the advantages of laser assisted periodontal therapy in details. The present study with complete clinical evaluation of before and after treatment recorded in different time periods reached to this rational that the bactericidal effects of Nd:YAG laser with 100 micro-second pulsed width can improve the healing process of chronic and aggressive periodontal diseases in more established stages. This statement is a lot more solid in evidence than previous studies because clinical and bactericidal effects are significantly improved by only adding the laser treatment to conventional methods in patients with similar problems.

It is also important to note that the clinicians mostly do not take into account the detailed features of the Nd:YAG dental laser instruments and also not consult physic specialists. Therefore they may propose statements that are not correct because reaching the correct output energies and pulse duration is not possible.

While running Nd:YAG laser in a pulse mode with the repetition rate of 20 Hz and only taking into account the energy of each pulse without considering the effects of pulse duration on the peak power, a lot of clinical problems my happen. Several investigators have reported that the output energy of lower than 200 mJ can be safe for such a procedure [2], but according to the pulse curve, the peak power with shorter pulse duration is very higher than a long pulse. While any pulse is about 100 microsecond time, the peak power with an average output energy will be about 200 mJ, which is about 2000 W. Therefore the average power of 2 watt(W) will be measured but the maximum power will be dependent directly on the pulse duration. In order to decrease the side effects of this high peak power, technology has come to help by providing longer pulses. It means that with 300 microsecond pulse we will be able to have more irradiation time and at the same time 3 times lower peak power and also fewer side effects. Although both of these pulse lengths are safe but performing a clinical trial for establishment of this treatment modification is necessary.

Pulse duration which in this study was 100 us, has a direct effect on "duty cycle" [14]. This term is discussed as the real dose of laser light that reaches to the exposed volume when you imagine the tissue three dimensionally. Therefore the report of energy density when the penetration depth is high could be physically described as joule per cubic centimetre (J/cm³). The accurate estimating of light penetration volume in different tissues in periodontal ligament elements concluding bone, connective tissue, tooth dentin and all the liquids and proteins need very accurate three dimensional calculations. With the

distal side of fiber was measured 100 mJ, but the output peak power of any pulse with the 100 microseconds (us) pulses, is about 1000 Watt (W) (For calculating the pulse peak power, the easiest way that is not accurate but could be acceptable is dividing the pulse energy to pulse duration. So, if we divide 100 mJ to 100 us, the peak power of pulse will be 1000 Watt [12]).

All experimental pockets were exposed to Nd:YAG laser for 120 seconds with the modified method of Gutknecht [11]. The 300 micrometer in diameter fibre was introduced into the pocket parallel to long axis of the tooth till it reached the bottom of the pocket (Fig-1).

The laser light was irradiated while moving the fibre from bottom of the sulcus to cervical part by circular movements screening whole the area.

Clinical indexes including probing depth (PD), Periodontal Index (PI), Gingival Index (GI), Bleeding Index (BI), clinical attachment level (CAL) and recession (Rec.) were measured before and after treatments. Also, one day, one month and two month after treatments, all the measurements were repeated.

The day after treatment microbiology samples were collected from pockets by inserting the sterilised paper cones inside the pockets (Fig-2). These samples in less than 3 hours were transferred to laboratory and cultured in pre-prepared anaerobic plates. Two kind of non-specific and specific anaerobic clutter environments were used. In the first type all anaerobic specimens could be grown. But in second group because of additional antibiotics only *Prevotella* and *Bacteriodes* were able to grow. The method of culturing was streak plate method (Fig-3). In this study gas pack system was used, jars where incubated for 4 days in 37° Celsius. Then, the colonies regarding their need to oxygen were divided into five groups as following:

- 1- Aerobic bacteria.
- 2- Microaerophilic parasites.
- 3- Mandatory anaerobic bacteria.
- 4- Oxygen bearing bacteria.
- 5- Facultative anaerobic bacteria.

Results

Clinical indexes

The Mann-Whitney test was applied in order to analyse the clinical variables statistically. There were logically significant improvement in indexes before and after treatment in both groups, but the laser treated groups after one month and two month demonstrated better attachment. (P= 0.031 and P= 0.047).

Regarding bleeding index, the least bleeding in laser treated sulcuses was reported in one month

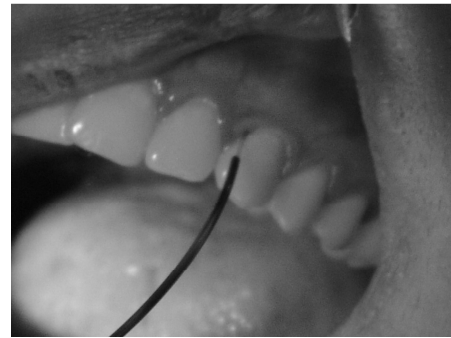


Fig-1- The fiber is inserted into the socket and is screening all



Fig-2- Collection of flora of the sulcus by the sterilised paper cone

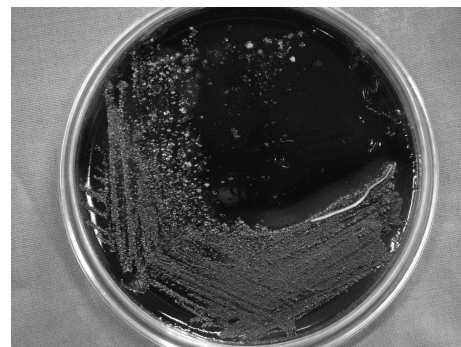


Fig-3- The paper cone is cultured and bacterial colonisation is took place.

and two month after treatment measurements (P= 0.006). The same happened in PD [Probing Depth] (P= 0.001) (Table-2).

Also GI was significantly less in laser group from the time the data were collected immediately after treatment and two months later (P= 0.03).

Microbiological evaluations

The Wilcoxon test was used to analyse the significance of decrease in reported amount of *Porphyromonas gingivalis* (PG) and *Prevotella intermedia* (PI) before and after treatment.

After one month significant reduction of PG was statistically observed (P < 0.05). It meant that the

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additional benefit was found when laser treatment was used secondary to traditional SRP therapy [7]. Several researches have focused on periodontal ligament elements like human periodontal fibroblasts (hPF) after Nd-YAG laser irradiation with different energy densities and exposure times [8]. In the most recent report, Chen et al stated that in a long term investigation while energy density is less than 12.5 mJ/cm², there is no significant decrease in total collagen content [8].

In another current investigation, conducted by Grassi et al. in Italy, carried out on 5 patients suffering from serious periodontal diseases, treatment results were more accurately evaluated with a sub-gingival check-up every week [9]. The selected patients presented either a relapse after the raising of a traditional flap, or showed acute phenomena and were therefore, treated with a laser with the propedeutic intention of surgery; or in cases that patients refused traditional surgical therapy or presented systematic pathologies that could not be included in the appropriate protocol. Pockets were chosen which had been probed between 5-8 mm, an optic fibre probe was pushed as far as possible into the probed pockets, for about 1 minute per site, 5 times with 30 second intervals. The samples, were taken in order to calculate bacteria colonies three times, first of all before using the laser and then immediately after and also 1 week later. In this study all the sites showed a slight reduction in the quantity of colonies forming immediately after the Nd:YAG laser application, while a week later, if clinical inflammation appeared to be resolved, the bacteria forming colonies seemed to be reduced. Authors concluded that the use of the Nd: YAG laser represents, in mild and average periodontitis, a therapeutic aid to scaling and root planing and additionally in the same way, in serious periodontitis for traditional surgical therapy [10].

Also according to Wang and his colleagues in China, laser appeared to have a stronger bactericidal effect in vivo especially on the dark-pigmented G-rods associated with periodontal disease [10]. Such conclusions were earlier suggested by Miyazaki research group in Japan, as Nd: YAG laser and ultrasonic scaling treatments showed significant improvements regarding the clinical parameters and subgingival micro flora compared to the baseline [11]. These are the reasons that in this study we have conducted the investigation according to the same study plan but with more accurate characteristics for the laser applied. The purpose of this study is to look at the effects of 100 microseconds (us) Nd: YAG laser on the clinical indexes and two of most important periodontal pathogenic germs

Fifty two periodontal pockets of 6 patients with chronic periodontitis were selected for this study. The selection criteria were as the following:

- 1- No systemic disease.
- 2- No report of taking interfering medicines like antibiotic in the last three months before the visit.
- 3- Good oral health and favourable co-operation.
- 4- No report of periodontal treatments in the last three months before the visit.
- 5- Not smokeing.
- 6- Moderate periodontitis diagnosed and at least two pockets with more than 5 mm depth present symmetrically.
- 7- Bleeding on probing (BP) obvious.

To create comparable conditions oral health education was performed for patients before starting the study. Modified bass method was recommended for all patients 5 minutes every night and dental flossing procedure was also demonstrated practically.

The depth of all the selected sulcuses were measured and recorded, they were about 4-6 mm before the treatments. The Michigan O probe was used for measurement. The sites were randomly divided into two experimental and control groups, each consisting of 26 pockets (Table-1). The pockets were coded randomly and first selected as laser treatment group and second for non-laser group and so on. For example in first patient, the pocket code 1,3,5 and 7 were lased and pockets 2,4,6 and 8 used as non-lased. The advantage of this method of randomizing was that the patients could not understand which pocket is lased and which is not lased.

All the pockets in both groups were treated with conventional methods of scaling, curettage and root planning while the experimental group received laser assisted periodontal treatment, the Nd: YAG laser (Fidelis, FOTONA, Slovenia) on 2 Watt and 20 Hz. Short pulses as short as 100 micrometers were investigated in this study. The output energy on the distal side of fiber was measured 100 mJ, but the output peak power of any pulse with the 100

Table-1- The selections of patients were randomized by number of the quadrants.

atient ID	Lased pockets	Non-lased pockets
1	4	4
2	4	4
3	5	5
4	4	4
5	5	5
6	4	4
Total	26	26

Materials and Methods

The clinical and microbiological evaluation of 100 micro-second pulsed Nd: YAG laser irradiation effects on Periodontal pocket healing after scaling and root planing.

Abstract:

Appropriate application of the Nd: YAG laser has been shown to reduce bacteria associated with periodontal pockets and improve the health of the diseased periodontal pocket tissue [1&2]. The objective of this *in-vivo* study is to investigate the effects of the 100 micro-second pulsed width (length) Nd: YAG laser on bacteria and pocket depth associated with periodontal disease. An Nd: YAG laser (Fidelias, FOTONA, Slovenia: 2 Watts and 10 Hz, 320 Micrometer beam diameter with a pulse duration of 100 micro-seconds) was used to irradiate the gingival pockets after the removal of calculus and appropriate root planing with hand instruments. Fifty-two teeth from six patients diagnosed with chronic periodontitis were used in this study. The samples were divided into two groups (control and experimental). All teeth were treated by normal hand instrumentation but in the experimental group the Nd: YAG laser was used to irradiate the pocket following hand instrumentation. Bacteria cultures were obtained from the gingival pockets prior to treatment, immediately after treatment, one and two months after treatment. The laser group of teeth demonstrated a significant reduction in bacteria, Probing Index (PI) and Bleeding Index (BI) compared to the control group of teeth.

Key words: Nd: YAG laser, periodontal treatment, pocket sterilisation, 100 micro-second pulse duration.

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Introduction

Appropriate application of lasers (Argon, Diode and Nd: YAG) has been shown to reduce bacteria associated with periodontal pockets and improve the health of the diseased periodontal pocket tissue [1-5]. The bactericidal effect of these wave lengths is dependent on several different factors which one of them maybe is the pulse duration while we are using the pulsed lasers like Nd:YAG (1064 nanometre). In-vivo reports on the effects of pulse duration are rare.

Gutknecht et al, made the 16S-rRNA and genomic DNA probes of germs cells and demonstrated lower amounts of *Prevotella intermedia*, *Porphyromonas gingivalis* and *Actinobasillus actinomysetem-comitans* in the group that Nd:YAG laser was used as assistance comparing to non lased control group[1]. Noguchi et al, have shown the clinical effects of Nd: YAG with 2 W and 10 Hz (200 mJ energy per pulse) with and without antibiotic therapy which was

statistically significant in reducing pathogenic micro-organisms. Probing depth and mean clinical attachment level were also significantly decreased by using laser after 3 months in a study which the authors have not mentioned which pulse length was applied [6].

Liu et al carried out a study with 8 patients in order to compare the effects of Nd: YAG laser treatment versus scaling/root planing (SRP) treatment on crevicular IL-1beta levels in 52 sampled sites [7]. They suggested that laser therapy appeared to be less effective than traditional SRP treatment. Of the 4 treatment modalities, inclusion of SRP was found to have a superior IL- 1beta response, when compared to other therapies without SRP. Furthermore, no additional benefit was found when laser treatment was used secondary to traditional SRP therapy [7]. Several researches have focused on periodontal ligament elements like human periodontal fibroblasts (hPF) after Nd-YAG laser irradiation with different energy densities and exposure times [8]. In the most