

# Study on the Effect of Low Density Power Laser Radiation in Treating Gingival Inflammation — Clinical, Microbiological, Histological Study —

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## I. INTRODUCTION

One of the most significant discoveries in the present century in the field of medical science is laser. It involves new, almost inconceivable perspectives in the fields of biological research and application in medical practice. There is, at present, a wide spectrum of possible applications.

High energy photon radiation of lasers can destroy normal and neoplastic tissues. The conversion of the absorbed energy into heat in the irradiated tissue is the most apparent mode of action for this destruction. Lasers proved useful in cutting biologic materials and in producing the coagulation necrosis in target tissues with a consequent reaction in surrounding tissues.

Low power densities of laser radiation have been used to study the wavelength-dependent physiologic effects of light. In this application, lasers serve as the most precisely defined and stable sources of monochromatic light. There were many investigations on the effects of low-power density of laser radiation without destroying the carriers of the biologic function. Enzyme activation and/or induction with light have been demonstrated,<sup>9, 23)</sup> as have enhancement of metabolic

processes in vitro following ruby (694.3nm) and helium-neon(632.8nm) laser radiation. Coherent red (ruby and helium-neon laser) and near infrared (neodymium laser, 10.60nm) light caused an enhanced proliferation of fibroblasts in vitro<sup>22, 50, 52, 53, 67)</sup> and in vivo<sup>63, 77)</sup>. A series of experiments has been arranged to examine the influence of laser radiation on the wound healing process.<sup>5, 6, 24, 45, 63, 73, 77)</sup>

The purpose of this investigation was to examine the effects of laser therapy within the same mouth in patients with clinical gingivitis, using BIOTHERAPY DENTAL LASER 3DL.

## II. METHODS AND MATERIALS

### A. Selection of subjects

Thirteen subjects (Males), aged between 20 and 24 years, were included in this study. On the basis of chronic gingivitis and/or clinically healthy gingiva and no overt evidence of alveolar bone loss, they were selected from a pool of students who are attending School of Dentistry, Chosun University. For participation in this study, the subjects were required to meet the following criteria:

- 1) no oral prophylaxis or periodontal therapy in the past 12 months,
  - 2) no antibiotic and antiinflammatory therapy in the past 3 months, and
  - 3) no history of systemic disease.
- The premolars and their gingiva were examined in each subject and selected according to the following: 1) presence of adequate zone of attached gingiva, 2) no cervical or interproximal carious lesions, 3) no interproximal and cervical restorations.

### B. Methods

THE BIOTHERAPY DENTAL LASER 3DL (OMEGA UNIVERSIAL TECHNOLOGIES LTD, England) was used as the low out put laser unit, which uses a GaAlAs laser as its source.

It is a near infrared laser apparatus with a wave-length of 820nm and an out put of 13mW (fig. 1.). By varying pulses, BIOTHERAPY 3DL has 12 out puts with different frequencies, which were reported that each of these different pulse lasers had its own unique effect. The frequency mode used was F12 (5000Hz). This experiment was a split-mouth design. In each subject two premolars in different upper quadrants were selected and randomly assigned as test and control sites. In using the BIOTHERAPY 3DL, the nozzle of the laser gun was placed as closed to the gingival sulcus as possible. The test regions were radiated for 4 minutes in each lingual or buccal side for a total of 8 minutes at each visit. Laser radiation were made on the first day, 3rd day, 5th day and 7th day. The control regions were manipulated in the same way except the Laserbeam off. Examinations were made on the first visit and the 4th visit (7th day). But Histologic assessment were made on only the 4th visit.

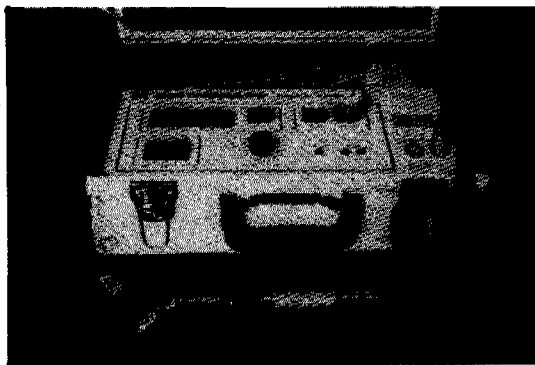


Fig. 1. Biotherapy Dental Laser 3DL (Omega Universal Technologies LTD., England) Radiation probe is seen in the front.

All examinations performed are as follow;

1. Clinical Assessment

- a) plaque index (PI, Silness and Loe) – Table 1.
- b) pocket depth (PD)

Clinical pocket depth was measured and recorded to 0.5mm, using a probe.\*

- c) Sulcus bleeding index (SBI, Mühlemann and Son) – Table 2.

Bleeding upon probing was assessed within 30

seconds of conventional probing during assesment of PD.

Table 1. Plaque Index (Silness and Loe)

- 
- 0; no plaque in the gingival area.
  - 1; a film adhering to the free gingival margin and adjacent area of the tooth, recognizable by running a probe across the tooth surface.
  - 2; moderate accumulation of soft deposits within the gingival pocket on the gingival margin and/or adjacent tooth surface which can be seen by the naked eye.
  - 3; abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.
- 

Table 2. Sulcus Bleeding Index (Mühlemann and Son)

- 
- 0; healthy appearance of gingiva, not bleeding on sulcus probing
  - 1; apparently healthy gingiva showing no change in color and no swelling, but bleeding on sulcus probing
  - 2; bleeding on probing and change in color and no swelling
  - 3; bleeding on probing and change in color and slight edematous swelling
  - 4; bleeding on probing and obvious swelling
  - 5; bleeding on probing and spontaneous bleeding, change in color, and marked swelling with or without ulceration
- 

2. Supragingival and subgingival Bacterial Evaluation. Microbial samples were obtained from the examined area by using a sterile gracey 11/12 curette\*\*.

The examined areas were at the distobuccal area of each upper 2nd premolar before radiations and at the mesiolingual area after radiations.

After the curette was removed with the sample, it was suspended in 0.15ml of a sterile physiologic saline solution containing 1% gelatin. In order to minimize clumping and the loss of bacterial motility, samples were prepared and the examination

were completed within half an hour of collection. The bacterial suspension was dispersed just prior to the examination by aspiration through a disposable insulin syringe equipped with a 25 gauge needle.

One drop of suspension was applied to a microscopic slide and coverslipped.

Excess fluid was removed by applying the finger pressure to the inverted slide against the absorbent surface. The slide was examined by dark field microscopy\*\*\* at the magnification of 1000 (fig. 2).

\* 3N HU-FRIEDY

\*\* American dental Mfg. Co.

\*\*\* American Optic Co.



Fig. 2. Photograph illustrating microorganism by dark field microscopy. (C=Coccoid cell, F= Filament, R=Rod and S=Spirochete)

From 5 random fields, 50 - 100 bacteria were counted and classified into 3 categories modified from other studies<sup>32)</sup>

- 1) non motiles,
- 2) spirochetes and
- 3) motiles.

Bacterial population was expressed as percentage distribution.

### 3. Histologic Assessment

Under local anesthesia (infiltration with 2% lidocaine containing 1: 50,000 epinephrine), gingival biopsy was performed from the distobuccal aspect of 1st premolar and mesiobuccal aspect of 2nd premolar, by making vertical incision at the center of interdental papillae and along the surface of tooth, and then horizontal incision 0.5mm apically from the clinical pocket base.

The biopsied specimens were immediately fixed in

10% neutral buffered formalin and embedded in paraffin, sectioned 5µm in thickness dento-orally and stained with Harris' hematoxylin and eosin.

From each specimen, 3 representative serial sections, 150µm apart, were selected for evaluation of density and extent of inflammatory infiltration. The connective tissue area was divided into dental and oral aspects (fig. 3), and evaluated separately according to the histologic criteria modified from Tagge's index<sup>69)</sup> (Table 3).

Median value was recorded from 3 sections for each specimen.

### 4. Statistical Anglysis

All the data were analyzed in order to determine whether laser radiation has significant relationship with plaque index, pocket depth, sulcus bleeding index, percentage of bacterial morphotype, and histologic status, using paired t-test.

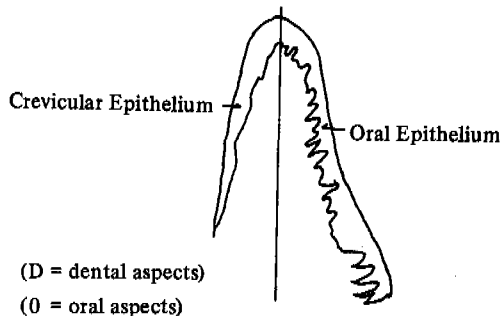


Fig. 3. Diagram illustrating the division of the histologic section into 2 zones for scoring inflammation

## III. RESULTS

### A. Clinical Assessment

The data for the mean clinical measurements using all tooth surfaces & gingiva are given in table 4. Except SBI scores ( $p < 0.01$ ), the paired t-test indicated no significant differences between controls and experimentals for PI and PD.

### B. Bacterial Evaluation

The mean percentages of the 3 microbial categories monitored by dark field microscopy are listed in Table 5 and 6.

**Table 3.** Scoring system for the density and extent of inflammatory infiltration (modified from Tagge's histologic index)

Dental Aspect	Score	Oral Aspect
none or scarce infiltration around sulcus buttom area	0	none or scarce perivascular infiltration
dense focal infiltration adjacent oto sulcular epithelium	1	dense focal infiltration around blood vessel
dense infiltration forming a continuous band along gingival sulcus	2	dense infiltration confined to the dental half of oral area
severe infiltration extending to the junction of dental and oral aspect	3	severe infiltration extending toward oral epithelium

**Table 4.** Mean Values ( $\pm$  SD) of clinical parameters.

	Before Radiation		After Radiation	
	C	E	C	E
plaque index	4.30 $\pm$ 1.75	4.69 $\pm$ 1.43	4.07 $\pm$ 1.65	4.00 $\pm$ 1.58
		n.s.		n.s.
pocket depth	2.32 $\pm$ 0.28	2.21 $\pm$ 0.27	2.02 $\pm$ 0.25	1.98 $\pm$ 0.14
		n.s.		n.s.
sulcas bleeding index	0.41 $\pm$ 0.21	0.38 $\pm$ 0.18	0.27 $\pm$ 0.17	0.13 $\pm$ 0.27
		n.s.		p < 0.01

n.s = nonsignificant

C = control group

E = experimental group

**Table 5.** Mean ( $\pm$  SD) Percentage of Each Microbial Morphotype In Supragingival Plaque

Group	Before Radiation		After Radiation	
	C	E	C	E
Motiles	52.20 $\pm$ 7.26	54.80 $\pm$ 8.28	58.00 $\pm$ 12.03	56.50 $\pm$ 8.84
		n.s.		p < 0.05
Nonmotiles	45.90 $\pm$ 8.04	43.30 $\pm$ 7.35	43.70 $\pm$ 8.98	45.40 $\pm$ 8.86
		n.s.		p < 0.05
Spirochetes	4.20 $\pm$ 15.87	3.00 $\pm$ 17.68	1.10 $\pm$ 6.45	1.10 $\pm$ 7.77
		n.s.		p < 0.01

A paired t-test of these data indicated that there were no significant differences in the mean proportions of any bacterial group between the controls and the experimentals before radiations. On the other hand,

there were significant differences in the mean bacterial proportions between the controls and experimentals after radiations.

**Table 6.** Mean ( $\pm$  SD) Percentage of Each Microbial Morphotype in Subgingival Plaque

Group	Before Radiation		After Radiation	
	C	E	C	E
Motiles	56.70 $\pm$ 14.28	54.40 $\pm$ 12.20	61.60 $\pm$ 6.70	52.30 $\pm$ 8.14
		n.s		p < 0.01
Nonmotiles	46.20 $\pm$ 13.14	45.30 $\pm$ 11.00	37.30 $\pm$ 6.29	46.50 $\pm$ 7.67
		n.s		p < 0.01
Spirochetes	10.43 $\pm$ 29.33	11.43 $\pm$ 24.19	14.57 $\pm$ 30.45	7.76 $\pm$ 23.20
		n.s		p < 0.01

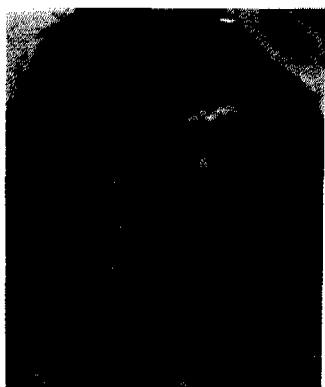
n.s = nonsignificant

C = control group

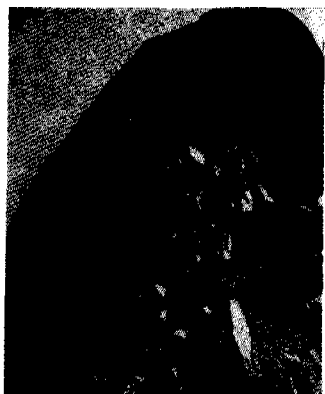
E = experimental group

### C. Histologic Assessment

Thirty two areas were examined under the light microscope and evaluated (figs 4, 5).



**Fig. 4.** Micro-photograph (X100) of a gingival specimen in control site illustrating score 3 on both dental and oral aspects.



**Fig. 5.** Micro-photograph (X100) of a gingival specimen in experimental site illustrating score 2 on both dental and oral aspects.

Dental and oral histologic inflammation index score were presented in Table 7.

Both, on the dental and oral aspects, the inflammatory cell infiltration decreased in the extent and density with radiation, and this change was statistically significant ( $p < 0.01$ ).

**Table 7.** Mean Value ( $\pm$  SD) of Histologic Inflammatory Cell Infiltration.

	After Radiation		
	C	E	
Oral	1.46 $\pm$ 0.97	0.68 $\pm$ 0.64	p < 0.01
Dental	1.78 $\pm$ 1.11	1.04 $\pm$ 0.93	p < 0.01
Total	2.90 $\pm$ 2.92	1.54 $\pm$ 1.57	p < 0.01

C = Control group

E = Experimental group

## IV. DISCUSSION

The discovery of the laser has opened up new prospects for both biological research and medical use.

In 1960 Maiman produced the first solid instrument from a synthetic ruby crystal. Since then, numerous other solid, gas, semiconductor and other lasers have been developed<sup>39)</sup>. McGuff was the first in 1964 to apply the laser in surgical practice.<sup>42)</sup> Early studies mainly concentrated on the deleterious effects obtained from irradiation.<sup>16, 17, 18, 41)</sup> Endre Mester discovered the biostimulative effect of laser light when he noticed growth of hair in the irradiated areas of depilated mice.<sup>44)</sup>

As a scoring system of clinical gingival inflammation,

sulcus bleeding index has been used for the diagnosis of early gingivitis and the evaluation of effectiveness in periodontal therapy. However, for clinical changes to be objective and reproducible, microscopic bacterial evaluation and histologic study have been suggested.<sup>43)</sup> Clinically uncomplicated gingivitis was seldom graded above 3, therefore, SBI = 0 to 3 were included. There were some discolored gingivae which did not bleed on probing. These were scored as non inflamed (SBI = 0).<sup>10)</sup>

This study showed that in clinical assessment, SBI had significant difference but others had no significance between two groups (table 4). Such findings were not consistent with previous longitudinal studies.<sup>34, 36, 68, 71)</sup> These results may be due to: (1) Most of samples were healthy that they might not be able to show clear differences between two groups. (2) the size of sample was not large enough to show the significant difference.

Numerous investigations have documented the fact that bacterial plaque is the etiologic agent in most forms of periodontal disease.<sup>8, 15, 21, 35, 56, 58, 72)</sup>

The initial development of gingivitis is thought to be a major consequence of bacteria associated with an increase in supragingival plaque formation.<sup>4, 38)</sup>

The subgingival flora at healthy and diseased sites in subjects with chronic periodontitis was studied by differential dark field microscopy.<sup>31)</sup> The findings demonstrated clear-cut difference in the microbial composition of healthy and periodontally diseased areas in the same individuals. The proportion of motile rods and spirochetes was significantly higher at diseased sites as compared to healthy sites.<sup>28, 29, 32, 37)</sup> The major findings from the studies by some researchers<sup>30, 54, 65)</sup> are the demonstration of clear-cut differences in the subgingival microbial flora before and after treatment and the fact that the microbial proportions are shifted from those generally associated with untreated periodontal defects toward those seen at periodontally healthy sites.<sup>31)</sup>

In supra and subgingival bacterial evaluation using dark field microscopy, 3 morphological categories were assessed in percentage distribution. Non motiles included all forms that did not have motility and total motiles included motile rods, spirochetes and other motile cells. This morphologic classification was performed to evaluate the types of cells easily. On the basis

of data from previous studies,<sup>30, 54, 65)</sup> it was expected to find a decrease in the proportion of spirochetes and motiles with radiation, a corresponding increase in the proportion of non motiles, as well as some evidence of clinical improvement temporally related to the microbial shifts.

Non motiles included all forms not showing motility and exhibited the increase with radiating motiles and spirochetes exhibited the decrease with radiating. They had significant differences between two groups. It seems, therefore, that the beam of the low out put laser caused the change in the bacterial composition of plaque and had a therapeutic effect on gingivitis. At control sites, the proportions of motiles and non motiles were much higher than other studies.<sup>3, 10, 27, 31)</sup> It is likely that higher proportion as compared to other studies was due to individual variation and the subjectivity on the evaluation of motility, which was distinguished from Brownian movement or motion impacted by fluid stream.

In addition, it may be the result from that all forms of cells not showing motility were included in non-motiles. Since the periodontal status of individual tooth surface may vary within the same mouth, and since the subgingival microbial flora in different states of periodontal health and disease differs both culturally<sup>64, 70)</sup> and microscopically,<sup>31)</sup> the microbial sampling technique used in this study may be open to criticism. Diagnostic precision achievable with the previous clinical changes is variable.

The assessment of visual changes relies on subjective interpretation of color and size.<sup>1, 11)</sup> Objective clinical criteria are needed which reliably reflect histopathologic changes associated with gingivitis and periodontitis. Previous histologic studies have characterized the pathologic changes in periodontal disease as an increase of inflammatory cells associated with a loss of collagen,<sup>57 61, 62)</sup> proliferative and degenerative epithelial changes.<sup>33, 59, 76)</sup>

Qualitative<sup>12, 20, 25, 33, 59, 76)</sup> and quantitative<sup>2, 26)</sup> changes in vasculature have been reported associated with gingival inflammation. Bleeding associated with inflammation may be due to structural alterations of the vessel walls and environs.<sup>59, 62, 78)</sup> It is possible that these structural alterations, which result in increased capillary fragility and permeability, may predispose to bleeding upon probing. In histologic

assessment, the area and density score was revealed to be highly correlated with the histometric evaluation.

In addition, the area and density score could be obtained more quickly and easily and required less equipment than histometric assessment.<sup>69)</sup> Therefore, in this study, histologic score was used which was modified from Tagge's histologic index to separate the specimen into dental and oral aspects, in order to make this index more sensitive and determine which aspect is more representative of clinical inflammatory sign. This study was, therefore, designed to histologically characterize the gingival lesion associated with the radiation of laser beam. This histologic assessment is coincident with the fact that studies attempting to define associations between clinical and histological characteristics of periodontal disease have reported both weak<sup>74, 76)</sup> and strong<sup>19, 57, 61)</sup> correlations. Control sites had a significantly larger score of inflamed connective tissue compared with radiated sites and the finding that inflammatory lesion was located primarily subjacent to the sulcular and junctional epithelium is in agreement with previous studies.<sup>59,62,75,76)</sup> The limitation in tissue obtainment made it difficult to evaluate and accurate change in sulcular epithelium and subjacent vasculature.

Although the actual mechanism of laser action on a biological system is still unclear, a majority of early researchers believe that it is related to the laser thermal energy.<sup>40)</sup> This may be true for the high dose effects. However, a large number of other results can not be explained on this basis alone.

Many studies, using low dose laser, have been performed investigate a different mechanism of laser action.

Such treatment actually increases the amount of intercellular material between the pigment epithelium and the visual cells. Although the exact origin of this intercellular material is dispute,<sup>13, 14)</sup> the low dose laser was expected to increase the production of intercellular material through increase in RNA synthesis in the epithelium. In addition, increased phagocytosis of leukocytosis and blood circulation by laser radiation were reported.

It was assumed that the laser influences the enzymatic process of wound healing during the early stage of effect. The effect of the ruby laser on the macromolecular (RNA, DNA, albumin) synthesis of human fibro-

cyte culture was studied. It was assumed that laser radiation increases the number of cells in the process of division.<sup>49)</sup>

The low power density laser of helium-and-neon was found to have a significant anti-exudative effect on inflammatory edemas of different genesis and at the same time it did not inhibit the reparatory process.<sup>55)</sup> The emission of the helium-and-neon laser was found to have a beneficial effect on the functional state of the microcirculatory bed, as shown by the increased rate of blood flow, increased diameter of the microvessels and larger number of functioning capillaries, as well as by a higher arteriolo-venular coefficient.<sup>7, 51, 60)</sup> On the basis of the above considerations, it is evident from these results that significant differences can be consistently demonstrated between the control and the experimental sites. According to this study, it is suggested that the low density power laser has not only a therapeutic effect on gingival inflammation but also an influence on the bacterial composition, although the direct effect of laser beam on bacteria could not be confirmed. But is its believed that laser therapy should be preceded by a removal of local factors to obtain a complete therapeutic effect on gingival inflammation.

For several years now, laser has shown its worth in the field of medicine, where laswe beams which have a thermal effect on the tissues are employed in surgery. Along side this type of laser, a "cold" laser, producing no heat and no trauma, has been investigated and deployed in general medicine and in the field of dentistry. In this second type the actual laser effect is generally obtained in a semiconductor medium, which produces the characteristics of the cold laser. BIOTHERAPY DENTAL LASER 3DL, which uses "Ga Al As" as semi-conductor, the source of the laser beam, is just this kind of laser.

The fundamental criterion in the choice of GaAlAs lasers for BIOTHERAPY DENTAL LASER 3DL was the wave length if the beam emitted which combines recognized therapeutic effect with very low ray absorption by the living tissue. This has the great advantage of not generating any heat during treatment, which is most desirable since the object of treatment is not destroy the cell (un-like the CO<sub>2</sub> laser, the So-called "Surgical laser")

Recently the quality of laser dio'des had increased dramatically, and accompanying the advancement of

fiber electronics, it is now possible to have a miniature diode, with a wide selection of 12 frequencies from 2.5HZ to 5,000HZ while Stomalaser\*\*\*\* has 7 frequencies from 47.5HZ to 3040HZ. Relating this the research will be performed to seek out the most suitable frequency for different diseases of each organ in the body, based on the tissue, or the tissue-penetrating power of the laser. The BIOTHERAPY DENTAL LASER 3DL emits a beam of 820nm. This frequency is very difficult for water to absorb. In other wards it has tissue-penetrating power and is considered a frequency that will give better treatment results. In the future there should be research carried out on the effective frequency or pulse modulated frequency of the low out put laser in treating facial or oral diseases. From the results of the study, it could be thought that the low out put laser is a treatment application with a bright future. It is expected to expand the application of the low out put laser to the treatment of tumors, bone fractures, TMJ dysfunctions and other diseases.

## V. CONCLUSION

This study was undertaken to determine whether the beam of the low out put laser has therapeutic effect on chronic gingivitis using the clinical examination of plaque index, pocket depth, sulcus bleeding index, supra and subgingival microbial morphotype and histologic inflammatory infiltration. The following conclusion was elicited.

- 1) SBI score decreased significantly with laser radiation, but there were no significant changes within PI and PD.
- 2) There was decrease in proportion of motiles and spirochetes, and concomittant increase in non-motiles with laser radiation.
- 3) After laser radiation, histologic inflammatory infiltration decreased in density and extent both on dental and oral aspects.

The results of this study indicate that the beam of the low out put laser is effective for the treatment and prognosis of gingival inflammation.

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# 저출력 레이저 조사가 치은염증에 미치는 영향에 관한 연구

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## 국 문 초 록

레이저의 의학적 이용은 고출력 레이저의 외과적 이용과 더불어 많은 진전을 하게 되었다. 근래에는 인체에 손상을 주는 고출력 레이저 외에도 조직의 파괴없이 인체조직에 유용한 효과를 줄 수 있는 저출력 레이저에 대한 연구가 적극적으로 시도되고 있다. 본 연구는 치은염을 가진 13명의 대상에 대해 Split-mouth design으로 대조군 및 실험군을 설정하여 실험군의 치은에 저출력 레이저(Biotherapy dental Laser 3DL)를 격일로 4회 조사한 후 plaque index, pocket depth, sulcus bleeding index, supragingival과 subgingival plaque의 bacterial morphotype의 비율 및 광학현미경을 통한 조직학적 염증 침윤상을 측정 평가하여 다음과 같은 결과를 얻었다.

- 1) 레이저 조사후 SBI( $p < 0.01$ )는 감소하였으나 PI는 별다른 변화가 없었다.
- 2) 레이저 조사에 따라 spirochetes( $p < 0.01$ )를 포함하여 motiles( $p < 0.05$ ,  $p < 0.01$ )은 감소된 반면 nonmotiles( $p < 0.05$ ,  $p < 0.01$ ) 증가하였다.
- 3) 레이저 조사 치은에서는 염증세포 침윤이 Dental( $p < 0.05$ ) 및 oral( $p < 0.01$ ) 양측 모두 감소되었다.