# 치의과학박사 학위논문

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# 임플란트주위염 처치에 있어서 다이오드 레이저 치료 효과

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김위붕 (Wei-Peng Jin)

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지도교수 이 종 호

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김위붕( Wei-Peng, Jin)

Wei-Peng Jin의 박사학위논문을 인준함

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위 원 장 김명진 (인)

부 위 원 장 이종호 (인)

위 원 임영준 (인)

위 원 김성민 (인)

위 원 안강민 (인)

**Abstract**

# Effects of diode laser therapy for the management of peri-implantitis

**Wei-Peng Jin**

**(Directed by Professor: Jong-Ho Lee, DDS, MSD, PhD)**

Department of Oral and Maxillofacial Surgery,
Graduate School, Seoul National University

**Purpose of Study** The efficacy of diode laser treatment for peri-implantitis remains poorly defined. Surface change, heat increase, and bacterial control following irradiation of titanium implants by diode lasers need to be investigated. The purpose of this study was to evaluate the effectiveness and safety of diode laser on peri-implantitis through laboratory and biological experiments.

**Materials and Methods** A diode laser of 808nm wavelength which could output on both continuous and pulse modes was used. Titanium implant of sandblast large grit and acid-etching (SLA) surface was placed on cow bone, and the laser temperature was measured by connecting a sensor on the mesial, distal, buccal and lingual sides. Peri-implantitis was induced by placing SLA-treated titanium screw(SLA-TS) ligated with floss silk on the palate of a 7-weeks-old Sprague Dawley rat. The experimental groups were untreated group, SLA-treated titanium screw implant group, peri-implantitis induction group, diode laser treatment on induced peri-implantitis group. The groups were observed via scanning electron microscope (SEM) and the bacterial count was measured. The level of oral bacterial growth was examined through polymerase chain reaction (PCR). Three premolars were extracted from the mandible of an adult, male beagle, and pretreated implant was placed on the extraction area after a 3 months' recovery period. The experimental groups were group with unpretreated implant surface and bone defect site unfilled with Bio-Oss®, group with unpretreated implant surface but bone defect site filled with Bio-Oss®, group pretreated with laser then filled with Bio-Oss®, group pretreated with 0.5% chlorhexidine and filled with Bio-Oss®, group

pretreated with iBrush® and filled with Bio-Oss®. The groups were observed and measured by index measurement on implant stability, microtomography and histomorphometry with undecalcified ground sections.

**Results** When the temperature was measured on the cow bone, 808nm diode laser showed an increase by an average of 3.1℃. When PCR was observed after diode laser treatment on the peri-implantitis SD rat model, bacterial reduction by the diode laser treatment was confirmed on the 4th day. As a result of bacterial counting, the number of bacteria decreased effectively in the groups treated with 808nm diode lasers. In the beagle bone regeneration experiment, Untreated but filled Bio-Oss® group better than all the groups. In the experiment, not much re-osseointegration was observed in the laser irradiation group as the untreated group in the implant stability index, microtomography, and histomorphometrical analysis. However, the diode laser treatment showed similar results as chlorhexidine and iBrush® treatment. When the untreated group which was not filled Bio-Oss® was compared to the filled group, there was a significant difference in bone regeneration effect, but there was no statistical significance.

**Conclusion** When the 808nm diode laser was irradiated on the titanium screw, no physical change was observed on the SLA surface. The temperature of the surrounding bone tissue was increased 2.3-3.4℃ when it was irradiated. Bacterial elimination from the SLA-TS surface of SD rat was effective. As a result of comparison between each experimental groups after 808nm diode laser treatment, bone regeneration was observed same as other experimental groups. It was considered that the 808nm diode laser may be effective in peri-implantitis treatment.

Keywords: Peri-implantitis, Diode laser, 808nm wavelength, SD rat Peri-implantitis model, Bone regeneration

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# Contents

# I. Introduction

The principle of how laser works was first theoretically proven by Einstein in 1917 where the emission process could be induced in the interaction of light and matter.[1] This theory has been used in medical field from 1960 onwards, and the fact that laser has an influence on dentin was first announced in dentistry by Stern and Sognnaes in 1965.[2] Since then, laser has brought huge development in dental treatment. Lasers in use could be largely divided into surface-absorptive ($CO_2$ laser, Er:YAG laser, etc.) and tissue-penetrative (diode laser, Nd:YAG laser, He-Ne laser, Argon laser). Among those, diode laser, Nd:YAG laser, Er:YAG laser and water drop laser (Er,Cr:YSGG laser) were most universally used for periodontal treatments.[3]

The increased use of implants in prosthodontic treatment has led to an increase in peri-implant diseases.[4] Appropriate and effective treatment modalities for peri-implantitis continue to be investigated. Current treatments can be divided into surgical and non-surgical treatments.[5,6] Non-surgical treatment modalities include antibiotics, curettage, ultrasonic scaling, and laser therapy.[7,8] However, effective and easy-to-use treatments for peri-implantitis are still lacking.[9] Peri-implantitis is a symptom similar to periodontosis; bacterial distribution on the mucous membranes with peri-implantitis was reported to be similar to the ones observed on tooth surface. In clinical dentistry, Nd:YAG laser has been used for disinfection within periodontal pockets and soft tissue incision for its low

absorption rate to water and good tissue penetration. However, Radvar[3] et al. stated that microbial count within the pocket was not observed to be improved when Nd: YAG laser was irradiated into the pocket. Another researcher reported that Nd:YAG laser removes subgingival bacteria uniformly but gives damage to the surrounding tissue when it was irradiated onto them. Er,Cr:YSGG laser, also known as water drop laser for its easy absorption in water, requires cooling water in treatment process and is used for soft tissue excision, bone tissue deletion and root canal treatments. However, as implant peri-implantitis is a chronic inflammation that accompanies bone resorption, Er,Cr:YSGG laser, which removes bone tissue, may give rise to secondary damage. A diode laser could easily convert electrical energy into light energy through medium such as arseniuretted helium (GaAs) and is also easily absorbed into haemoglobin, so it is relatively suitable for use in environment with blood (inside periodontal pockets). Diode laser is mostly used for periodontitis treatment and showed positive influence on clinical data and treatment results.[10,11] Nonetheless, use of diode laser in peri-implantitis lack experimental data on treatment effects and also lack study on heat increase, Sandblasted, Large-grit, Acid-etched (SLA)-treated implant surface change and bacterial population level when diode laser was irradiated onto titanium metal.

The purpose of this study is to evaluate the effectiveness and safety of a newly developed diode laser (wavelength 808 nm) *in vitro* and *in vivo* as it is considered to be most suitable for peri-implantitis treatment.

# II. Materials and Methods

## 1. Diode laser system

Diode laser system of 808nm wavelength capable of both continuous wave and pulse wave laser output was designed with the help of laser company(Bison, Seoul, Korea)(Fig. 1). The pulse width and pulse repetition ratio can be adjusted as desired when using the pulse wave laser, which changes the output power. The laser output consists of a hand-piece and an optic fiber with a 200-μm core.

The output power characteristics were compared with those of an existing 810-nm diode laser, the Picasso Lite (Dentsply international, Sarasota, FL, USA). The output power was measured from the tip of the hand-piece at a distance of approximately 5 mm using a laser power meter (Gentec-EO Inc, Quebec, Canada).

### Implant preparation and surface changes following implant irradiation

Commercially available implants (3.5 × 8.0 mm, Dentium Co., Ltd., Suwon, Korea) and 1.2 × 4 mm sandblast large grit and acid-etching (SLA)-treated titanium screws (SLA-TS) were prepared for implantation into white rats.

The SLA-TS surface was irradiated with the 808nm or the 810nm diode laser, and physical changes in the surface were observed using scanning electron microscopy (SEM).[12] Untreated SLA-TS surfaces and SLA-TS surfaces that had been irradiated with the diode laser for 15 second in the pulse mode at a power of 1.5, 2.0, or 2.5 W were examined by SEM ( × 100 and × 50,000 magnification).[13,14]

### *In vitro* measurements of heat generation

A 6.0mm deep, 3.5mm diameter hole was drilled in bovine bone. Then, a 3.8 × 8.0 mm SLA-TS was implanted so that the upper 2 mm was exposed outside the bone. After SLA-TS implantation, four holes with a diameter of 0.5 mm and depth of 1 mm were drilled medial, distal, superior, and inferior to the laser irradiation site. A temperature sensor (Omron, ZR-RX40, Kyoto, Japan) was implanted in each hole to measure the temperature. A graph was drawn in real time on a heat-transfer temperature recorder and the temperature range was set at 20–60° C (Fig. 2). A 2mm section of the dental neck was considered the location of peri-implantitis. The 810nm diode laser and the 808nm diode laser were used to irradiate the implant surface for 15 s each, using a surround method in continuous mode and pulse mode with a non-initiated tip at powers of 0.5, 1.0, 1.5, 2.0, and 2.5 watt. The heat occurred during irradiation was recorded by each sensor and compared.

## 2. Inducing peri-implantitis in Sprague-Dawley rats

The study was approved by the Animal Care and Use Committee of Seoul National University (SNU-150305-3). Male Sprague-Dawley rats (250g) (Orient Bio, Gapyeong, Korea) were reared in a specific-pathogen-free laboratory with a constant temperature (21 ± 1° C), humidity (55%), and 12 hour light cycle (light: 07:30–20:00; dark: 20:00–07:30). The rats were given ad libitum access to standard feed (Purina Rodent Chow, Purina Co., Ltd., Seoul, Republic of Korea) and distilled water and underwent a 1 week quarantine period. After the acclimation period, the rats were intraperitoneally injected with a mixture containing 3mL pentobarbital (Hanlim Pharm. Co., Ltd., Gyeonggi, Korea) and 100 mg/kg chloral hydrate (Sigma-Aldrich. Co., Ltd., ON, Canada) to rapidly induce deep anesthesia. The 1.2 × 4 mm SLA-TS were then implanted into the right and left maxillary palate.[15]

Floss silk or a 26G wire was ligated to the head of the left SLA-TS to form plaque induced peri-implantitis. Healthy saliva and gingival cervicular fluid (GCF) was collected from around the untreated right SLA-TS and treated left SLA-TS using a paper point for root canal treatment (Absorbent Paper Point, META BIOMED Co., Ltd., Cheongju, Korea). The paper point samples were tested by polymerase chain reaction for total bacterial value at 4, 7, 10, and 14 days (Fig. 3).[16]

**DNA extraction from SD rat**

The extraction and purification of bacteria and sample DNA were performed with DNA Extraction kit(QIAGEN, Hiden, Germany). The concentration of sheared DNA was determined at 260nm using the Nanodrop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE, USA). The reference strains to be showed by 4 Pannels (A Pannel: Aggregatibacter actinomycetemcomitans KCTC3698, Porphyromonas gingivalis KCTC5352, Tannerella forsythensis KCTC5666, B Pannel: Treponema denticola KCTC15104, Prevotella intermedia KCTC3692, Fusobacterium nucleatum KCTC2640, C Pannel: Parvimonas micra ATCC33270, Campylobacter rectus KCTC5636, Eikenella corrodens KCTC15198, D Pannel: Prevotella nigrescens KCTC 5407, Eubacterium nodatum KCTC 15015, Total bacteria 16s rDNA control )were used as controls for real-time PCR assay.

**Quantification using Multiplex Real-Time PCR**

The quantification of 11 bacteria related to periodontal diseases was determined on real-time PCR at the Periogen analysis facility (Microis Co., Ltd., Seoul, Korea). A four-tube multiplex real-time PCR assay was developed for the detection of 11 periodontal pathogens and total bacteria in oral cavity. Primers and probes for periodontal bacteria were based on three functional genes and

they were designed for specificity using RealTimeDesign Software (Life technology, Singapore).

Multiplex real-time PCR amplification was performed in a total reaction volume of 20 $\mu\ell$ mixture. The reaction mixtures contained pathogen-specific primers, pathogen-specific probes and extracted DNA from saliva samples. All the reactions were performed on ABI 7500 Fast real-Time PCR System (Life technology, Singapore ). The cycling conditions were one cycle at 95℃ for 10min, 45 cycles at 95℃ for 15s, 55℃ for 15s, 72℃ for 30 second. Appropriate negative and positive controls were included. The critical threshold cycle (Ct) was defined as the cycle in which fluorescence becomes detectable above the background fluorescence and was inversely proportional to the logarithm of the initial number of template molecules.

**Calculation of cell numbers**

Amplification profiles for each target were generated in duplicates with standard DNA dilutions ($10^5$-$10^1$). The computer-assisted second derivative maximum algorithm was used for crossing point inference for each dilution. A standard curve was generated for each marker by linear regression analysis and used as a basis for further quantification of target DNA from the clinical samples. Amplification efficiencies were inferred according to the following formula: $E = 10(^{-1}/s)$, where s is the slope of the standard curve .[17, 18,19]

**Efficacy of diode laser treatment for peri-implantitis in a rat model**

The implanted titanium screws were irradiated for 15 seconds at 0.5 W in the continuous mode by the diode laser, and then, GCF was collected from around the SLA-TS using a paper point for root canal treatment.[20] GCF samples were then analyzed by PCR (PerioGen Co., Ltd., Seoul, Korea).[21] Finally, the SLA-TS was removed from the rat and fixed in 4% paraformaldehyde solution for 24 hours. After fixation, bacteria on the screw head were examined and counted using SEM.

**3. Bone regeneration of peri-implant defect in beagles**

The study was approved by the Animal Care and Use Committee of Seoul National University (SNU-140707-5-1). Adult beagle dogs (15kg, n = 5) were used for the experiments.[22] After general anesthesia with an intravenous injection of Zoletil® ( tiletamine+ zolazepam 25 mg/kg)(Virbac korea Co., Ltd. Seoul, Korea), and Rompun® (xylazine 5 mg/kg)(Bayer KOREA Co., Ltd. Seoul, Korea) was induced to minimize stress and suffering, three mandibular premolars were extracted on each side. The animals were then supplied with soft feed twice per day for 1 week until restoration. For postoperative care, they were administered

anti-inflammatory analgesics and antibiotics twice per day for a total of 4 days, starting on the day of surgery, to minimize the stress caused by tooth extraction. The animals were closely observed for the first 48 h after surgery to monitor feeding problems or complications caused by tooth extraction and then allowed to heal for 8 weeks.

Twelve weeks after the extraction, anesthesia was induced and six 3.5 × 8 mm SLA surface implants were implanted bilaterally in the mandible under anesthesia (right = 3; left = 3). After implantation, alveolar bone was removed from the platform to a depth of 4 mm and breadth of 2 mm to mimic an alveolar defect caused by peri-implantitis. 808nm diode laser, 0.5% Chlorohexidine (CHX), and iBrush® (Neobiotech Inc., Seoul, Korea) were used as experimental materials (Fig. 4), and the bone defect site was filled with Bio-Oss®(Geistlich, Wolhusen, Switzerland) )which is a bone graft material. The experiment was performed with Group A ( Defect ) : implant surface not pretreated and bone defect site unfilled with graft material, Group B( Defect+Bios ):Defect filled with Bio-Oss®: implant surface not pretreated but bone defect site filled with Bio-Oss®, Group C( Laser treatmant): preatreated with laser then filled with graft material, Group D( Chemical treatmant ): pretreated with 0.5% CHX then filled with graft material, Group E( Mechanical treatment ): pretreated with

iBrush® then filled witgraft material. In the Group C, the implant surface was irradiated for 60 seconds (power 0.5 watt, continuous mode). In the Group D and E, the implant surface was washed for 3 minutes with 0.5% chlorhexidine or for 3 min with an iBrush® (low-speed angled hand-piece 1,500 RPM) before implantation. All implants were measured three times in the buccal-lingual and mesial-distal orientations using an Osstell Mentor (Integration Diagnostics AB, Diateborg, Sweden) prior to submerging and suturing.[23]One of these five groups was selected randomly for the final implant on the left side (Fig. 5, Table. 2).

**Animal sacrifice and ISQ measurement**

Twelve weeks after implantation, the animals were sacrificed by inducing cardiac arrest using potassium chloride (KCl, 0.5 mL/kg, total 7.5 ml per animal, Huons, Korea) after anesthesia with an intravenous injection of Zoletil® and Rompun® ISQ values of all implants were measured using the same method as that at implantation (Fig. 6).

**Micro-CT imaging and observation**

The part of the mandible containing the implants posterior to the canine teeth and anterior to the molars was excised manually from all five sacrificed animals using a saw, and fixed for 24 hours in 4% PFA. Skyscan1171 (Skyscan, Kontich, Belgium) was used to conduct a non-destructive examination of the interior structure of the bone implant. First, the tissue was placed in a micro-CT tube filled with PBS solution to cover the tissue sample and imaged after identification of the distal, center, and mesial sites. The tissue and bone volumes were measured from the images.

**Fabrication of non-decalcified tissue samples**

After micro-CT imaging, the tissue was fixed for 48 hours in a neutral 10% formalin solution. The gross tissue was soaked in water for 3−6 hours in a cassette or brown glass bottle. After the tissue was dehydrated in 80−100% ascending alcohol, it was placed in meta-acrylic resin and penetrated for sufficient time. The block was cut in the middle and ground to approximately 40 −50 μm to produce the sample.

**Histomorphometric analysis**

After H/E staining tissue sample was examined a light microscope (BX51, Olympus, Japan), Images were obtained at ×40 magnification using

Kappa Imagebase Metro Program (KAPPA, Opto-Electronics, Gremany), and the portion obtaining the implant and peri-implant structures was digitalized. The bony part in contact with the implant was measured to calculate the bone to implant contact ratio (BIC). The bone volume was measured by calculating the BIC of head from three screws with bone union.

**Statistical analysis**

Data were analyzed by one- or two-way (q-PCR, Bacterial count, ISQ, Micro-CT, BIC) analysis of variance (ANOVA), using StatView software 5.0.1 Ver (Abacus, Berkeley, CA, USA). The difference between means was considered significant when $p$ values were less than 0.05.

# III. Results

## 1. Diode laser output characteristics

Diode laser was used in the continuous mode. When the 808nm diode laser was set to its maximum output power of 7 watt, it produced a power of 6.34 watt, representing an efficiency of 90.6%. When set to an output power of 2.5 watt, the maximum output of the 810nm diode laser was 2.35 watt, representing an efficiency of 94%. When set to a power of 1.5 watt, it produced an actual output power of 1.4 watt, representing an output efficiency of >93%. This indicated a high coupling efficiency between the diode laser and the optic fiber and limited loss of laser light. When the 810nm diode laser was set to its maximum output power of 2.5 watt, it produced an actual output of 1.98 watt, corresponding to an efficiency of approximately 80%(Fig. 4A,B). Fig. 4A shows the output power of the newly developed 808nm diode laser, and Fig. 4B shows the output power of the existing 810nm diode laser.

### Implant surface changes

To examine the effects of diode laser irradiation on the surfaces of SLA-treated titanium screws, the surfaces were irradiated with the 808nm diode laser for 15 seconds at 1.5, 2.0, or 2.5 watt and the surfaces were analyzed by SEM.

No clear differences were observed between the control and experimental groups (Fig. 7).

### *In vitro* measurements of heat generation

Irradiation by the 810nm diode laser increased the temperature by a mean of 3.7℃ in 15 seconds (25.3-29℃). Irradiation by the 808nm diode laser increased the temperature by a mean of 3.1℃ (22.7-25.8℃). When the mesial part was irradiated for 15 seconds at 0.5 or 1.0 watt in the pulse mode, the 810nm diode laser showed a temperature increase of 2.3℃ (25.2-27.5℃), while the 808nm diode laser showed a temperature increase of 2.3℃ (22.8-24.5℃). Thus, although the temperature increase was similar for the two devices (+2.3℃), the 808nm diode laser maintained a lower temperature than the 810nm diode laser (Fig. 8).

## 2. Efficacy of diode laser treatment in a peri-implantitis a rat model

Infection was quantified by PCR detection of bacteria in SLA-TS implant groups with and without a diode laser treatment for peri-implantitis. The diode laser treatment reduced the amount of bacteria 4 days after implantation (Fig. 9). Fewer bacteria were detected in animals treated with the 808nm diode laser (Fig. 10B).

Observation of SLA-TS implants in the rat hard palate by SEM revealed the presence of bacteria (Fig. 11B, F). More bacteria were observed in SLA-TS implants from the peri-implantitis group (Fig. 11C, G). Irradiation of the implant surface with the 810nm and 808nm diode lasers reduced the bacterial levels after peri-implantitis induction (Fig. 11D, H). The number of bacteria were counted in each group, and treatment with the 810nm diode laser or the 808nm diode laser effectively reduced the number of bacteria (Fig. 11 I).

### 3. ISQ measurement

The mean ISQ values immediately after implantation versus 12 weeks after implantation were 73.5 ± 9.6 and 61.1 ± 6 in the Group A, 70.3 ± 4.5 and 69.1 ± 8.2 in the Group B, 72.8 ± 3.9 and 68.9 ± 9.1 in the Group C, 76 ± 2.4 and 71.1 ± 5.6 in the Group D, and 72.1 ± 3.5 and 68.2 ± 5.6 in the Group E, respectively. Immediately after implantation, the mean ISQ was highest in the Group D and lowest in the Group B. The ISQ decreased in all groups after 12 weeks, with the Group A showing a significantly lower value after 12 weeks than the other groups (Group B, $p < 0.0001$; Group C, $p < 0.0001$; Group D, $p < 0.0001$; Group E , $p < 0.0001$) (Fig. 12).

**Micro-CT**

Twelve weeks after implantation, bone volume was highest in the Group B (4.54 ± 1mm$^3$), followed by the Group A (4.22 ± 1.13 mm$^3$), Group C (4.13 ± 1.13 mm$^3$), Group D (3.97 ± 1.42 mm$^3$), and Group E (3.89 ± 0.31 mm$^3$). There were no significant differences between the groups (Fig. 13).

**Histomorphological analysis**

In the Group B, the BIC 12 weeks after implantation was 64% and the BIC in regions with good bone union was 92.5%. In the Group A, the BIC was 53.5%, while the BIC in regions with good bone union was 82.7%. In the Group C, the BIC was 59.1% and the BIC in regions with good bone union was 96.6%. In the Group D, the BIC was 60.1% and the BIC in regions with good bone union was 99.4%. For the Group E, BIC was 53.2%, while the BIC in regions with good bone union was 75.8%. There were no significant differences between the groups (Fig. 14).

## IV. Discussion

Diode lasers of 800-810 wavelengths have been actively used in periodontitis treatments, and there were cases of peri-implantitis treatments. [10,11] However, studies on how the 808nm laser affects the surrounding tissues of the implant during peri-implantitis treatment remain insufficient. There were especially lack of in vitro studies such as studies on heat generation laser irradiation for safety and the change in rough surface of the implant during laser irradiation.

No surface changes were found when the SLA-TS implants were irradiated with diode lasers in the continuous mode at wavelengths of 808nm and 810nm. Although an increase in temperature was observed, the change in temperature at a power of 0.5 watt was small. In the pulse mode, the temperature increased, but then declined. The effect of temperature measured on the cow bone showed the temperature increased only on the mesial side, and no temperature change was observed in distal, buccal and lingual sides of implant.

No implant loss was observed for 4–7 days after SLA-TS was implanted into the hard palate of rats. We irradiated the screw with a diode laser on these days and detected a significant reduction in the number of bacteria. Successfuldecontamination was inducedat the peri-implantitis induced by ligatinf floss silk around the SLA-TS. Both an 808nm and 810nm diode laser reduced the

number of bacteria in the peri-implantitis model, but the bacteria were more efficiently reduced by the 808nm diode laser.

Conner et al. [24] conducted an implant bone defect model in a beagle dog. In the mandible of an adult dog, the bone defect site was created at a surrounding radius of 2mm from a 5mm depth implant, and 10mm implants were placed on the defect site. ISQ was not measured when placing the implant, so it was considered that confirming the initial stability would be difficult.Because this study created the bone defect on only either the mesial or distal side, it tried to increase initial stability by making the cortical bone area completely in contact with the placed implant as it has high bone density. Also, the implant was firmly in contact with the alveolar bone to be similar to the characteristics of peri-implantitis bone defect, and artificial vertical resorption was made for a similar environment as peri-implantitis.

Schwarz et al.[22] found similarities between naturally occurring peri-implantitis lesions in 24 patients and induced peri-implantitis lesions in five beagles. Hanisch[25] and Schou et al.[26] reported no differences between peri-implantitis in the maxilla and mandible. In this study, mandibular peri-implantitis was treated with a diode laser, CHX, and iBrush in beagles. Schwarz et al.[27] reported better re-osseointegration following treatment with an Er:YAG laser than an ultrasonic scaler and plastic curettes. Deppe et al.[28] demonstrated that treatment with a $CO_2$ laser showed better outcomes than mechanical cleaning,

although no long-term differences were observed. Wetzel et al.[29] found that TPS treatment of SLA surfaces was better than a machined surface. Persson[30] and Sennerby et al.[31] reported that SLA treatment promoted better regeneration of the implant surface than a machined surface. Roos-Jansaker et al.[32] reported no effect of treatment with an absorptive membrane after bone transplantation, and a study by Khoury and Buchmann[23] supported these findings. In our study, however, diode laser treatment did not show superiority of re-osseointegration than chlorhexidine or mechanical therapy using iBrush®.

All 3 treatment methods showed difference in bone regeneration effect compared with Group A Among the treated groups, Group E was observed to show lower bone regeneration effect compared with other 2 groups. It was considered that the laser, CHX and iBrush® would both physically and chemically change the SLA-treated surface. The iBrush®, which is used on the implant surface in peri-implantitis, physically grinds the SLA-treated surface to remove the attached bacteria and changes the surface into a machined surface.

Until the iBrush® was developed, high or low speed dental hand-piece was used to grind the implant surface. However, a lot of grinding on the implant surface was required as the SLA-treated area was not ground well. It was thought to maintain cleanliness thus not reduce the implant strength as the

implant thread could be evenly ground. Although there's no reference found, the iBrush® is thought to be more effective compared to the conventional treatment method and to increase the treatment effect as it evenly grinds the implant into a machined surface in peri-implantitis treatment. However, it was observed to show lower bone regeneration effect compared to the implant surfaces treated with diode laser or CHX. This may be because the iBrush® grinds the SLA-treated surface and may cause more than diode lasers or CHX.

In this study, horizontal bone absorption was observed in the beagle bone tissue around the implant. Horizontal bone absorption was considered to be caused as it is not possible to secure 1mm or more of supporting bone tissue when 3.5mm implant was placed on the mandibular lingual side of a beagle which is around 5 mm. Better results without horizontal bone absorption is predicted if mini-implant of 2.5−3.0mm was placed on the beagle mandible.

Considering the above experiment results, the 808 nm diode laser was able to effectively regulate inflammatory tissues and bacteria in the peri-implantitis treatment without causing heat or surface changes in the implant. Re-osseointegration in terms of regeneratieve peri-implantitis was not superior to mechanical or chemical methods, but at least it was not feasible and its utility would be sufficient

# V. Conclusions

When the 808nm diode laser was irradiated on the titanium screw at 1.5w continuous mode for 15 seconds, no physical change was observed on the SLA surface. However, the temperature of the surrounding bone tissue was increased by 2.3-3.4℃ when it was irradiated at 0.5-1.0 watt pulse mode for 15 seconds. Bacterial elimination from the implant surface of SD rat was effective at 0.5watt continuous mode for 15 seconds. As a result of comparison between each experimental groups after 808nm diode laser treatment, bone regeneration was observed as the SLA surface was not destroyed. It was considered that the 808nm diode laser may be effective in peri-implantitis treatment.

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# VII. Tables

**Table. 1.** Demographic and bacterial q-PCR and counting data of the SD rat peri-implantitis model.

| | Group | | | |
|---|---|---|---|---|
| (n=6, mean±SD) | Normal | SLA-TS | Peri-implantitis | Laser-Tx |
| Bacterial qPCR | 6.50±2.14 | 46.38±41.55 | 233±12.97 | 16.88±5.73 |
| Bacterial counting | 0.3±0.16 | 3.02±0.32 | 6.5±0.47 | 1.83±0.65 |

**Table. 2.** Demographic and micro-CT and BIC data of the beagle dog bone defect regeneration model.

| | **Group** | | | | |
|---|---|---|---|---|---|
| (n=6, mean±SD) | A (Defect) | B (Defect+Bios) | C (Laser) | D (CHX) | E (iBrush) |
| micro-CT | 4.23±1.13 | 4.97±1.45 | 4.58±0.98 | 4.38±1.13 | 4.13±0.53 |
| BIC | 53.49±25.23 | 63.96±18.29 | 59.12±22.42 | 60.1±30.03 | 53.17±23.29 |

## VIII. Figures and figure legends

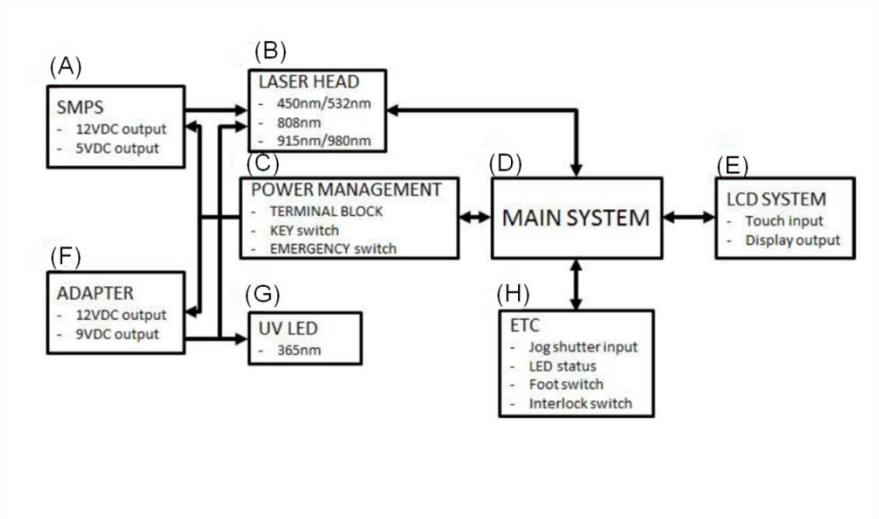


***Fig. 1.*** **The 808nm diode laser device** (A) Laser output by stable power supply on the laser head, (B) Selective laser irradiation of desired wavelength via laser head with multiple wavelengths. (C) Laser output by stable power supply on the laser head, (D) Main control device, (E) Control of Input-Output by RS232 system through USART1, (F) Stable power supply on the laser head and UV LED, (G) LED emission by power supply onto LED of 365nm wavelength, (H) Foot switch, interlock switch, LED input. Jog shutter input.

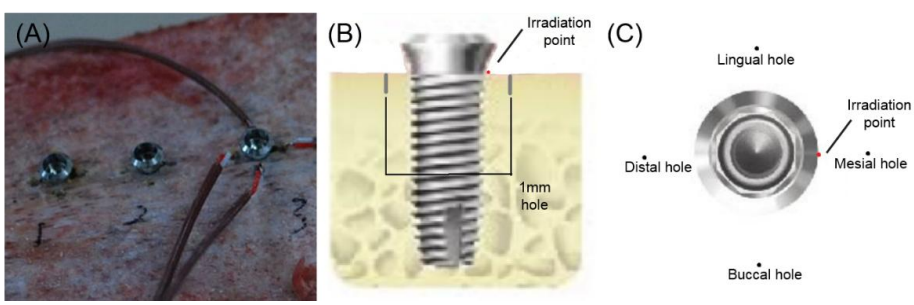


***Fig. 2.*** **In vitro measurements of heat generation**. (A) The temperature sensors placed into the bovine bone after SLA-implant installation. (B), (C) SLA- implant installation into the bovine bone and a schematic graph showing the location of laser irradiation. Starting from the right, in a clockwise direction, the mesial, distal, buccal, and lingual aspects are indicated; the diode laser irradiates the mesial part.

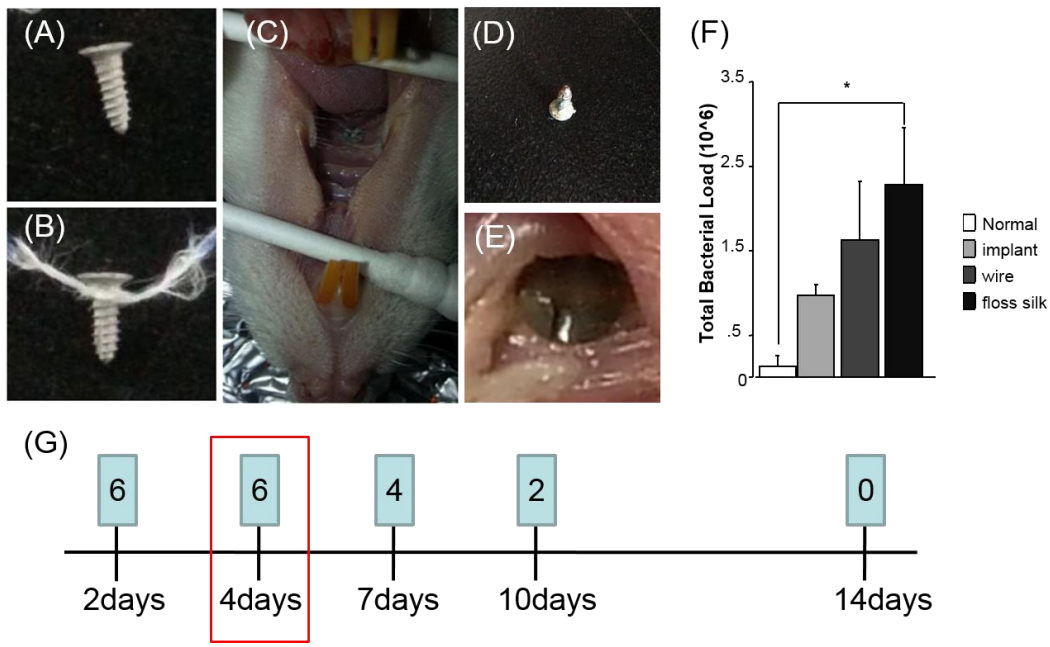


***Fig. 3.*** **Peri-implantitis induced in rat**. (A) An untreated SLA-TS. (B) An SLA-TS wrapped in floss silk. (C) After implantation of (A) and (B) into the hard palate of a rat. (D) Extraction of implanted SLA-TS. (E) Induction of peri-implantitis around an SLA-TS implanted in the hard palate. (F) Wire or floss silk is used to cause peri-implantitis (n = 3, $p < 0.0001$). (G) A schematic graph of the peri-implantitis induction period.

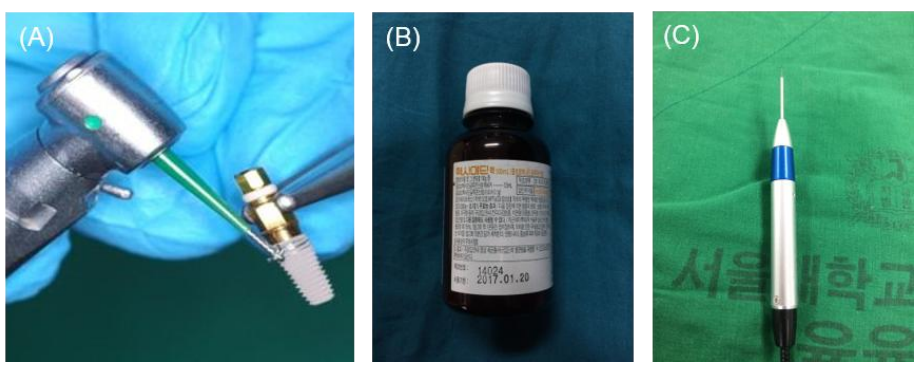


***Fig. 4.*** **Instruments and reagents for the different experimental groups**. (A) iBrush®, (B) 0.5% chlorhexidine treatment, (C) 808nm diode laser handpeace.

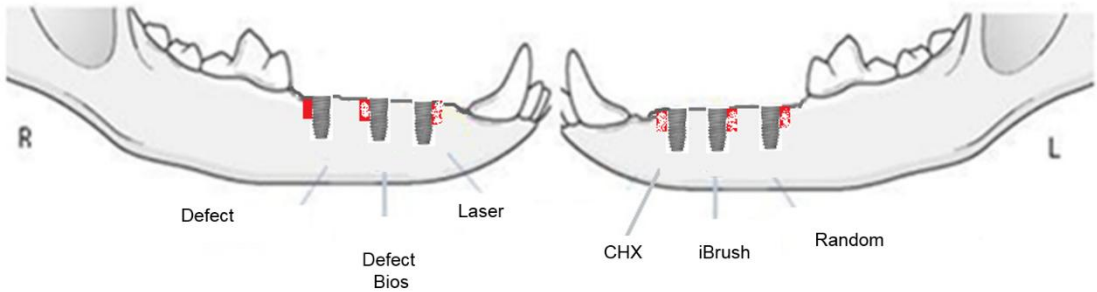


***Fig. 5.*** **Tooth extraction sites in beagles**. Three teeth each are extracted on the left and right sides. Implants from different experimental groups are implanted in the three sites on the right side and two sites on the left; one of the five groups is selected randomly for implantation into the remaining site on the left side.

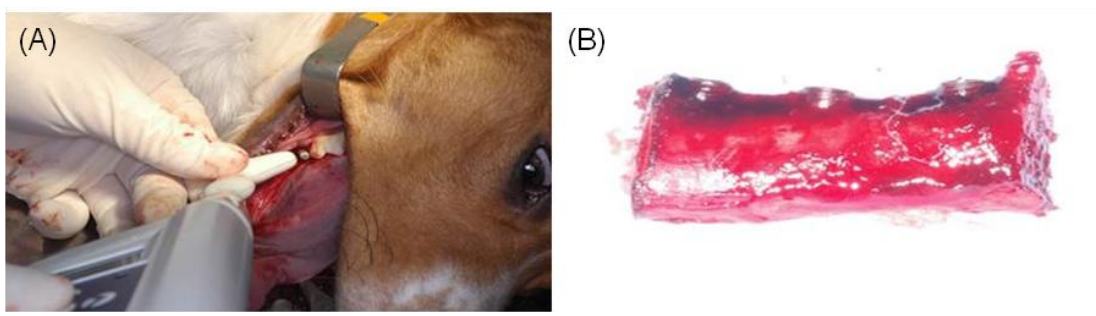


***Fig. 6.*** **ISQ measurement and extraction sites.** (A) Measurement of ISQ after implantation in beagles. (B) The extracted portion including the implantation sites after animal sacrifice.

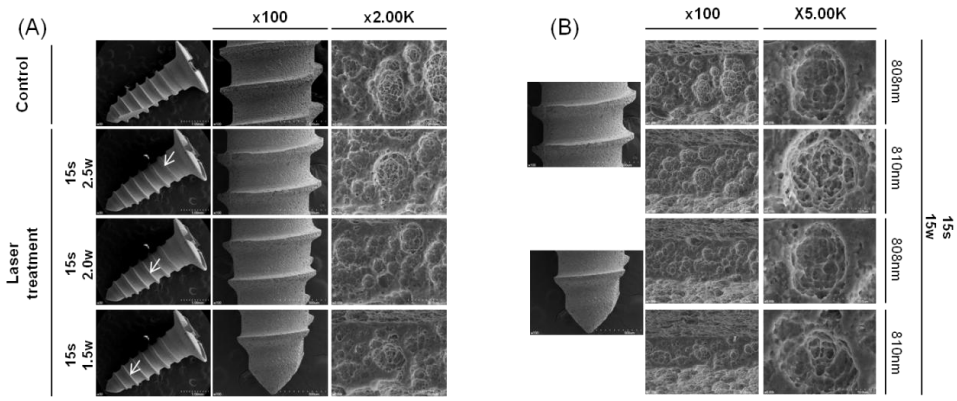


***Fig. 7.*** **SLA-TS surface observed by SEM**. (A) The point marked by the white arrow is irradiated by the diode laser for 15 s at 1.5, 2.0, or 2.5 W. The SLA-TS is observed at ×100 and ×5,000 magnification using SEM, but the diode laser does not appear to cause any surface changes. (B) The 810nm diode laser and the 808nm diode laser irradiate the SLA-TS, but no surface changes are observed.

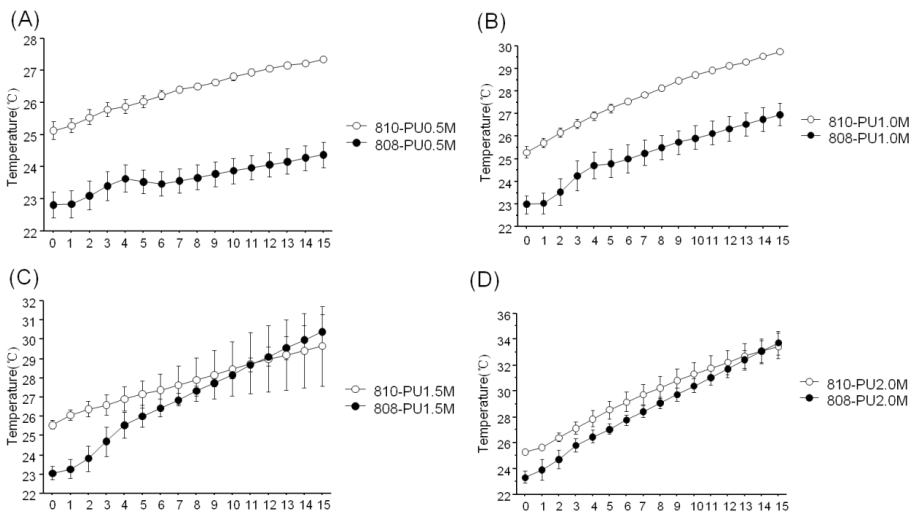


***Fig. 8.*** **Measurements of heat occurrence in bovine bone**. After SLA-TS implantation in bovine bone, the diode laser irradiates the mesial part of the implant. (A)–(B) Temperature changes when the 810nm laser and 808nm laser irradiate the mesial part for 15 s at 0.5 or 1.0 W in the continuous mode ($p < 0.001$). (C)–(D) Temperature changes when the lasers irradiate the implant in the pulse mode, as described in (A) and (B) ($p < 0.001$).

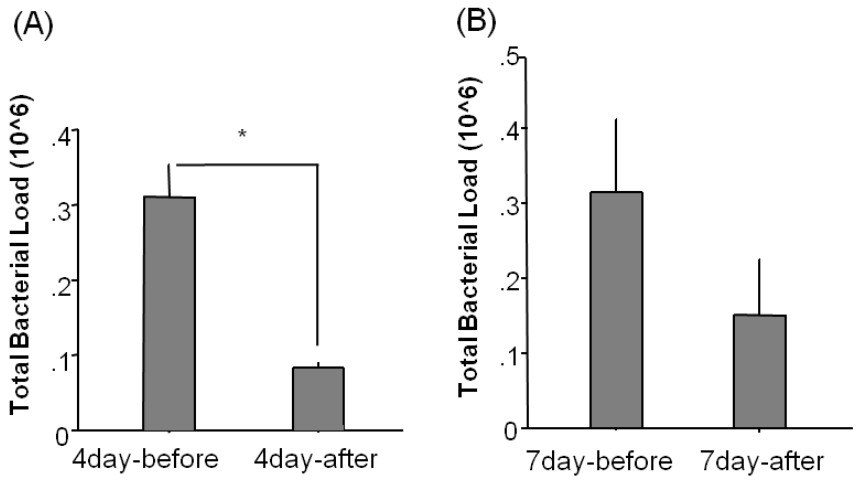


***Fig. 9.*** **Induction of peri-implantitis**. (A) q-PCR results for pre- and post-diode laser irradiation 4 days after SLA-TS implantation into the hard palate of rats. (B) Comparison of pre- and post-diode laser irradiation 7 days after SLA-TS implantation (n = 6, p < 0.001).

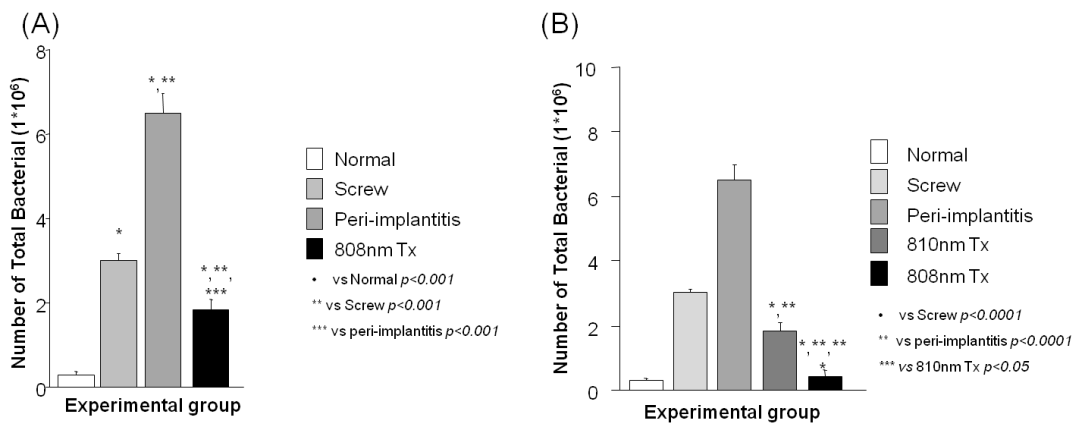


***Fig. 10.*** **Bacterial qPCR.** (A) Graph showing the bacterial PCR results after diode laser treatment of peri-implantitis (n = 6, Unit = $1*10^{6,}$ ***** Normal vs. Screw (p<0.001), ****** screw vs. peri-implantitis and 808nm Tx (*p<0.001*), ******* peri-implantitis vs 808m Tx (*p<0.001*), **(B)** Results of q-PCR performed to verify the bacteria-eliminating effects of the 810nm and 808nm lasers (n = 6, Unit = $1*10^{7}$, * Screw vs 810nm Tx (*p<0.001*), ** Peri-implantitis vs 810nm (*p<0.001*), *** 810nm Tx vs 808nmTx (*p<0.001*).

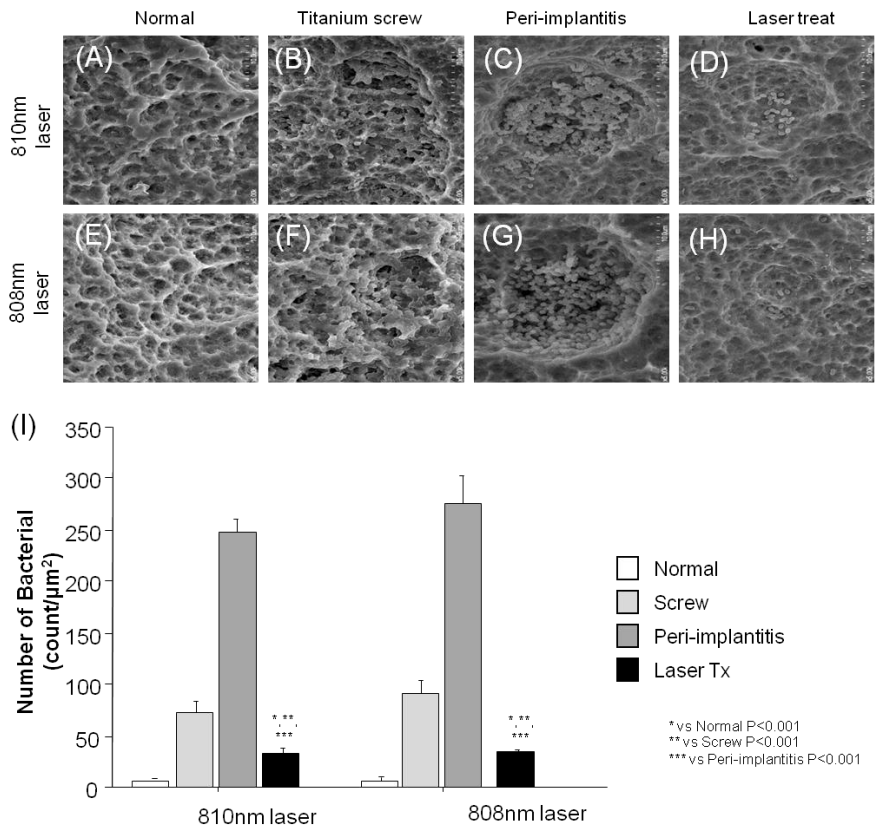


***Fig. 11.*** **Bacterial counts.** (A), (E) control, (B), (F) SLA-TS, (C), (G) Peri-implantitis, (D), (H) laser treatment group. The heads of the SLA-TS implanted in rats are examined by SEM. (I) Diode laser irradiation reduces the bacterial count (n = 6, * vs. control (*p<0.001)*, ** vs. SLA-TS (*p<0.001)*, *** vs. peri-implantitis (*p<0.001*).

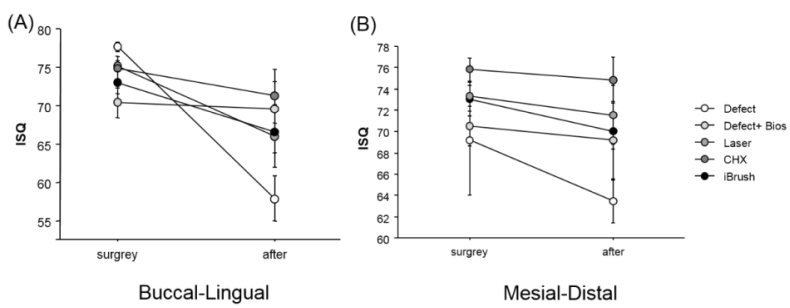


***Fig. 12.*** **Measurement of implant stability.** (A) Buccal-Lingual (B) Mesial-Distal

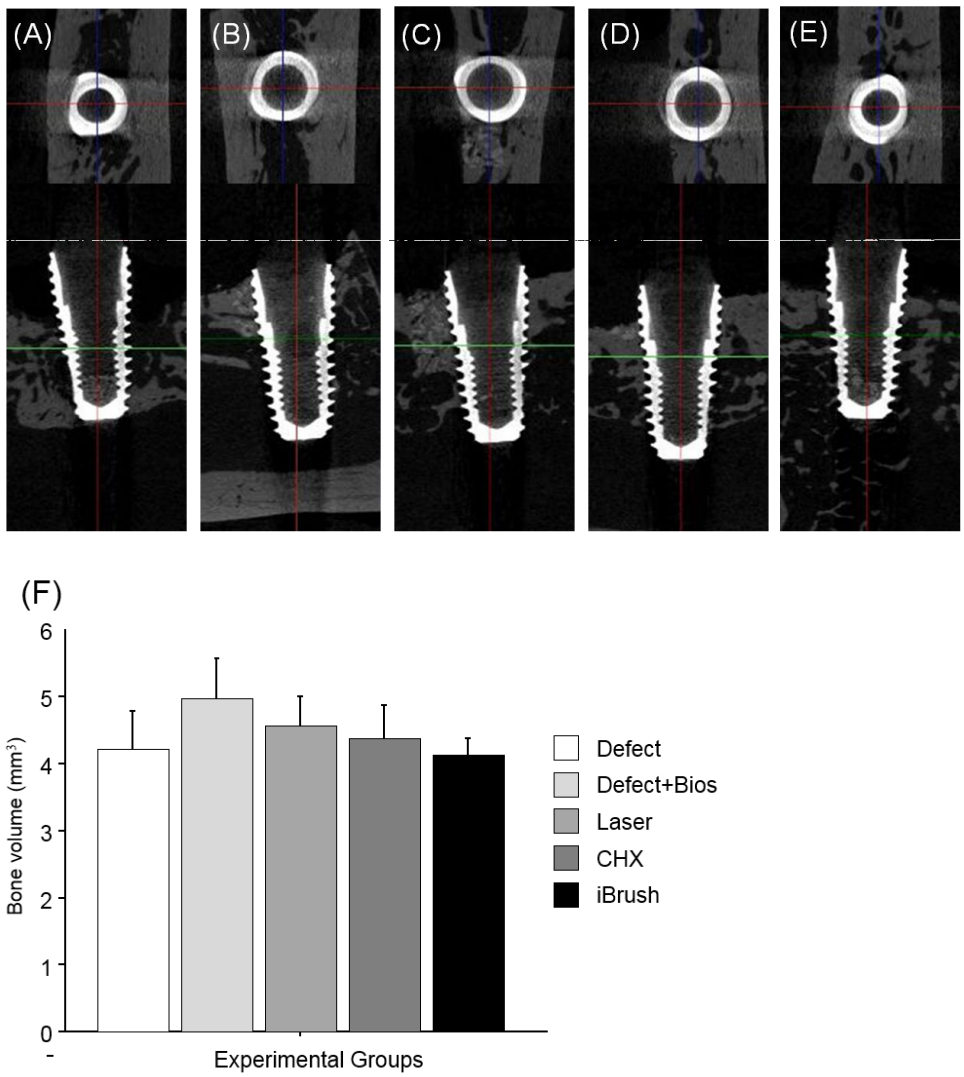


***Fig. 13.*** From the CT image of bone volume after 12 weeks, absorption of bone tissue around the implant was observed for each group. This was most clearly expressed in the Group A (A). Unabsorbed bios were observed in the groups filled with B (B), C (C), D (D), E (E)).

**Measurement of bone volume using micro-CT**(F)**.** Bone volume is the highest for the Group B, followed by the Group C, D and E.

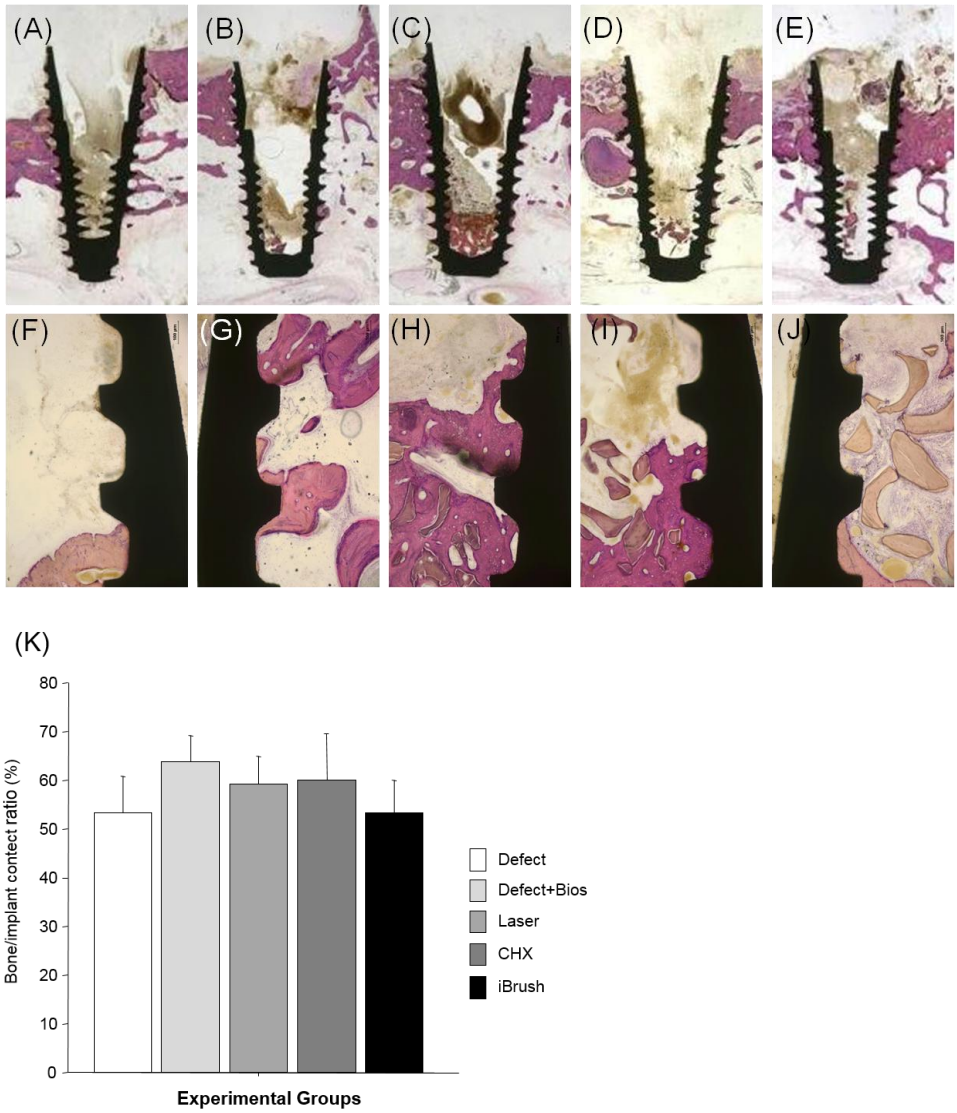


***Fig. 14.*** When the tissue slide with H/E staining was observed after 12 weeks, few bone regeneration was observed for up to 3 screw lines in the Group A (A) and (F) groups. However, bone regeneration was observed in Group B(B)(G) group due to Bio-Oss. Few bone regeneration was observed in (C)(H), Group D(D)(I) and E(E)(J) groups. (K) From the statistical results, Group B was the highest in bone-implant contact ratio, followed by Group D, C, E and A, however, there was no statistical significance.

## IX. Abstract in Korean

# 임플란트주위염 처치에 있어서 다이오드 레이저 치료 효과

서울대학교 대학원 치의과학과 구강악안면외과 전공

(지도교수 이종호)

김위붕 (Wei-Peng Jin)

**연구목적** 다이오드 레이저 치료 방법은 치주염 치료에서 많이 이용되었으며, 임상적 데이터와 치료 결과에 긍정적 영향을 가지고 있다. 본 연구는 실험실 및 생체실험 연구를 통하여 임플란트주위염에 다이오드 레이저를 이용하여 치료가 얼마만큼 안전하고 효과적인지 평가하는데 목적을 두었다.

**재료 및 방법** 연속 모드와 펄스 모드의 레이저 출력이 모두 가능한 808nm 파장의 다이오드 레이저를 이용하여 소뼈에 SLA 표면 처리된 티타늄 임플란트를 식립 한 뒤, 근심, 원심, 협측, 설측 부위에 센서를 연결하여 레이저에서 발생하는 온도를 측정하였다. 7 주령 백서 입천장에 면사가 감긴 SLA 표면 처리된 티타늄 스크류를 식립하여 임플란트 주위염을 유발하였다. 실험 그룹은 처치하지 않은 그룹, SLA-표면 처리된 티타늄 스크류 식립 그룹, 임플란트 주위염 유발 그룹, 임플란트 주위염 유발 후 다이오드 레이저 처치 그룹으로 실험하였다. 주사전자현미경으로 관찰 후 박테리아 수를 측정하였고

중합효소연쇄반응을 통해 구강내 박테리아 증식 정도를 검사하였다. 수컷 성견의 하악 소구치 치아 3 개를 발치 후, 3 개월 간의 회복기간을 가진 뒤 발치 부위에 전처리한 임플란트를 식립하였다. 실험그룹은 임플란트 표면에 전처리를 하지 않고, 골 결손 부위에 골 이식재를 충전하지 않은 그룹, 임플란트 표면에 전처리를 하지 않고, 골 결손 부위에 골 이식재를 충전한 그룹, 레이저로 전처리 후 골이식재를 충전한 그룹, 0.5% 클로로헥시딘으로 전처리 후 골이식재를 충전한 그룹, iBrush®로 전처리 후 골이식재를 충전한 그룹으로 실험을 진행하였다. 임플란트안정성지수 측정, 미세단층촬영, 비탈회연마표본으로 관찰 및 평가 하였다.

**연구결과** 소뼈에서 온도측정 시, 808nm 다이오드 레이저 치료시 평균 3.1℃ 상승하였다. 백서 임플란트주위염 모델에서 다이오드 레이저 처치 후, 중합효소연쇄반응을 관찰 한 결과, 4 일째에서 다이오드 레이저 처치에 의한 박테리아 감소를 확인하였다. 수컷 성견 실험에서는 임플란트 안정성지수, 미세단층촬영시스템, 조직형태학적 분석결과에서 레이저를 조사한 군에서 임플란트 표면에 아무런 처치를 하지 않은 그룹과 같이 재골유착이 많이 일어나지는 않았으나, 다이오드 레이저 처치는 클르로헥시딘 처치, iBrush®로 처치한 그룹과 유사한 결과를 보였다. 임플란트 표면에 처치 안하고 골이식재를 넣지 않은 그룹과 골이식재를 넣은 그룹을 비교했을 때 골재생효과가 차이를 나타냈으나 통계적 유의성은 없었다.

**결론** 808nm 다이오드 레이저를 이용하여 티타늄 스크류에 조사 후 SLA 표면에 물리적 변화를 관찰할 수 없었고 주위 뼈조직 온도가 2.3-3.4℃ 로 상승하는 것을 확인하였다. 808nm 다이오드 레이저 조사 후 백서 구강 내에서 유발된 임플란트 표면에 있는 박테리아를 제거하는데 효과적이 였다. 808nm 다이오드 레이저로 처치 후 각 실험

그룹간에 비교 결과, 유사한 골재생이 관찰되었다. 808nm 다이오드 레이저가 임플란트 주위염 치료에 효과가 있을 것이라고 판단되었다.

**주요어:** 임플란트 주위염, 다이오드 레이저, 808 파장, 백서임플란트주위염 모델, 골재생

**학번:** 2013-30830