Research & Reviews: Journal of Dental Sciences

e-ISSN: 2320-7949
p-ISSN: 2322-0090

Effect of Bacterial Decontamination in SD Rats Peri-Implantitis Model by 808 nm Diode Laser Irradiation

Sung-Ho Lee[1†], Wei-Fung Jin[1†], Ryun-Kyung Kim[2], NaRi Seo[1], Kyung Won Ju[1], Ho-Kyung Lim[1,3], Soo-Hwan Byun [1,4], Young-Joon Lim[5], Soung-Min Kim[1,6], Jong-Ho Lee[1,6*]

[†]These authors contributed equally to this study

[*]This author is corresponding author to this study

[1]Department of Oral and Maxillofacial Surgery, Clinical Trial Center, Seoul National University Dental Hospital, Seoul, Korea

[2]College of Information and Communication Engineering, Sungkyunkwan University, Suwon, Korea

[3]Department of Oral and Maxillofacial Surgery, Korea University Medical Center, Guro Hospital, Seoul, Korea

[4]Department of Oral and Maxillofacial Surgery, Dongtan Sacred Heart Hospital, Hallym University Medical Center, Hwaseong, Korea

[5]Department of Prosthodontics, Seoul National University Dental Hospital, Seoul, Korea

[6]Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Korea

Research Article

Received: 17/08/2017
Accepted: 28/08/2017
Published: 06/09/2017

***For Correspondence**

Jong-Ho Lee, DDS, MSD, PhD, Professor, Department of Oral and Maxillofacial Surgery, Director, Clinical Trial Center and Oral Cancer Center, School of Dentistry, Seoul National University, 03080, 275-1, Yeongun-Dong, Jongro-Gu, Seoul,
Tel: +82-2-2072-2630;
Fax: +82-2-766-4948.

E-mail: leejongh@snu.ac.kr

Keywords: Peri-implantitis, Bacterial infection, Decontamination, Lasers

ABSTRACT

Peri-implantitis is an inflammatory condition that affects soft tissues and alveolar bone adjacent to dental implants. This condition leads to agitation or even loss of implants. Bacterial infection caused by contamination of implant site is one of the known causes of peri-implantitis. Infective bacterial agents include *Aggregatibacter actinomycetemcomitans*, Capnocytophaga sp., and *Fusobacterium nucleatum*. Various noninvasive methods have been evaluated for the elimination of these contaminants. The present study investigated the efficacy of diode laser irradiation for decontamination of implant surfaces and adjacent areas in a peri-implantitis model. Twelve-week-old Sprague-Dawley rats (minimum weight, 300 g) were divided into a control and three implantation groups. Sandblasted and acid-etched titanium screws (SLA-TS; 1.2 × 4.0 mm) were implanted in the hard palate of rats of the three implantation groups: uninfected SLA-TS implant, peri-implantitis infection, and laser-treated peri-implantitis infection groups. Peri-implantitis in inflamed soft tissues and alveolar bone was treated by irradiation with 808 nm diode laser in the continuous mode for 15 s at 0.5 W. The degree of contamination on implant surfaces was evaluated by scanning electron microscopy (SEM), and the proliferation of periodontopathic bacteria in each group was analyzed by quantitative-polymerase chain reaction. The efficacy of laser treatment was evaluated in terms of increase in temperature of the mouth, surface changes of implants, and decrease in bacterial load. Our results indicated that laser irradiation resulted in a significant decrease of periodontopathic bacterial titer, without causing denaturation of implant surfaces.

INTRODUCTION

The incidence of peri-implantitis has increased with the increased use of implants in prosthetic dentistry [1]. Although treatment methods for this condition have been extensively investigated, there is still a need for an effective noninvasive treatment method. Current treatment methods for peri-implantitis are categorized as noninvasive [2] or invasive [3] methods. Noninvasive treatments include antibiotics, curettage, ultrasonic scaling, and laser treatment [4,5]. However, an effective and feasible noninvasive treatment method for peri-implantitis has yet to be reported [6]. Although diode laser irradiation for the treatment of periodontitis has yielded positive clinical data and treatment outcomes [7], few studies have investigated its efficacy in peri-implantitis [8-10]. Moreover, current research regarding heat generation, surface changes and degree of bacterial decontamination by diode laser irradiation

e-ISSN: 2320-7949
p-ISSN: 2322-0090

of titanium implants is inadequate. The present study aimed to evaluate the efficacy and safety of diode laser treatment in peri-implantitis through *in vitro* and *in vivo* studies with 808 nm diode laser irradiation.

METHODOLOGY

Diode Laser System

The 808 nm diode laser device used in this study (Dental 5, Bison Medical Co., Seoul, Korea) allows for both continuous and pulse-wave outputs. In the pulse-wave mode, the pulse width and repetition ratio can be controlled at will, in accordance with the manufacturer's specifications, in order to vary the laser output. The treatment hand-piece for laser output is composed of 200 μm-OD core fiber optic cables. The output power of the device is measured at a distance of approximately 5 mm from the end of the laser hand-piece using a laser power meter (Gentec-EO Maestro, Gentec-EO Inc., Quebec, Canada).

Surface Changes during Implant Preparation and Irradiation

Sandblasted and acid-etched titanium screws (SLA-TS; 1.2 × 4.0 mm) were prepared for implantation in rats. To investigate the effect of diode laser irradiation on the surface of SLA-TS, an implant was irradiated with 808 nm diode laser beams in continuous mode for 15 s at 1.5, 2.0, and 2.5 W. Physical changes on the surface of the irradiated SLA-TS were then assessed by scanning electron microscopy (SEM; magnification 100–5000 X) [11-13]. An untreated SLA-TS was also evaluated by SEM under the same conditions for comparison with the irradiated implant.

Measurement of *In Vitro* Heat Generation

An SLA-TS (1.2 × 4.0 mm) was implanted into a hole in a cow bone. The hole was 1.0 mm in diameter and 2.0 mm in depth, and it allowed the top 2 mm of the SLA-TS implant to be exposed. After implantation, four adjacent holes (0.5 × 1.0 mm) were drilled at medial, distal, superior, and inferior points adjacent to the irradiation site in order to install temperature sensors (ZR-RX40, Omron, Kyoto, Japan). The sensors were then inserted into each hole, and the temperature was recorded in a real-time graph using a thermoelectric temperature recorder. The temperature ranged from 20–60°C **(Figure 1)**. A tooth neck area of 2 mm was considered as indicating peri-implantitis. The implant surface was irradiated by 808 nm diode laser radiation for 15 s at 0.5, 1.0, 1.5, 2.0, and 2.5 W in the continuous and pulse wave modes. Heat generation data from each sensor was recorded for comparative analysis.

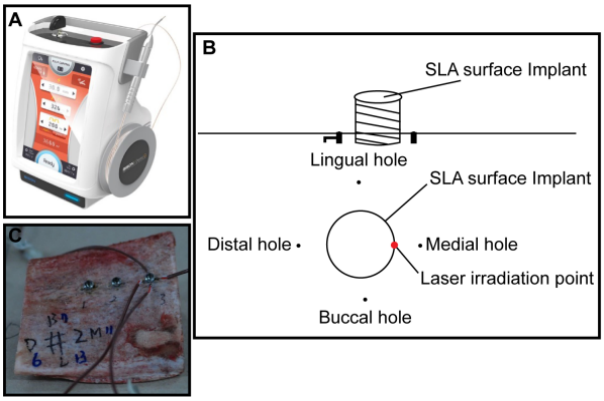

Figure 1. Measurement of *in vitro* heat generation. (A) An 808 nm diode laser device developed by Bison Medical. (B) Schematic sketch of the sandblasted and acid etched-titanium screw (SLA-TS) implant (top) and mimetic diagram of a cow bone and target locations for laser irradiation (bottom): clockwise from the top: the lingual, medial, buccal, and distal regions are simulated, and the medial region is irradiated with diode laser radiation. (C) A temperature sensor is installed after SLA-TS implantation on cow bone.

Induction of Peri-implantitis in Sprague-Dawley Rats

In this study, all methods using experimental animals complied with the instructions of the author's institution and were approved by the concerned Ethics Committee.

Male white Sprague-Dawley (SD; 300–350 g) rats (Orient Bio, Gapyeong, Korea) were bred in a specific-pathogen-free animal experiment laboratory under conditions of constant temperature (21°C ± 1°C) and humidity (55%) with 12 h light-dark cycles (light, 07:30–20:00; dark, 20:00–07:30). Food (Purina Rodent Chow, Purina Co., Seoul and Republic of Korea) and purified water were provided ad-libitum. The animals were divided into a control and three implantation groups: uninfected SLA-TS implant, peri-implantitis infection, and laser-treated peri-implantitis infection groups. All animals were quarantined for a period of 1 week. After the adjustment period, animals in the implantation groups were each injected with a 3-mL solution (100 mg/kg) of pentobarbital

e-ISSN: 2320-7949
p-ISSN: 2322-0090

(Hanlim Pharm. Co., Ltd., Gyeonggi, Korea) and chloral hydrate (Sigma-Aldrich. Co., ON, Canada) in the abdominal area for anesthesia, following which, SLA-TS were implanted on the hard palate [14].

In order to encourage plaque formation and induction of peri-implantitis, the heads of the SLA-TS were wrapped in cotton-based floss silk or 26G liners prior to implantation. The animals were evaluated for signs of infection on days 2, 4, 7, 10, and 14 post-implantation. Implants wrapped with floss silk were observed to induce peri-implantitis effectively within 4 days post-implantation **(Figure 2A)**. Samples of saliva from the control and uninfected SLA-TS implant group and samples of gingival crevicular fluid (GCF) from areas adjacent to the SLA-TS in the peri-implantitis infection groups were extracted using absorbent paper **(Figure 2B)** (Meta Biomed Co., Ltd. Cheongju, Korea). These samples were evaluated via quantitative-polymerase chain reaction (qPCR; CytoGen CO., Ltd, Seoul, Korea) for quantification of bacterial infection [15] **(Figure 2C)**.

Post-irradiation Bacterial Load in a Peri-implantitis Rat Model

Following irradiation of implant-adjacent regions in peri-implantitis induced rats with diode laser beams in the continuous mode for 15 s at 0.5 W, [16] **(Figure 2D)** samples of GCF were extracted from the irradiated areas using absorbent paper points (Meta Biomed Co., Ltd. Cheongju, Korea) **(Figure 2E)** and analyzed by qPCR (CytoGen CO., Ltd, Seoul, Korea) [17]. Then, the implanted SLA-TS were removed and stored in 4% paraformaldehyde for 24 h, following which, bacterial population on the screw heads were observed and enumerated by SEM [15] **(Figures 2F and 2G)**.

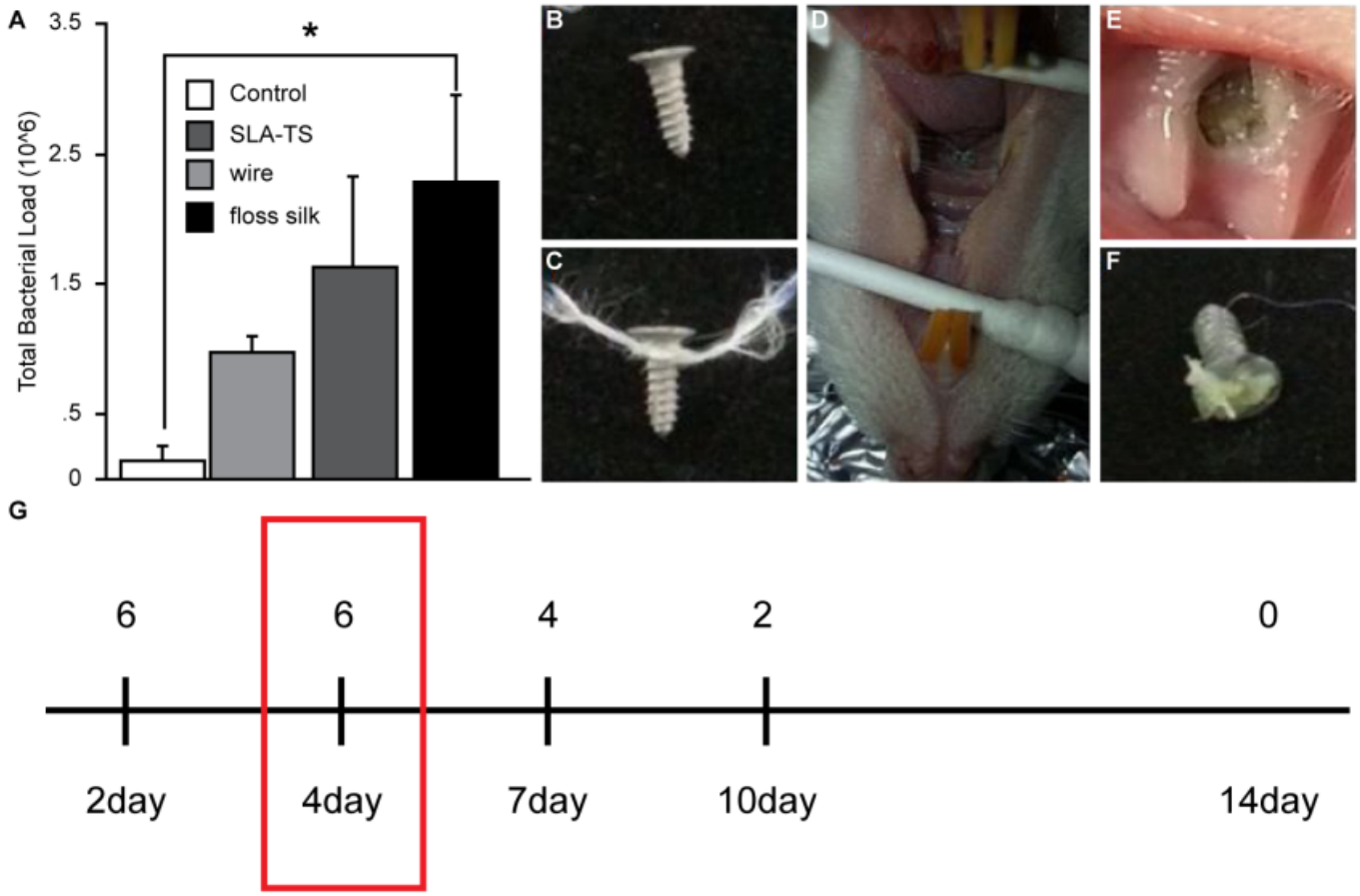

Figure 2. Induction of peri-implantitis in a rat. (A) Wire and floss silk were used to induce peri-implantitis in a rat model (n=3; p<0.0001). (B) An untreated sandblasted and acid etched-titanium screw (SLA-TS). (C) An SLA-TS with floss silk coiled. (D) Peri-implantitis in the palate of rats implanted with (B) and (C). (E) Peri-implantitis induced in a rat. (F) An SLA-TS removed from the palate of a rat in the peri-implantitis group. (G) Timeline of induction of peri-implantitis. Values above and below the line represent the number of preserved SLA-TS implants in each rat and duration since SLA-TS implantation, respectively.

Statistical Analysis

Data analysis was performed using Stat View software (version 5.0.1, the SAS Institute, Cary, NC, USA). All data were presented as mean values with standard errors of mean and the level of significance was set at $p<0.05$. One-way analysis of variance followed by Fisher's PLSD test, when necessary, was used to analyse data.

e-ISSN: 2320-7949
p-ISSN: 2322-0090

RESULTS

Surface Changes of Implants

No physical changes of SLA-TS surfaces irradiated with 808 nm diode laser radiation for 15 s at 1.5 W were observed upon SEM analysis **(Figure 3)**.

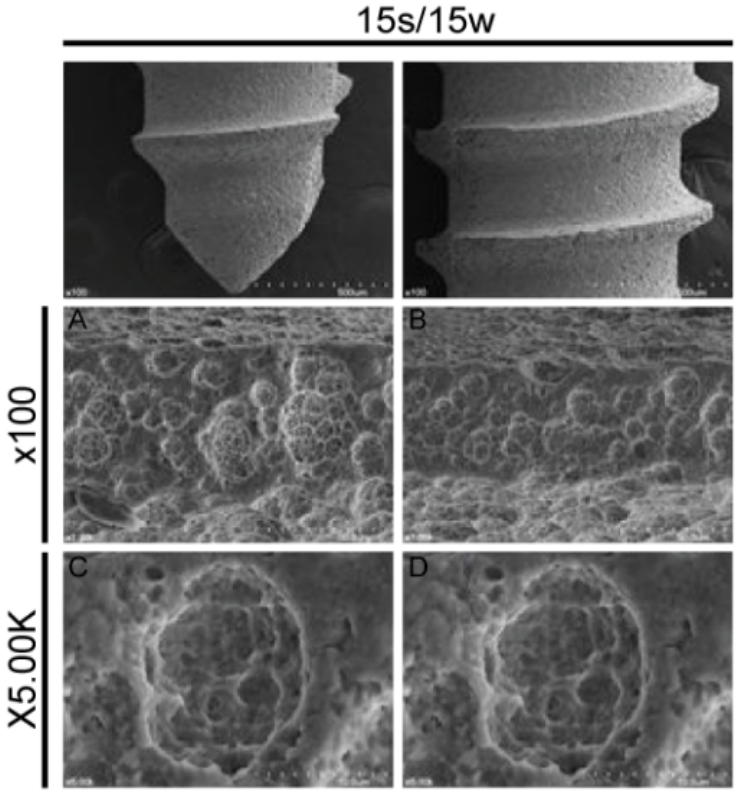

Figure 3. Scanning electron microscopy (SEM) findings of surface changes in sandblasted and acid etched-titanium screws (SLA-TS). The mid and end regions of SLA-TS were irradiated by an 808 nm diode laser for 15 s at 1.5 W, and the resulting surface changes were evaluated by at 100 X and 5000 X magnifications by SEM. There are no differences in SEM findings from the surface evaluation between untreated (control; A and C) and irradiated (B and D) SLA-TS.

In Vitro Heat Generation Measurement

To investigate the effects of laser irradiation of implant surface on oral temperature, an SLA-TS implanted in cow bone was irradiated on the medial side with 808 nm diode laser radiation in continuous mode for 15 s at 0.5, 1.0, 1.5, 2.0, and 2.4 W and the resultant temperature changes were recorded **(Table 1)**.

Table 1. Measurement of heat generated by cow bone.

Continuous Mode	Pulse Mode				
Out-put power (w)	Standard	Increase	Out-put power (w)	Standard	Increase
0.5	23°C	24.5°C	0.5	23°C	24°C
1	23.8°C	25.2°C	1	23.8°C	26.8°C
1.5	23.8°C	32.0°C	1.5	23.8°C	29.4°C
2	23.8°C	36°C	2	23.8°C	31.2°C
2.4	23°C	42°C	2.4	23°C	33°C
Mean increase	3.1°C		Mean increase	1.7°C	

Effect of 808 nm Diode Laser Irradiation on Bacterial Count

Bacterial samples were extracted from the two SLA-TS-implanted infection groups on days 4 and 7 after induction of peri-implantitis. While one of these groups was untreated (peri-implantitis infection group), the other had been irradiated in the implant-adjacent areas with 808 nm diode laser radiation (laser-treated peri-implantitis infection group). Bacterial titer in the samples collected from the two groups was then evaluated by q-PCR. The results indicated a decrease in bacterial titer in samples collected from the laser-treated peri-implantitis infection group on day 4 **(Figures 4A and 4B)**.

e-ISSN: 2320-7949
p-ISSN: 2322-0090

Comparison of bacterial titer among samples collected from the control, uninfected SLA-TS implant, and peri-implantitis infection groups revealed a decrease in bacterial titer in the group that received laser treatment after peri-implantitis induction **(Figure 4C)**.

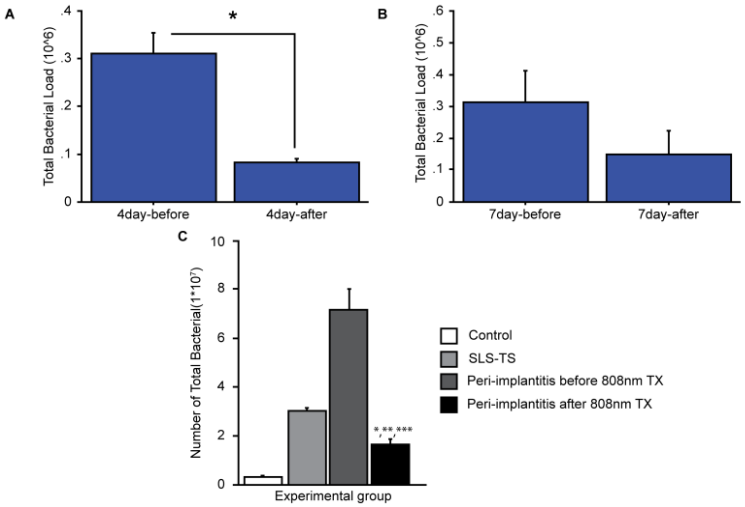

Figure 4. Bacterial load in sandblasted and acid etched-titanium screws (SLA-TS) of peri-implantitis induced rats. Quantitative polymerase chain reaction (qPCR) findings of bacterial load in SLA-TS implanted in the palate of rats (A) 4 days and (B) 7 days before and after irradiation (n=6; p<0.001). (C) Graphical representation of bacterial q-PCR findings (n=6; unit = 1 x 107; *control vs. all groups, p<0.001; **SLA-TS implant group vs. peri-implantitis groups before and after treatment, p<0.001; ***peri-implantitis groups before vs. after treatment, p<0.001).

Bacterial Counting

Implant heads coiled with floss silk prior to implantation were retrieved from rats in the two peri-implantitis infection groups in order to evaluate the effects of diode laser treatment by SEM examination. The bacterial count in the uninfected SLA-TS implant group **(Figure 5A)** was higher compared with that in the control group **(Figure 5B)**. The bacterial count in the peri-implantitis infection group **(Figure 5C)** was greater compared with those in the uninfected SLA-TS implant and laser-treated peri-implantitis infection **(Figure 5D)** groups. This implied a reduction in bacterial population upon laser irradiation after induction of peri-implantitis **(Figure 5E)**.

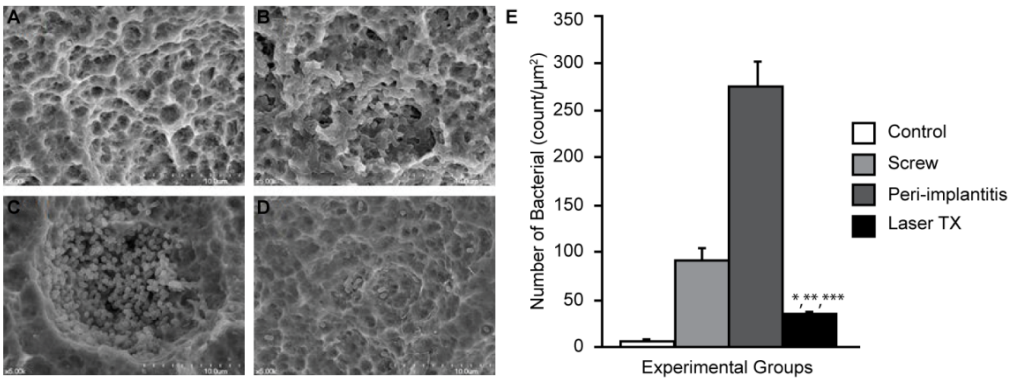

Figure 5. Bacterial titer. (A) Control group; (B) sandblasted and acid etched-titanium screw (SLA-TS) implant group; (C) Peri-implantitis-induced group; (D) Laser-treated peri-implantitis-induced group; (E) Bar graph of bacterial count. SLA-TS were implanted the palate of rats and irradiated with 808 nm diode laser. Bacterial populations in irradiated and untreated SLA-TS were then evaluated by scanning electron microscopy. The bacterial load in peri-implantitis-induced rats treated with laser irradiation is lower compared with those in uninfected rats with SLA-TS implants and peri-implantitis-induced rats. (n=6; * vs. Control, p<0.001; ** vs. SLA-TS, p<0.001; *** vs. Peri-implantitis, p<0.001).

DISCUSSION

To investigate the effects of 808 nm diode laser irradiation on implant surfaces, SLA-TS implant surfaces were irradiated for 30 s at 1.5, 2.0, and 2.5 W, and evaluated by SEM. There were no changes in the structure of the laser-exposed surfaces. Evaluation of temperature changes in implant-adjacent regions of cow bone due to laser irradiation at incrementally increasing power levels in the continuous and pulse modes revealed increases in mean temperature of 3.1°C and 1.7°C, respectively. The increase in temperature at 0.5 W was only minimal in either mode. Laser irradiation of SLA-TS implanted in the hard palate of SD rats on days 4 and 7 post-implantation did not result in implant loss. The results of qPCR analysis revealed that laser irradiation resulted in decrease reduction of bacterial populations.

e-ISSN: 2320-7949
p-ISSN: 2322-0090

Peri-implantitis was induced by introducing contamination using a foreign substance (wire or floss silk) coiled around the SLA-TS. The bacterial count in the floss silk-induced peri-implantitis infection group was found to be higher compared with that in the uninfected SLA-TS implant group. Bacterial count in the peri-implantitis infection group decreased after implant irradiation by laser. The results of SEM examination of implant heads in the palate of rats in each group revealed that the bacterial count in the peri-implantitis infection group was higher compared with that in the uninfected SLA-TS implant group; in contrast, the group that received treatment by laser irradiation following peri-implantitis induction exhibited a significantly lower bacterial count.

These findings confirm that 808 nm diode laser irradiation is an effective method of bacterial decontamination for sites affected by peri-implantitis.

CONCLUSION

Peri-implantitis in soft tissues and alveolar bone was treated by 808 nm diode laser irradiation in continuous mode at 0.5 W for 15 s. Laser treatment reduced the bacterial load on implant surfaces, without causing excessive heat generation or denaturation of the surface. Therefore, 808 nm diode laser irradiation is a safe and effective treatment for peri-implantitis.

ETHICAL APPROVAL

In this study, all methods using experimental animals complied with the instructions of the Seoul National University Committee and were approved by the Ethics Committee (approval number: SNU-140707-5-1).

ACKNOWLEDGEMENTS

This study was supported by a grant of Ministry for Trade, Industry and Energy, (10047615) and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI15C1535).

REFERENCES

1. Zitzmann NU and Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol. 2008;35:286-291.
2. Schwarz F, et al. Clinical evaluation of an Er: YAG laser for nonsurgical treatment of peri-implantitis: A pilot study. Clin Oral Implants Res. 2005;16:44-52.
3. Azzeh MM. Er, Cr:YSGG laser-assisted surgical treatment of peri-implantits with 1-year re-entry and 18-month follow-up. J Periodontol. 2008;79:2000-2005.
4. Mailoa J, et al. Clinical outcomes of using lasers for peri-implantitis surface detoxification: A systematic review and meta-analysis. J Periodontol. 2014;85:1194-1202.
5. Figuero E, et al. Management of peri-implant mucositis and peri-implantitis. Periodontol 2000. 2014;66:255-273.
6. Park SH and Lee JK. Nonsurgical interventions for treating peri-implantitis and prognosis]. Journal of Korean Dental Association Dental Ass. 2014;52:396-401.
7. Slot DE, et al. The effect of the thermal diode laser (wavelength 808-980 nm) in non-surgical periodontal therapy: a systematic review and meta-analysis. J Clin Periodontol. 2014;41:681-692.
8. Roncati M, et al. Non-surgical treatment of peri-implantitis with the adjunctive use of an 810 nm diode laser. J Indian Soc Periodontol. 2013;17:812-815.
9. Ashnagar S, et al. Laser treatment of peri-implantitis: a literature review. J Lasers Med Sci. 2014;5:153-162.
10. Pai BSJ, et al. Adjunctive use of diode lasers in the treatment of peri-implantitis: A case series. J Dent Implants. 2014;4:91-97.
11. Castro GL, et al. Scanning electron microscopic analysis of diode laser-treated titanium implant surfaces. Photomed Laser Surg. 2007;25:124-128.
12. Yamamoto A and Tanabe T. Treatment of peri-implantitis around TiUnite-surface implantis using Er: YAG laser microexplosions. Int J Periodontics Restorative Dent. 2013;33:21-30.
13. Freire MO, et al. Development of an animal model for Aggregatibacter actinomycetemcomitans biofilm-mediated oral osteolytic infection: a preliminary study. J Periodontol. 2011;82:778-789.
14. Abtahi J, et al. Effect of local vs. systemic bisphosphonate delivery on dental implant fixation in a model of osteonecrosis of the jaw. J Den Res. 2013;92:279-283.
15. Takasaki AA, et al. Er: YAG laser therapy for peri-implant infection: a histological study. Lasers Med Sci. 2007;22:143-57.
16. Ebadian AR, et al. Bacterial analysis of peri-implantitis and chronic periodontitis in Iranian subjects. Acta Med Iran. 2012;50:486-492.
17. Guo M, et al. Kgp, rgpA, and rgpB DNA vaccines induce antibody responses in experimental peri-implantitis. J Periodontol. 2014;85:1575-1581.