Effect of Low Level Laser Irradiation on Osteoblast Cell Proliferation and Differentiation after Implant Placement

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• Abstract

Objective : The purpose of this study was to evaluate the effects of low level lasers on bone healing and new bone formation around titanium dental implants in canine models. 18 oxidized surface treated implants and a Dens-bio laser were used.

Study design : Low level lasers were irradiated with a total of 8J for 4 minutes by pulse wave type and 1 minute by continuous type. For the experimental group, a low level laser was used to irradiate the first premolar implant's insertion area at the time of insertion, a low level laser was used to irradiate the second premolar implant's insertion area daily for one week after implant insertion, and a low level laser was used to irradiate the third molar implant's insertion area daily for 2 weeks postoperatively. At the conclusion of the study, sacrificed tissue sections were made from investing tissue and observed under an optical microscope.

Results : The rate of new bone formation around the implant showed no significant difference between the control group and the experimental group. New bone formation rates of the control and experimental group 2 weeks following implant placement were higher than that of immediately after implant placement and 1 week after implant placement.

Conclusions : Based on these results, a low-level laser showed no statistically significant increase in bone formation following implant placement.

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Introduction

With the use of the therapeutic application of lasers, tissue is revived due to biological stimulation and the methods of obtaining the treatment effect. How about, "The use of therapeutic application of lasers has been found anecdotally to revive tissue, possibly due to biological stimulation This has been intriguing. Such clinical effects as anti-inflammatory responses, analgesic effect, improved wound healing and promoting bone healing have also been confirmed[1,2]. Particularly regarding bone tissue, there have been recent reports of a positive effect on bone remodeling. In bone tissue, exposure to low level laser has been seen to regulate the inflammatory response, promote cell division[3] and accelerate the healing process[4-6].

Successful implants in jaw bones depend upon the wound healing process and the potential effect of bone-derived cells that are present in the peri-implant. Bone formation within the interface between the implant and bones is a complex physiological process that is controlled by systemic hormones and local factors produced by cells in the bone. This is associated with a series of events such as the attachment of cytoplasm, enlargement, differentiation, and deposition.

Recent in vitro studies have shown that low-level laser therapy (LLLT) increases the rate of attachment and proliferation of human gingival fibroblasts in titanium implant material[7]. According to the regeneration of bones around the implant, however, a greater extent of clinical associations includes osteoblasts and their responses. When the implant surface is contacted, cells (like osteoblasts) behave in a different manner from oral fibroblasts[8,9]. Little is known about the mechanisms by which LLLT is associated with the effect of titanium implants on the attachment and proliferation of osteoblasts.

The purpose of this study was to evaluate the effects of low level lasers on bone healing and new bone formation around titanium dental implants in a canine models.

Materials and Methods

Experimental materials

1) Experimental animals

In the current study, the experimental animals consisted of three adult dogs. All three dogs were 12 months old, with weights ranging from 10 to 12 kg, and were bred under the same conditions. No sex differentiation was involved and their health status was good.

2) Implant

In the current study, 18 Osstem GSII implant (Osstem, Seoul, Korea) surfaces were treated with oxidation. Implants were 3.5mm in diameter and 10mm long.

3) Low-level laser

A Dens-bio laser (TMC Korea, Seoul, Korea) was used throughout the study. This model has a wave length of 904nm and its frequency is 5~10,000㎐ for pulse wave and 10~100㎑ for continuous wave. The output was 27㎽(≒ 0.3W).

4) Experimental devices

Following the implant placement, radiation with a low-level laser was performed depending on the experimental group. Following the sacrifice, the embedded tissue was placed into the tissue sample and then examined with an optical microscope.

Experimental methods

1) Anesthesia

For general anesthesia, Xylazine (Rompun®, Bayer Vetchem-Korea Co.) and Ketamine (Ketara®, Yuhan Corp.) 2cc were injected intramuscularly. Then, to control the bleeding and pain at the site of tooth extraction, an infiltration anesthesia was performed using 2% lidocaine.

2) Tooth extraction

In each adult dog, the mandibular premolars 1, 2, 3 and 4 were extracted. Then, a 10-week recovery period was given. In all groups, a 5-day course of daily IM injections of 2cc of gentamicin was used to prevent postoperative infection following the extraction and the implant placement.

3) Implant placement and laser irradiation

A conventional crestal incision was made. A flap elevation was performed to such an extent as to make it possible to implant the tooth without any involvement of soft tissue on the buccal and lingual sides. Then, the implant placement was performed under the infusion of saline. All the implants

were submerged and the wound was sutured with black silk. A pulse wave, low-level laser was used to irradiate the area for 4 minutes. Then, irradiation with a continuous wave laser was performed for one minute for a total of 8J of laser.4 The right mandible served as the experimental group. In the first premolar area, the site of implant was irradiated with a low-level laser at the time of implant placement. In the second premolar area, a low-level laser was irradiated daily for 2 weeks following the implant placement. In the third premolar area, a low-level laser was irradiated daily for 2 weeks following the implant placement. The left mandible served as the control group. The implant placement was performed at the same time as the experimental group. Following regular monitoring, adult dogs were sacrificed at the same time (Table I).

Table I. Study protocol

	Left side on mandible	Right side on mandible
The 1st premolar	Control group 1 (Sacrifice after immediate implant placement)	Experimental group 1 (LLLT application during implant placement)
The 2nd premolar	Control group 2 (Sacrifice after 1 week)	Experimental group 2 (LLLT application during 1 week)
The 3rd premolar	Control group 3 (Sacrifice after 2 weeks)	Experimental group 3 (LLLT application during 2 weeks)

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4) Sacrifice

All experimental subjects were sacrificed following a 7-day period of cellular differentiation after the experiment was performed. In accordance with the experimental schedule, adult dogs were sacrificed immediately after the implant placement, 1 week after the implant placement, and 2 weeks after the implant placement. To collect the tissue sample, the implanted mandible was resected.

Experimental assessment

1) Histomorphometric analysis

Following the implant placement, the bone around the implant was examined immediately after the implant placement, 1 week after the implant placement and 2 weeks after the implant placement. Implant specimens were immediately immersed in a 70% alcohol-based solution. Dehydration was attempted with the use of alcohol washing. Following this, the specimen was formatted using glycolmetacrylate resin (spurr low-viscosity embedding media, Polyscience, Warrington, PA, USA). The polymerized specimen was sectioned off in the longitudinal direction at a thickness of 200um using a high-precision diamond disc (low speed diamond wheel saw 650, SBT, San Clemente, CA, USA). Finally, using a lapping and polishing machine (OMNILAP 2000, SBT, San Clemente, CA, USA), the polishing was done at a thickness of 30um. One slide was prepared for each implant. Afterwards, they underwent a Villanueva osteochrome bone stain (San Clemente, CA, USA). This was followed by light microscopy (Olympus BX50, Tokyo, Japan). For histomorphometric analysis, the new bone formation rate was calculated using the following formula:

New bone formation rate = areas where new bones were formed / areas outside of the thread × 100%.

2) Statistical analysis

Statistical analysis was performed using SPSS11 for Windows 98. A significant difference between the control and the experimental group was assessed using analysis of variance (ANOVA). Statistical difference was considered significant if $P < 0.05$.

Results

Histopathologic findings

1. Control group

a. Week 1 (Fig. 1)

Inflammatory connective tissue was involved between the bone tissue and the implant, but there were no findings suggesting of increased activity of osteoblasts or fibroblasts.

b. Week 2 (Fig. 2)

Acute and chronic inflammatory connective tissue and premature fibroblasts were observed. In the areas of bone defects, osteoblasts and new bones were mildly identified.

c. Week 3 (Fig. 3)

Inflammation of the connective tissue was reduced and the density of fibroblasts was increased. In the areas of bone defects, the formation of new lamellar bones and osteoblasts was significantly increased.

2. Experimental group

a. Week 1 (Fig. 4)

Acute and chronic inflammatory connective tissues were involved between the bone and the implant. Furthermore, mild fibroblasts were emerged.

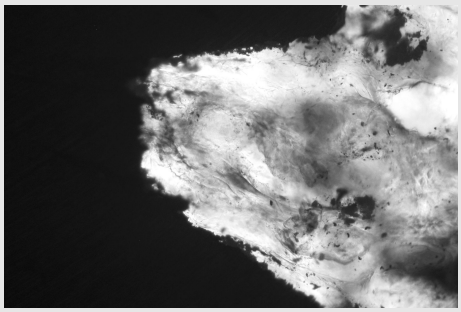

Fig 1. Control group 1. Between implant to bone area engaged inflammatory connective tissue, no increasing activity in osteoblast and fibroblast. Villanueva osteochrome bone stain, ×100.

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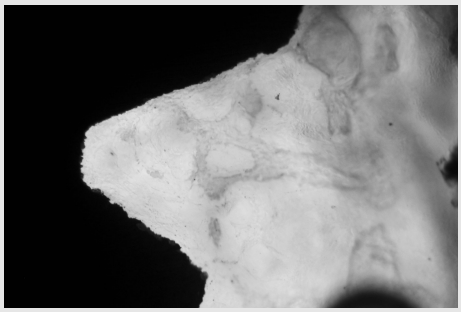

Fig 2. Control group 2. Acute and chronic inflammatory connective tissue was seen in the peri-implant area. A few new bones were located in the bone defect area. Villanueva osteochrome bone stain, ×100.

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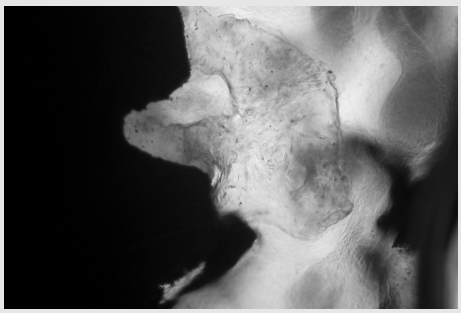

Fig 3. Control group 3. Decrease of connective tissue inflammation and increase of density of fibroblast were noted. Increasing trabecular pattern at bone defect area. Villanueva osteochrome bone stain, ×100.

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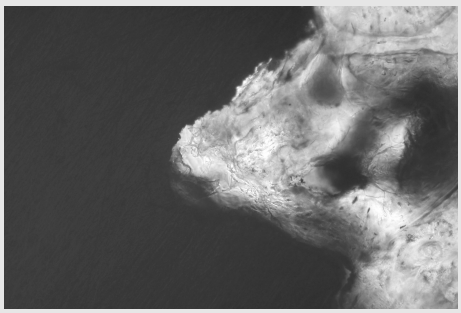

Fig 4. Experimental group 1. Between implant to bone area engaged inflammatory connective tissue, no increasing activity in osteoblast and fibroblast. Villanueva osteochrome bone stain, ×100.

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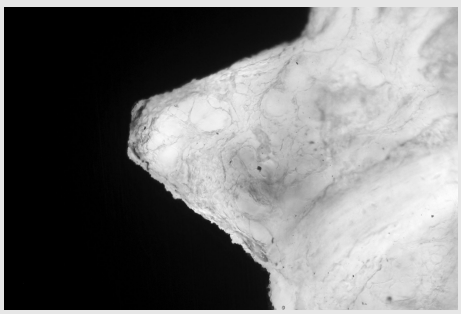

Fig 5. Experimental group 2. Chronic inflammatory cells were seen in the bone defect area and osteoblasts were seen in peripheral area. Villanueva osteochrome bone stain, ×100.

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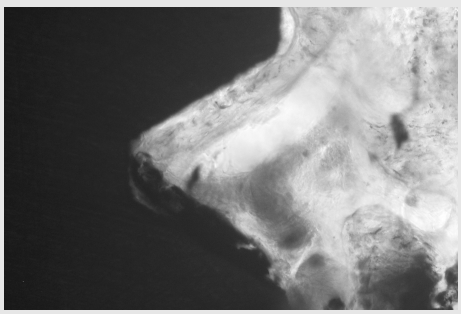

Fig. 6. Experimental group 3. New bone trabecullae were seen in the bone defect area, osteoid was seen between implant to bone defect area. Villanueva osteochrome bone stain, ×100.

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b. Week 2 (Fig. 5)
Chronic inflammatory cells were observed in the areas of the bone defects. Premature fibroblasts were mildly present in the connective tissue.
c. Week 3 (Fig. 6)
In the areas of the bone defects, new lamellar bones were observed. In the adjacent area, osteoblasts were observed. Osteoid was noted to be present between the implant and the bone defect.

The rate of new bone formation

Between the control group and the experimental group, there was no significant difference in the rate of new bone formation. New bone formation rates of the control and experimental group after 2 weeks were higher than after immediate implant placement or 1 week following implant placement (Table II).

Table II. New bone formation rate (Unit : %)

Classification	Number	Classification	Number
Control group 1	0.0283±0.0236	Experimental group 1	0.0297±0.0326
Control group 2	0.1567±0.0322	Experimental group 2	0.1750±0.0288
Control group 3	0.6024±0.2363	Experimental group 3	0.6550±0.1480

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Discussion

The growth and differentiation of bone-forming cells (osteoblasts) play a crucial role in the regeneration of bones around the dental implant. It has been proposed that biological stimulation due to LLLT enhances the possibility of regenerating bones. Despite the positive results seen on both in vitro and in vivo studies, controversial opinions exist regarding whether LLLT has a significant effect on the synthesis of bone matrix[10].
These controversial opinions are based on a wide variety of experimental models that were used for irradiation protocol[11].
From the biological viewpoint, the use of LLLT for human oral diploid cells as a test system is more reliable than the aneuploid cell lines of other tissue and species. One of the important differences between the primary cell culture and an established cell line is the possibility for unlimited growth of the latter. Powerful characteristics of certain cell lines can screen a laser-induced effect.
Boulton and Marshall[12] experimentally demonstrated that the effect of LLLT can be better proven in a slow-growing culture. According to various studies using established cell lines, however, the beneficial effect of LLLT has been demonstrated.
The use of this model, known to be a primary culture, can restrict the possibility of the change of expressions. According to the latent period due to 1.25(OH)2D3, compared to the basal level, the production of osteocalcin was significantly different.
To examine the responses of human osteoblast-like cells to LLLT, the cells were cultured using titanium implant material. A GaA1As diode laser was used to irradiate the cells. Selected variables include the early attachment, proliferation, differentiation and synthesis of TGF-β1. The early attachment following 1, 3 and 24 hours was significantly higher in the irradiated cells compared to the control group. The proliferation of cells following 48 and 72 hrs was not statistically significant. These results were confirmed by Ueda et al.[3] and Ozawa et al.[13]. In these two studies, however, there were no significant differences in the amount of laser irradiation. The amount of 3J/㎠ no longer effected the early attachment and proliferation of osteoblast-like cells.
Alkaline phosphatase (ALP) activity is considered to be an indicator for the differentiation of osteoblasts[14]. Early progenitor cells cannot express ALP activity. In addition, enhanced levels of expression of the osteoblast markers suggested that the extracellular matrix contributes to both the shutdown of proliferation and the development of the osteoblast phenotype. It can therefore be inferred that the effect of lasers on ALP activity reflects the effect of laser on bone formation[15]. This data shows no significant difference between the two groups where the laser was used. In the same manner as these results, Coombe et al.[10] reported that ALP activity was not affected by laser irradiation to a significant extent in a study with cells isolated from human osteosarcoma.
By contrast, other studies have shown that the ALP activity was markedly increased following LLLT[16,17]. Ueda et al.[3] reported a positive effect of pulse frequencies of LLLT on bone nodule formation in rat calvarial cells in vitro. Osteoblast-like cells isolated from fetal rat calvariae were

irradiated once with a low-energy Ga-Al-As laser (830 nm, 500 mW, 0.48-3.84 J/cm^2) in four different irradiation modes : continuous irradiation (CI), and 1-, 2-, and 8-Hz pulsed irradiation (PI-1, PI-2, PI-8). The effects on cellular proliferation, bone nodule formation, ALP activity, and ALP gene expression were then investigated. It was concluded that the pulse frequency of LLLT was an important factor effecting biological responses in bone formation.

The stimulatory effect of LLLT parameters could be obtained following a 3-day consecutive application, rather than a one-time application[14,18].

Several laser systems are currently applied to the stimulation of tissue regeneration. In selecting the most customizable laser therapy for basic and clinical use, a careful assessment of the characteristics of each machine is mandatory[19]. The current study showed that LLLT using GAA1A diode laser had a positive biological stimulatory effect on osteoblast-like cells and did not cause cellular injury. Cell viability was more than 90% in all the experimental groups. It is noteworthy that the application of laser irradiation did not induce any clear evidence of cell injury.

In addition to these in vitro studies, animal experiments that have been conducted up to the present showed that LLLT promoted the synthesis of factors associated with wound healing in the metabolism of osteocytes and thereby strengthened the integration between the hard tissue and implant.[4] These results will establish the optimal dose of laser irradiation for the areas between the tissue and the implant and will contribute to the development of a clinical model of a laser regimen for implant therapy.

Based on the results, a low-level laser showed no statistically significant increased bone formation after implant placement. The conclusions are based on a very small sample size and should be re-confirmed with follow-up studies using a larger sample size.

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