

Long-Term Histologic Analysis of Bone Tissue Alteration and Healing Following Er:YAG Laser Irradiation Compared to Electrosurgery

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Background: The erbium-doped:yttrium, aluminum, and garnet (Er:YAG) laser is reportedly useful for periodontal therapy. However, the potential thermal damage that Er:YAG laser irradiation can produce on bone tissue has not been fully clarified. The purpose of this study was to histologically examine the effects of the Er:YAG laser on bone tissue and subsequent wound healing compared to electrosurgery in a long-term study.

Methods: Calvarial bone from 30 rats was exposed to contact and non-contact Er:YAG laser irradiation (115 mJ/pulse, 10 Hz) without water coolant, or electrode contact. The treated surfaces were analyzed by scanning electron microscopy (SEM), and the healing process was histologically observed until 12 months post-surgery.

Results: Contact irradiation resulted in substantial bone ablation, whereas non-contact irradiation produced slight tissue removal. Histologic and SEM analyses of the lased surface showed no severe thermal damage, except for the production of a superficially affected layer with a microstructured surface. The layer did not inhibit new bone formation, and the ablated defect was repaired uneventfully. Although the thickness of the layer gradually decreased, it generally remained in the cortical bone through the observation period. Electrosurgery produced a large area of thermal necrosis without ablation, and the damaged area was not replaced with new bone.

Conclusions: Unlike electrosurgery, Er:YAG laser irradiation without water coolant easily ablated bone tissue, and thermal alteration in the treated surface was minimal. The superficially affected layer did not interfere with the ensuing bone healing, resulting in favorable repair of the defect. *J Periodontol* 2009; 80:82-92.

KEY WORDS
Bone; electrosurgery; Er:YAG laser; histology; lasers; wound healing.

Lasers are commonly used for oral soft tissue procedures because of their easy tissue ablation and strong bactericidal and hemostatic effects. Neodymium-doped:yttrium, aluminum, and garnet (Nd:YAG), carbon dioxide (CO₂), and diode lasers are the most common choices for soft tissue management.¹ However, these lasers have the potential to produce thermal damage to the underlying bone, particularly when applied to areas with thin soft tissues.^{2,3} In addition, delayed bone healing has been reported after osteotomy using these lasers.^{4,5} Such delays are most commonly due to the thermal side effects produced by lasers, e.g., the carbonized/charred layer with necrosis on the treated bone surface that is caused by strong heat effects during irradiation.^{5,6}

Electrosurgery is another generally accepted method of soft tissue management in periodontal surgery because of its easy tissue incision accompanied by strong hemostatic effects.⁷ Electrosurgery can achieve good results when certain variables, such as waveform, power, speed of instrument movement, and depth of tissue coagulation, are properly selected and controlled. However, the effects of electrosurgery seem to be relatively intense, and it is difficult to limit the thermal effects to the immediate surgical

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site. Direct contact of the electrode with the root surface frequently causes severe pain in the dental pulp when local anesthesia is insufficient. In addition, the major concern when using electrosurgery is the potential risk for damage to the root surface⁸ or underlying osseous structures, such as the periosteum and alveolar bone, by direct contact with the electrode during gingival tissue management; this can lead to necrosis and sequestration of bone tissue that results in delayed wound healing.⁹⁻¹¹ However, to the best of our knowledge, detailed investigations into histologic alteration and wound healing of bone tissue after electrode contact have not been reported.

Erbium-doped:YAG (Er:YAG) lasers are increasingly being accepted in the field of dentistry. The 2.94- μ m wavelength of this laser coincides with a large absorption band for water;¹² therefore, this laser is able to effectively ablate soft and hard tissues with fewer thermal side effects to the surrounding tissues compared to other hard lasers.^{13,14} The successful application of the Er:YAG laser in caries treatment¹⁵⁻¹⁸ led to its clinical application in periodontics. This laser is considered one of the most promising ones in periodontal therapy^{13,14} because of its favorable performance in periodontal soft tissue procedures,¹⁹⁻²¹ as well as hard tissue treatments, such as root surface preparation,²²⁻²⁷ osseous surgery,²⁸⁻³³ and osseous defect debridement.³⁴⁻³⁸

During soft tissue procedures, the Er:YAG laser is occasionally used without water coolant to enhance laser performance and tissue hemostasis. However, laser irradiation without water cooling presents a risk for thermal injury to the underlying bone tissue during soft tissue surgery. Therefore, to ensure the clinical safety of Er:YAG laser during periodontal soft tissue procedures, a thorough analysis of the actual maximal thermal damage produced to bone tissue in a clinical situation should be performed.

The purpose of this study was to investigate alterations in bone tissue after Er:YAG laser irradiation without water coolant, via scanning electron microscopy and histology, and to examine subsequent wound healing over a long-term period compared to electrosurgery, which is a conventional technique for soft tissue procedures using a thermal effect.

MATERIALS AND METHODS

Animals and Experimental Procedures

The protocol design and animal experiment procedures were approved by the ethics committee of the Animal Research Center of Tokyo Medical and Dental University. Thirty 10-week-old, male Wistar rats were used in this study. After general anesthesia and skin incision, the calvarial bone was exposed, and the periosteum was gently detached.³¹ Calvarial bone was exposed to Er:YAG laser irradiation under two sets

of conditions or electrode contact (Fig. 1A). A pulsed Er:YAG laser^{18||¶} was used, and laser irradiation was applied vertically in a straight line perpendicular to the parietal bone using a straight contact tip (Fig. 1B) at an energy output of 115 mJ/pulse (panel setting: 150 mJ/pulse) and 10 Hz. Contact focused irradiation (energy density: 40.7 J/cm²/pulse) was performed while moving the handpiece manually with a speed of \sim 1 cm per 3 seconds in a position vertical to the bone surface. Non-contact defocused irradiation (\sim 6.6 J/cm²/pulse) was performed in the same way, keeping the tip end 5 mm from the bone surface using a guiding gauge. Energy output was selected based on our previous studies^{30,31} and pilot experiments. Electrode contact was performed on the frontal bone in a similar manner as described for laser irradiation. An electrosurgical device[#] with a frequency of 1.2 MHz and a maximal power of 40 W was used. The bone surface was exposed to electrode contact (Fig. 1C) at an intensity setting of 5 (range, 0 to 9) in the electrocurrency mode of cutting, which is suitable for soft tissue incision. The time of instrumentation was \sim 3 seconds in all three treatment groups. Each rat received these three treatments.

Immediately after treatment, six of 30 rats were sacrificed. Surface changes of the bone were analyzed by scanning electron microscope (SEM) and histologic examination using three rats each. For the other 24 rats, the periosteum and the skin were replaced and sutured after treatment. The healing process was observed histologically at 1 and 3 days; 1 and 2 weeks; 1, 3, and 6 months; and 1 year after surgery. Three rats were sacrificed for each observation period, and bone samples were prepared.

Scanning Electron Microscopy

Three bone specimens were fixed, dehydrated, and critical-point dried in liquid CO₂. Then specimens were mounted, sputter-coated with gold, and observed with the SEM.** A secondary electron image was obtained at an accelerating voltage of 20 kV and a tilt angle of 50°.³¹

Histologic Examination and Histometric Analysis

Following fixation, the 27 specimens were decalcified with Plank-Rychlo's solution and cut in the center in the left-to-right direction; the left half was used for histologic analysis, avoiding the median suture between the parietal bones. After dehydration, the specimens were embedded in paraffin, and serial sections were cut in the nose-to-neck direction; specimens were stained with hematoxylin and eosin and subjected to light microscopy. Photomicrographs were taken,

|| Model ML22, Erwin, HOYA Photonics, Tokyo, Japan.

¶ J. Morita Manufacturing, Kyoto, Japan.

Operer DS-M, J. Morita Manufacturing.

** Model T-20, JEOL, Tokyo, Japan.

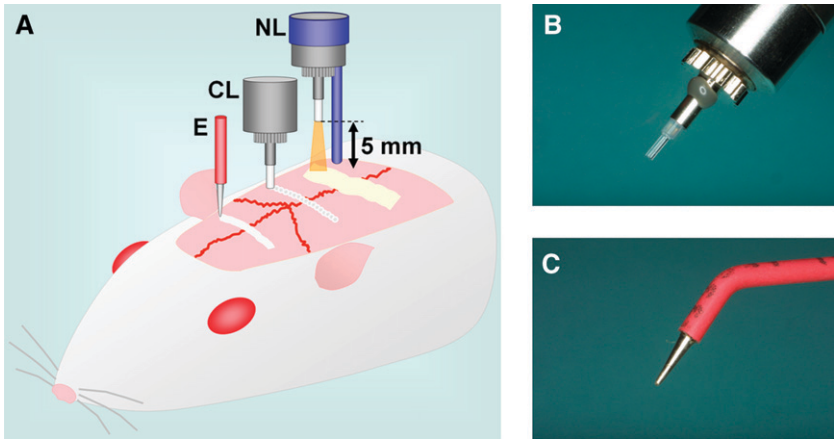


Figure 1. Experimental design and contact tips. **A)** Schematic location of treated sites on rat calvaria. Contact (CL) and non-contact (NL) Er:YAG laser irradiation was performed in a straight line on the parietal bone keeping the contact handpiece vertical. Electrode contact (E) was performed on the frontal bone in a similar manner. **B)** Straight Er:YAG laser contact tip with a 600- μ m diameter and 80% transmission rate, made of quartz glass, mounted on a handpiece. **C)** Single-pointed electrode probe with a tip diameter of 400 μ m.

and histomorphometric analyses were performed by a trained examiner, who was masked to the specific experimental conditions, using computerized image system software.^{††} Five histologic sections, at ~600- μ m intervals from the center, were selected by the examiner for the evaluation, for immediately after, 2 weeks; 1, 3, and 6 months; and 1 year post-surgery, and the following characteristics were evaluated.

Ratio of new bone formation within ablated defect of contact irradiation site. Areas of the original bone defect and newly formed bone were measured for each of five sections. The area of new bone was converted to a percentage relative to the area of each original defect. The average of five original values obtained from five sections was calculated and denominated as a representative value for each rat of each observation period. Next, three representative values from three rats were averaged and denominated as a representative value for each observation period.

Thickness of affected layer at contact and non-contact irradiation sites and width of affected area at non-contact site. The thickness of the affected layer was determined based on the extent of hematoxylin staining. Measurement was performed at three different points on the treated surface with equal intervals in the contact and the non-contact irradiation site for each section. The average of the three data points was used as a representative value for each of five sections. The representative value for each observation period was determined in the same way as the calculation of ratio of new bone. The width of the affected area following non-contact irradiation was also mea-

sured for each histologic section, and the representative value was calculated in a similar manner.

Area of affected region and area of tissue defect at electrode contact site. The entire affected area, as determined by the extent of hematoxylin staining, including the defect area and the area of tissue defect within the stained region, was measured for each of five sections. The representative value for each observation period was calculated in the same manner as for the area of the affected region and the area of each defect.

Statistical Analysis

The differences in new bone formation at the contact irradiation site, the width of the affected area at the non-contact irradiation site, and the areas of the affected region and the defect at the electrocauterization site among the observation periods were subjected to one-way factorial analysis of variance (ANOVA) followed by the Dunnett post hoc test for multiple comparisons. Differences in the thickness of the affected layer between contact and non-contact irradiation for each observation period were analyzed by the unpaired *t* test, and *P* < 0.05 was considered statistically significant.

RESULTS

Macroscopic Observation

Contact focused Er:YAG laser irradiation without water coolant easily ablated bone tissue without producing major carbonization and coagulation. The treated site showed a whitish, groove-like appearance with occasional bleeding, whereas non-contact defocused irradiation produced a wide zone of white and yellowish surface alteration with minimal tissue removal and no bleeding. Electrode contact produced a white band of coagulation on the bone surface without removing any tissue or producing bleeding (Fig. 2).

SEM Observation

At the Er:YAG laser contact irradiation site, a sequential crater formation was observed, resulting in a groove-like appearance with a clear border (Fig. 3A). At high magnification, the lased surface showed a scale-like or flaky microstructure, without evidence of melting (Fig. 3B). At the border of the ablation groove, thermal changes were represented by ~10- to 20- μ m-wide zone that was dark in color (Figs. 3C and 3D).

At the non-contact site, the irradiated area became wider and shallower, with a smoother surface texture

^{††} Image-Pro Plus, version 3.0.1, Media Cybernetics, Silver Spring, MD.

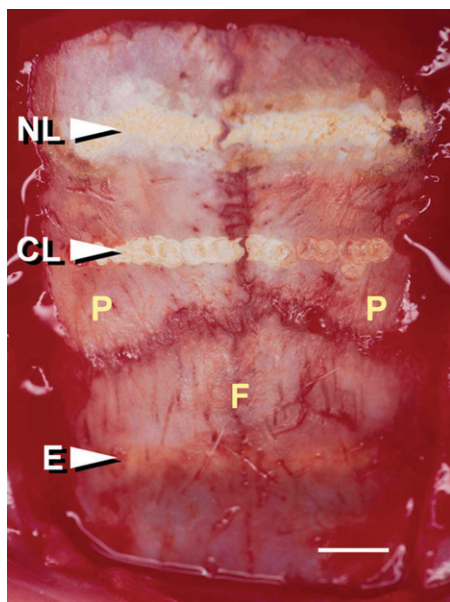


Figure 2.

Macroscopic view of representative treated bone surface immediately after Er:YAG laser irradiation or electrode contact. Contact focused Er:YAG laser irradiation (CL) without water coolant ablated bone tissue substantially without producing major thermal damage, such as carbonization and coagulation, and the treated site showed a groove-like appearance with a whitish color and occasional bleeding. Non-contact defocused irradiation (NL) produced a wide zone of white and yellowish surface alterations with minimal tissue removal and no bleeding. Electrode contact (E) easily produced a white band of coagulation on the bone surface, due to strong thermal effects, without removing any tissue or causing bleeding. F = frontal bone, P = parietal bone. Bar = 2 mm.

than observed at the contact site. The border between the irradiated and the non-irradiated area was clear, but a dark-colored zone with a width of 65 to 125 μm was observed, which was wider than that after contact irradiation (Fig. 3E). At high magnification, a microirregular structure, similar to that after contact irradiation, was observed on the surface (Fig. 3F).

At the electrode contact site, changes on the treated surface were seen as a dark band without any tissue ablation (Figs. 3G and 3H).

Histologic Examination

Contact Er:YAG laser irradiation. The contact irradiation site exhibited a dome-shaped defect that was $514.5 \pm 52.9 \mu\text{m}$ wide and $173.2 \pm 32.4 \mu\text{m}$ deep ($n = 3$ rats) (Fig. 4). An affected layer of $\sim 10\text{-}\mu\text{m}$ thickness, which was thermally denatured and deeply stained by hematoxylin, was observed on the ablated surface. No major thermal changes were noted around the ablation defect.

At day 1 post-surgery, the bone defect was filled with exudate composed of hemorrhage and fibrin. At day 3, hemorrhage had increased markedly, and

accumulation of numerous erythrocytes and newly formed small vessels were observed in the defect. At 1 week, granulation tissue formation with less hemorrhage was observed in the defect. A small amount of new woven bone was observed in a limited number of sections. After 2 weeks, the defect was increasingly filled with new bone, although detachment between the laser-treated surface and new bone was observed. After 1 month, the newly formed bone tissue within the defect was connected to the new bone formed on the original bone surface. The affected layer was still present, but it was discontinuous. By 3 months, the defect was almost completely repaired by new bone. Some sections revealed the presence of numerous osteocytes and blood vessels in the defect, representing a high activity of bone remodeling within the defect. At 6 months, the newly formed bone tissue still presented an active appearance. The affected layer was still observed after 1 year.

Non-contact Er:YAG laser irradiation. The lased area showed only a slightly concave or nearly flat surface that was $1,456.7 \pm 157.5 \mu\text{m}$ wide and $58.6 \pm 21.9 \mu\text{m}$ deep ($n = 3$ rats) (Fig. 5A). No obvious heat changes were detected under the irradiated surface, except for the thin affected layer with a thickness $\sim 15 \mu\text{m}$. At day 1 post-surgery, some hemorrhage was observed between the periosteum and the affected layer. At day 3, increased bleeding was noted in the periosteum. Bleeding had decreased after 1 and 2 weeks, and a discontinuous appearance of the affected layer was occasionally observed. At 3 and 6 months, discontinuous sites in the affected layer were observed more frequently, with new bone formation over the irradiated area, depending on rat growth. After 1 year, a large part of the affected layer remained, and it was embedded between the old and newly formed bone.

Electrode contact. Electrode contact produced a large hematoxylin-stained region with a semicircular shape and lacking tissue ablation (Fig. 5B). The stained region was $408.9 \pm 47.2 \mu\text{m}$ wide and $164.7 \pm 23.4 \mu\text{m}$ deep ($n = 3$ rats). Immediately after treatment, most of the tissue in the stained area was lost, resulting in a large defect hole. The defect was actually an artifact that resulted from the loss of severely damaged bone tissue during the decalcification process of histologic preparation. At day 1 post-surgery, some hemorrhage was observed between the periosteum and the stained region. After 1 week, bleeding was reduced. After 2 weeks, slight bleeding remained in the periosteum. After 1 month, the defect hole in the stained region tended to diminish. After 3 months, the defect hole in the region became markedly smaller, and the area of the stained region tended to decrease. New bone formation was observed over the stained region, and the region was embedded in the bone tissue. After 6 months, the defect hole in

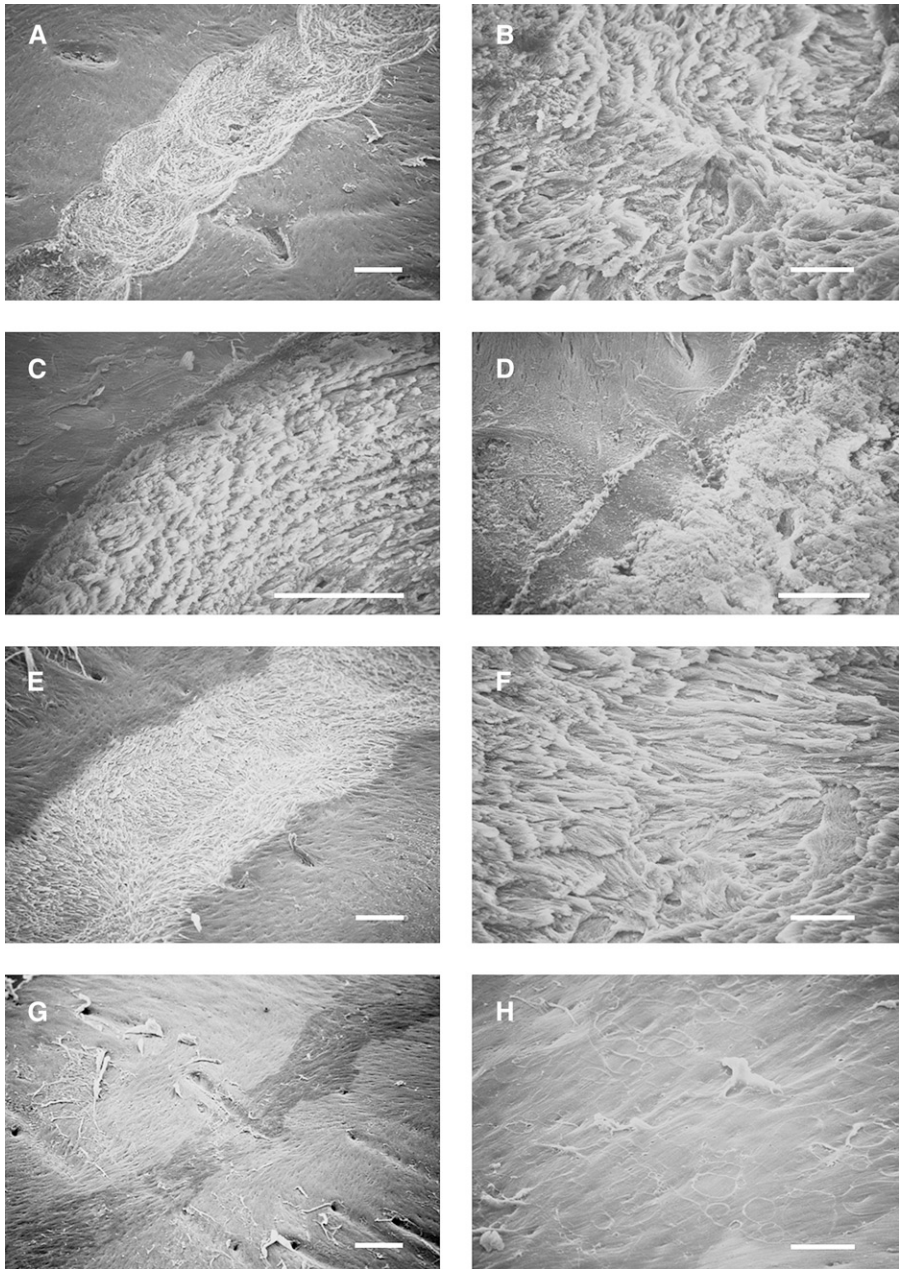


Figure 3. Scanning electron micrographs of the treated bone surface. Bone surface following Er:YAG laser contact irradiation without water coolant at low magnification shows sequential crater formation resulting in a groove-like appearance with a clear border. **A)** The width of the groove was ~500 to 650 μm . At the marginal area of the ablation groove following Er:YAG laser contact irradiation at low magnification **(C)** and high magnification **(D)**, a very narrow thermal alteration zone was observed as a dark colored zone of ~10 to 20 μm in width. At low magnification, the bone surface following non-contact irradiation without water coolant showed a wider (1,200 to 1,500 μm in width) and shallower ablation area **(E)** compared to that after contact irradiation. **G)** After electrocauterization, changes on the treated surface were seen as a dark band with a width of ~250 to 450 μm that was due to thermal alteration without tissue ablation. At high magnification, the contact **(B)** and the non-contact irradiation **(F)** surfaces showed a scale-like or flaky microstructure without major thermal damage, such as melting, whereas the electrode contact site **(H)** exhibited no structural changes. (Original magnification: A, E, and G, $\times 50$; B, C, F, and H; $\times 350$; D, $\times 1,000$; bar: A, E, and G, 250 μm ; B, F, and H, 50 μm ; C, 100 μm ; D, 25 μm .)

the region became minimal, although the stained area remained evident. After 1 year, the hole in the stained region was no longer evident, but the large stained region was generally still noted.

Histometric Examination

The thickness of the affected layer at the contact (Fig. 6A) and non-contact irradiation sites (Fig. 6B) is shown in Figure 6C. At 0 days; 2 weeks; 1, 3, and 6 months; and 1 year, respectively, the thickness was 9.5 ± 0.7 ; 10.2 ± 0.5 ; 10.5 ± 0.7 , 7.5 ± 0.2 , 9.1 ± 0.6 ; and $6.9 \pm 1.2 \mu\text{m}$ for the contact site and 15.0 ± 0.4 ; 16.2 ± 1.1 ; 14.4 ± 4.5 , 12.1 ± 0.4 , 11.7 ± 1.3 ; and $6.5 \pm 1.3 \mu\text{m}$ for the non-contact site ($n = 3$ rats). There was a statistically significant difference between the contact and non-contact modes immediately after, at 2 weeks, and at 3 and 6 months after surgery ($P < 0.05$). Over time, the thickness of the affected layer showed a significant change for each irradiation mode ($P = 0.0002$ for contact and $P = 0.0012$ for non-contact; ANOVA). The thickness decreased significantly from immediately after to 3 or 12 months after surgery for contact irradiation and from immediately after to 12 months after surgery for non-contact irradiation ($P < 0.05$).

Figure 7A shows the width of the affected area at the non-contact site. The width was 1.46 ± 0.07 ; 1.35 ± 0.07 ; 1.51 ± 0.09 , 1.28 ± 0.09 , 1.63 ± 0.07 ; and $1.35 \pm 0.05 \text{ mm}$ ($n = 3$ rats) at 0 days; 2 weeks; 1, 3, and 6 months; and 1 year, respectively. The width had not decreased significantly at 1 year. With regard to new bone area at the contact irradiation site, Figure 7B shows the ratio (% area) of new bone formation. The ratio was 0%; $60.9\% \pm 24.0\%$; $71.7\% \pm 20.5\%$, $77.7\% \pm 15.9\%$, $79.0\% \pm 19.6\%$; and $79.1\% \pm 16.0\%$ ($n = 3$ rats) at 0 days; 2 weeks; 1, 3, and 6 months; and 1 year after

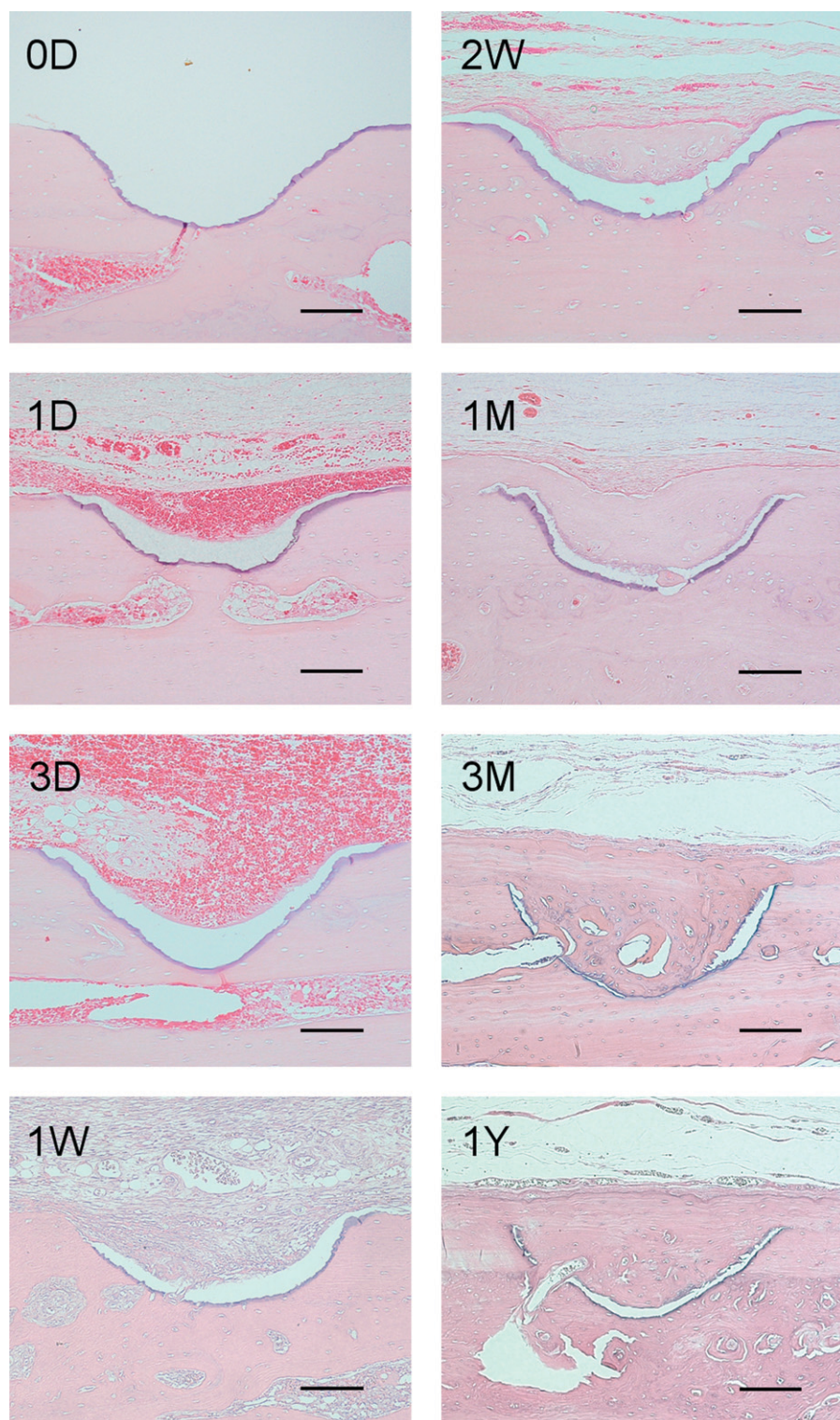


Figure 4.

Representative photomicrographs of histologic sections of contact irradiation site. Contact Er:YAG laser irradiation without water cooling ablated bone tissue effectively without major thermal damage. The irradiation site presented a dome-shaped defect with a thin affected layer having $\sim 10\text{-}\mu\text{m}$ thickness on the surface, which was deeply stained by hematoxylin. The affected layer did not inhibit new bone formation, and the bone defect was gradually repaired by new bone. Within 3 months, the defect was filled almost completely with new bone. A discontinuous appearance of the affected layer was occasionally observed, but the layer generally remained after 1 year and was embedded in the bone tissue. The detachment of newly formed bone from the lased surface was an artifact resulting from histologic preparation. 0D = 0 days; 1D = 1 day; 3D = 3 days; 1W = 1 week; 2W = 2 weeks; 1M = 1 month; 3M = 3 months; 1Y = 1 year. (Hematoxylin and eosin; original magnification $\times 100$; bar = $200\text{ }\mu\text{m}$.)

surgery, respectively. The ratio of new bone increased significantly with time after surgery ($P < 0.0006$) and reached a plateau after 3 months. Figure 7C shows the entire affected area at the electrode contact site. The affected area was 4.75 ± 0.90 ; 3.87 ± 1.98 ; 2.91 ± 1.47 ; 3.38 ± 1.13 ; 3.52 ± 2.04 ; and $2.83 \pm 1.65\text{ mm}^2$ ($n=3$ rats) at 0 days; 2 weeks; 1, 3, and 6 months; and 1 year, respectively. Although the mean area tended to decrease with time, there was no statistically significant difference among the observation periods. Figure 7D shows the area of tissue defect, which was 3.93 ± 0.81 ; 1.32 ± 0.42 ; 0.97 ± 0.20 ; 0.33 ± 0.08 ; 0.18 ± 0.06 ; and $0.13 \pm 0.09\text{ mm}^2$ ($n=3$ rats) at 0 days; 2 weeks; 1, 3, and 6 months; and 1 year, respectively. The defect area exhibited a statistically significant decrease with time after surgery ($P < 0.0001$).

DISCUSSION

Periodontal treatments using an Er:YAG laser are increasingly common because of its several advantageous properties for soft and hard tissue surgeries.^{14,18,20,22,26,27} However, these new applications of the Er:YAG laser have led to a need for a thorough and precise understanding of the potential damage that laser irradiation might cause in oral tissues. Particularly in periodontics, the potential risk of this laser to the underlying alveolar bone tissue during periodontal soft tissue applications^{14,19-21} should be precisely understood.

Several studies²⁹⁻³¹ also reported that the application of Er:YAG laser with water irrigation resulted

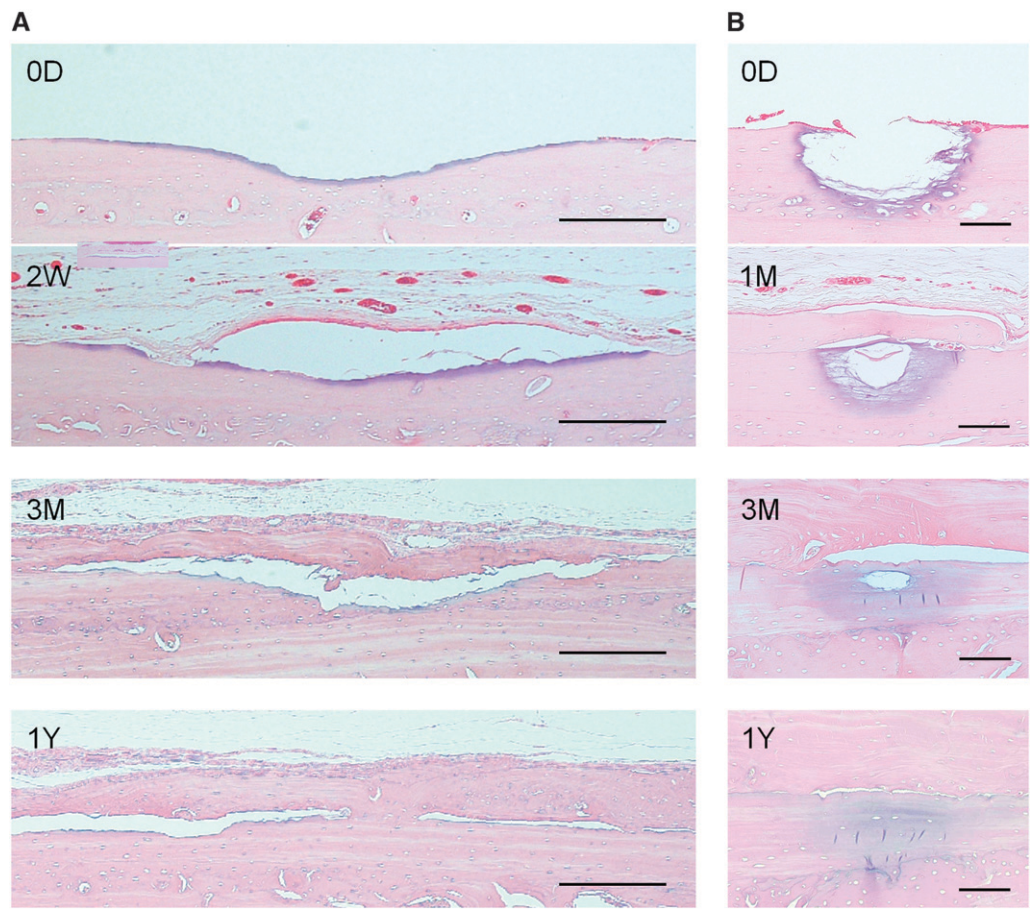


Figure 5. Representative photomicrographs of histologic sections of the non-contact irradiation site (A) and electrode contact site (B). Non-contact irradiation minimally ablated bone tissue and the lased area showed a slightly concave or nearly flat surface with a thin affected layer. A discontinuous appearance of the affected layer was occasionally observed, and the thickness of the stained layer decreased significantly; however, at 1 year after surgery, a large part of the affected layer remained and was embedded in the bone tissue. Electrocauterization produced a large hematoxylin-stained region with a semicircular shape and lacking tissue ablation. The large defect within the affected area was an artifact resulting from the loss of severely damaged bone tissue during the decalcification process of histologic sample preparation. The defect in the stained region tended to become smaller with time. After 1 year, the defect was no longer evident. However, the large stained region was still generally noted, although the area of staining was markedly reduced. 0D = 0 days; 2W = 2 weeks; 1M = 1 month; 3M = 3 months; 1Y = 1 year. (Hematoxylin and eosin; original magnification: A, $\times 40$; B, $\times 100$; bar = 200 μm .)

in excellent outcomes for bone ablation, followed by favorable wound healing. The clinical application of this laser to osseous surgery³² and other osseous procedures treating bone surfaces, such as diseased granulation tissue ablation within a bone defect during periodontal surgery^{14,35-37} or peri-implant surgery,^{38,39} has been investigated. For direct and indirect osseous procedures, water cooling has been advocated as indispensable in minimizing the thermal effects of Er:YAG laser irradiation.^{25,29,40} However, in clinical situations, inadequate water irrigation may occur during irradiation, and irradiation without water cooling may have deleterious effects on bone tissue. Thus, it needs to be clarified whether accidental Er:

YAG laser irradiation without water coolant is hazardous to bone tissue.

In the present study, non-contact irradiation without water coolant produced slight tissue removal with the formation of a thin affected layer without producing severe thermal damage. The contact irradiation caused substantial removal of bone tissue, resulting in a deep bone defect. Despite the increased energy density (fluence) associated with contact focused irradiation, the degree of thermal alteration of the treated bone surface was lower compared to non-contact irradiation, as confirmed by histologic examination. This might have been because larger amounts of weak energy remained on the bone tissue with non-contact irradiation, without being consumed during bone ablation.

With regard to changes on the bone surface, previous histologic examinations^{28-30,41-46} revealed a similar superficial, deeply stained layer on the lased bone surface with a thickness ranging from 4 to 15 μm following Er:YAG laser osteotomy at 5 to 141.5 J/cm²/pulse and 2 or 5 Hz without water cooling. Compared to these studies, the present study showed a comparable thickness of 10 or 15 μm after irradiation in contact or non-contact mode. In studies using almost the same irradiation conditions with water spray cooling, Sasaki et al.³⁰ and Pourzarandian et al.²⁹ reported that after contact irradiation, the thickness of the affected layer was $\sim 20 \mu\text{m}$ in non-decalcified specimens. The lower value of 10 μm observed in the present study may be due to differences in specimen preparation, i.e., decalcified or non-decalcified; however, the present results revealed that

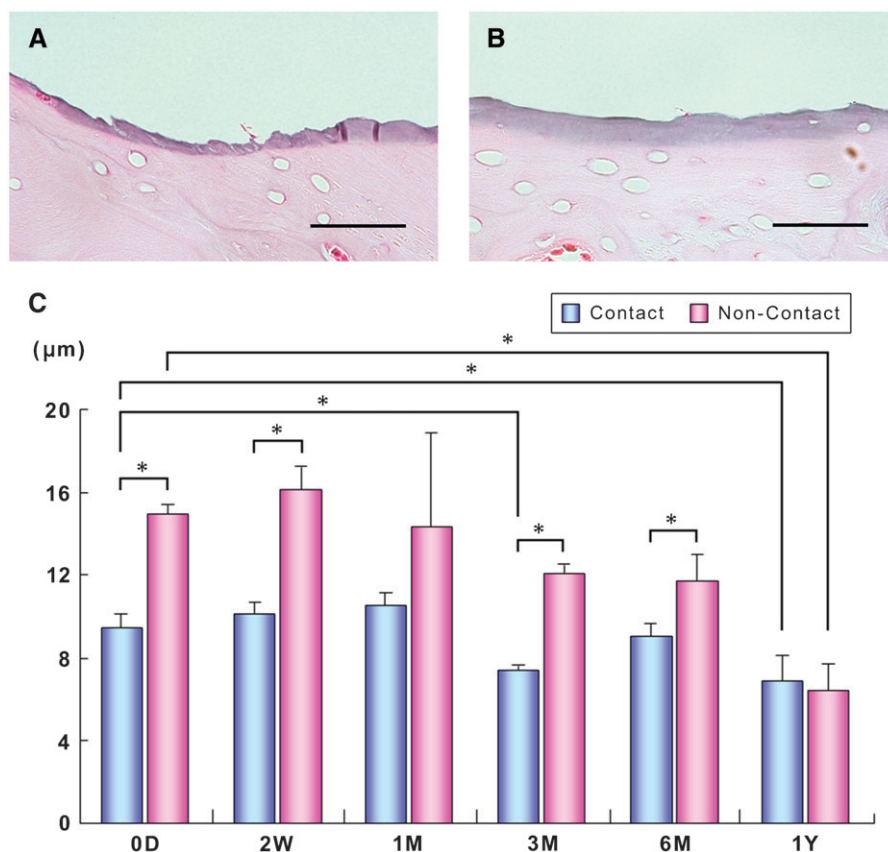


Figure 6.

Comparison of the affected layer on the bone surface following Er:YAG laser contact and non-contact irradiation. Representative photomicrographs of histologic sections of contact (A) and non-contact irradiation (B) sites at high magnification. Less thermal alteration of the treated bone surface was seen following contact irradiation than following non-contact irradiation. The thickness of the affected layer, which was deeply stained with hematoxylin, was $\sim 10 \mu\text{m}$ for the contact irradiation site and $15 \mu\text{m}$ for the non-contact irradiation site. (C) Histometric analysis of the affected layer following Er:YAG laser contact and non-contact irradiation. The affected layer produced by contact irradiation was significantly thinner than that produced by non-contact irradiation at 0 days, 2 weeks, and 3 and 6 months ($P < 0.05$; unpaired t test). Over time, the thickness showed a statistically significant change for each irradiation mode ($P = 0.0002$ for contact and $P = 0.0012$ for non-contact; one-way ANOVA), and the thickness was significantly lower at 1 year compared to 0 days for contact and non-contact irradiation (Dunnett post hoc test). *Statistically significant difference ($P < 0.05$). 0D = 0 days; 2W = 2 weeks; 1M = 1 month; 3M = 3 months; 6M = 6 months; 1Y = 1 year. (Hematoxylin and eosin; original magnification $\times 400$; bar = $100 \mu\text{m}$.)

even with contact focused irradiation with no water cooling, laser irradiation did not produce major thermal changes in the bone. SEM analysis confirmed the minimal thermal effect of Er:YAG laser irradiation without water coolant. No severe damage, such as melting and carbonization, which are common findings with Nd:YAG, CO_2 , and diode lasers,^{5,6,9,47} was observed. Conversely, Schwarz et al.⁴⁸ reported no signs of thermal damage on the bone tissue following Er:YAG laser application for implant bed preparation in an animal study. The difference in thermal damage between that study and the present study might be mainly due to the difference in the use or non-use of water spray

and partly due to differences in the other various experimental conditions, such as the kind of bone tissue (cancellous or cortical bone) and the direction of irradiation (parallel or vertical) to the remaining bone surface. In addition, in that study, the possibility that the loss of a slightly affected layer, which had existed immediately after irradiation, occurred as a result of the insertion of final diameter drills and fixture, should be considered.

The healing of bone defects produced by contact irradiation without water cooling progressed uneventfully, resulting in the repair of almost the entire bone defect by new bone formation. Some studies^{41-46,49,50} examined the healing process in the short-term after osteotomy using the Er:YAG laser. Nelson et al.⁴³ reported that there was a delay in healing after Er:YAG laser osteotomy compared to saw osteotomy, whereas Buchelt et al.⁴⁹ reported that Er:YAG laser osteotomy showed less callus formation than saw osteotomy at 4 weeks. However, Lewandrowski et al.⁴¹ reported that the healing rate following Er:YAG laser irradiation may be equivalent or faster than that following bur drilling. In addition, Pourzarandian et al.²⁹ reported that Er:YAG laser osteotomy using water spray may be advantageous for initial bone healing and that the laser-treated site exhibited faster new bone formation than the bur-treated site. Thus, the evaluation of bone tissue healing after Er:YAG laser irradiation

has not reached a consensus because of differences in experimental design and irradiation conditions. However, the results of the present study showed that Er:YAG laser bone ablation did not affect the ensuing bone-healing process.

One major shortcoming of Er:YAG laser treatment is the production of the thin affected layer on the lased surface. el Montaser et al.⁵⁰ described it as an amorphous, mineral-rich layer surrounding the Er:YAG lased bone defect. Sasaki et al.^{25,30,31} reported that the affected layer after Er:YAG laser irradiation with water coolant was basically non-toxic, although it showed structural alterations and minor compositional

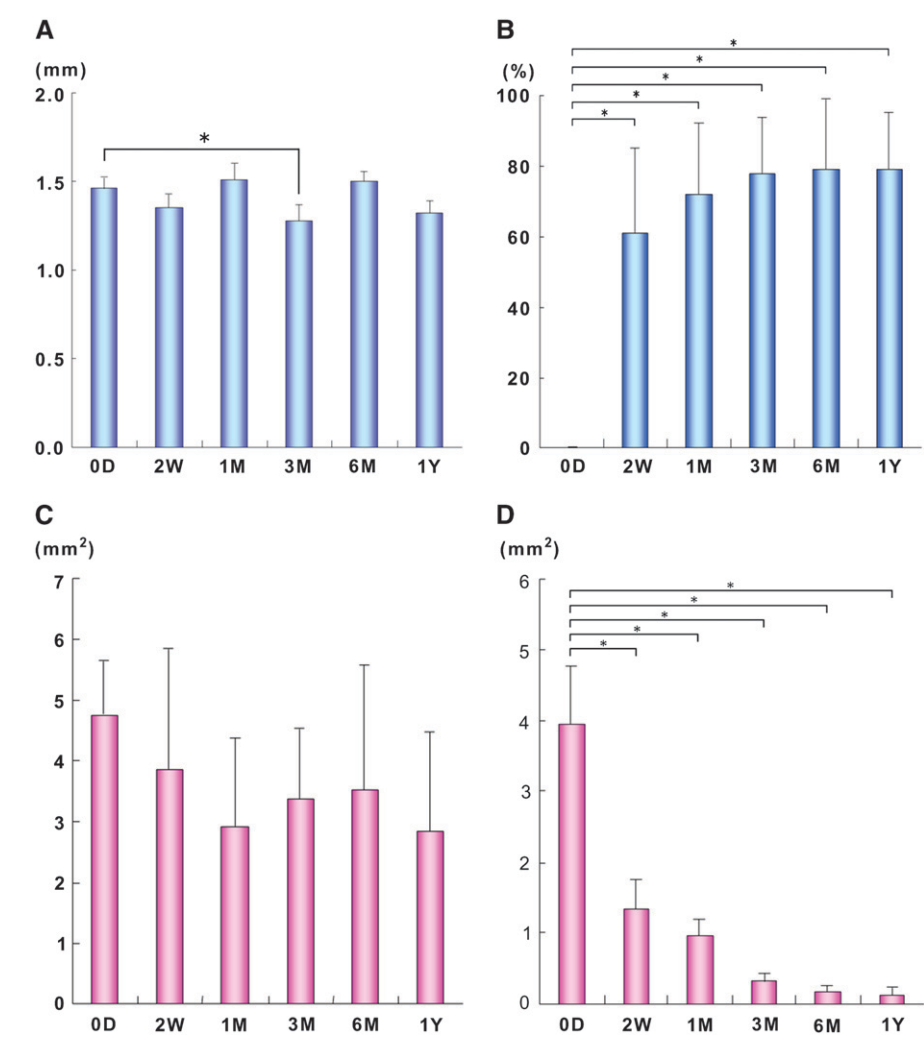


Figure 7. Histometric analyses of changes on the bone surface following laser irradiation and electrode contact. **A)** Width of the affected area at the Er:YAG laser non-contact irradiation site. The width showed no significant decrease at 12 months compared 0 days. **B)** Ratio (% area) of newly formed bone within the ablated defect in the histologic section after Er:YAG laser contact irradiation. The new bone formation increased significantly and reached a plateau after 3 months ($P < 0.0006$; one-way ANOVA). **C)** The entire area of the deeply stained region, including the defect area at the electrocauterization site. The stained area tended to decrease with time, but no statistically significant differences were noted. **D)** Area of the defect within the stained region at the electrode contact site, which is an artifact produced during specimen preparation. The defect area decreased significantly with time ($P < 0.0001$, one-way ANOVA). *Statistically significant difference ($P < 0.05$; Dunnett post hoc test). 0D = 0 days; 2W = 2 weeks; 1M = 1 month; 3M = 3 months; 6M = 6 months; 1Y = 1 year.

The direct deposition of new bone on the lased surface was generally observed, resulting in a favorable repair of the defect by new bone filling. However, detachment of new bone tissue from the affected layer was observed. In addition, after this layer was embedded in the cortical bone tissue, which does not show active bone remodeling, the layer generally remained in the tissue, although the thickness of the stained layer decreased significantly.

Conversely, electrode contact produced a wide range of thermal damage, i.e., electrocauterization, and this region generally was not replaced by new bone. The dark region observed on SEM analysis and the stained region observed on histologic analysis may have been necrotic tissue resulting from the thermal alteration of organic components. On histologic analysis, an oval-shaped defect of varying size was generally observed within the deeply stained region. It is believed that these defects are artifacts produced during the preparation of histologic sections. The decalcification procedure caused the loss of the severely damaged bone structure in which thermal coagulation and structural degradation of organic components occurred. The stained area remained in the bone tissue through the observation period of this study, although the size of the defect and the area of staining in the affected lesion gradually decreased, and no defect was observed in the stained region at 1 year post-surgery. The reduction of the defect area seemed to be partially due to the in vivo recovery

changes with a major loss of organic components and a slight loss of inorganic components. Even in the case of irradiation without water coolant, the production of toxic substances, such as cyanate and cyanamide, was reported to be very low compared to the CO₂ laser.²⁵ Therefore, the affected layer produced by Er:YAG laser irradiation is believed to be harmless with regard to bone healing. In the present study, the affected layer did not inhibit cell migration and proliferation or granulation tissue formation in the bone defect.

of the composition and structural strength of the affected and denatured bone tissue during the long-term observation period.

CONCLUSIONS

In the present study, we used rat cortical bone to investigate the basic effect of the Er:YAG laser. Considering the differences in the structure and metabolism between rat cortical bone and human alveolar bone, the results cannot be directly transferred to

the influence on alveolar bone tissue in clinical situations because alveolar bone is composed of cortical and cancellous bones. The results of this study provide limited, but important, basic knowledge about the effect of the Er:YAG laser without water cooling on cortical bone tissue; however, further studies should be performed using larger animals to clarify the more precise effects on cancellous bone as well.

Our results showed that, unlike electrosurgery, which produces a large area of thermal necrosis of bone without ablation, the thermal alteration of bone tissue produced by Er:YAG laser irradiation without water coolant was minimal, causing virtually no severe thermal damage to the surrounding tissue. Er:YAG laser treatment did not interfere with the ensuing bone-healing process, although the affected layer on the lased surface remained in the cortical bone 1 year after irradiation. Based on these findings relating to the clinical use of the Er:YAG laser without water coolant, accidental irradiation of bone tissue during gingival or osseous surgery would not cause severe problems, such as necrosis of bone tissue, or delay wound healing.

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