Morphological Changes of Bovine Mandibular Bone Irradiated by Er, Cr: YSGG Laser: An in Vitro Study


ABSTRACT

Objective: The purpose of this study was to investigate the morphological changes of bovine mandibular bone following Er, Cr: YSGG laser irradiation in different methods in vitro. Background Data: Recently, an erbium, chromium/yttrium, scandium, garnet (Er, Cr: YSGG) laser device that emits a laser beam at the wavelength of 2.78 μm was introduced. This type of infrared laser proved to ablate dental hard tissues effectively. However, the different effects of bone ablation by this laser in different irradiation methods were still unknown. Materials and Methods: Adult bovine mandibular bones were cut into 24 small pieces, 3–4 cm in length. The parameters of Er, Cr: YSGG laser irradiation were as follows: wavelength was 2.78 μm, pulse duration was 140–200 μsec, repetition rate was 20 pulse/sec, power was 4 W, spot size was $1.26 \times 10^{-3}$ mm$^2$, and energy density was 160 J/cm$^2$. Irradiation methods were different in four groups (six specimens in each group): group A, fixed position and contact mode; group B, fixed position and noncontact mode; group C, nonfixed position and contact mode; and group D, nonfixed position and noncontact mode. Results: Ablation depth in group A was significantly greater than in group B ($p < 0.01$). In group A, thermal damage was apparent. In group B, C, and D, thermal damage was minimal. Conclusion: Er, Cr: YSGG laser allows for precise surgical bone cutting and ablation with minimal thermal damage to adjacent tissue. Irradiation in different methods may achieve different ablation rates and thermal damage.

INTRODUCTION

Bone ablation with laser is an important issue in cavity preparation by lasers in the field of dentistry. Since the initial discovery of the ruby laser on dental hard tissue in the 1960s, continuous studies have been performed on bone ablation or cutting with different lasers, such as CO$_2$, Nd: YAG, and ArF: Excimer lasers. It has been demonstrated that the lasers of infrared wavelength were so strongly absorbed by water and hydroxyapatite that they could ablate bone tissue efficiently. A number of investigations proved the use of the CO$_2$ laser to produce experimental osteotomies in animals. However, results also confirmed a significant delay in the healing of the laser osteotomies compared with conventional mechanical methods. Thermal necrosis or the carbonization generated by the laser adjacent to the irradiated area is thought to be responsible for the delay and the foreign body reactions to charred material could complicate the healing process.

Recently, an erbium, chromium/yttrium, scandium, garnet (Er, Cr: YSGG) laser device that emits a laser beam at the wavelength of 2.78 μm has been made available. This type of infrared laser proved to ablate dental hard tissues effectively. It offers the advantages of straight, clean, and precise cutting by laser energy interaction with water at the interface of tissues. Furthermore, it caused minimal damage around the ablation area in laser stapes surgery. However, there have been few reports investigating the histological and morphological changes of mandibular bone irradiated by Er, Cr: YSGG laser. The different effects of bone ablation by this laser in different irradiation methods were still unknown.

The purpose of the present study was to investigate the morphological changes of bovine mandibular bone following Er, Cr: YSGG laser irradiation in different methods in vitro.
MATERIALS AND METHODS

Sample preparation

Adult bovine mandibular bones were obtained directly from a slaughterhouse and were wrapped in plastic to inhibit desiccation immediately after mortem. They were cut into small pieces of 3-4 cm in length using a low-speed cooled diamond saw and were put into small plastic bags individually. Then they were refrigerated to -70°C. Before used, they were carefully warmed to room temperature.

Laser device and laser irradiation

An Er:Cr:YSGG laser device (Millennium, Biolase, San Clemente, CA) emitting at 2.78 μm, pulsed with a duration of 140-200 μsec and a repetition rate of 20 Hz was employed. The power output can be varied from 0 to 6 W. The delivery system consisted of a fiberoptic tube terminating in a handpiece with a sapphire crystal tip bathed in an adjustable air-water spray. The beam spot size was 1.26 × 10⁻³ mm² (diameter 0.4 mm). Samples were irradiated at 4 W for 60 sec at the fixed position or moving at the speed of 2-3 mm/sec (nonfixed position). According to the relationship between the tip and the sample, there are two modes: contact mode (the sapphire tip lightly touched tissue) and noncontact mode (the sapphire tip was maintained at 1-2 mm from tissue). In both modes, the tip was maintained in a perpendicular position to the irradiated bone surface. Then, all the samples were divided into four groups: group A, fixed position and contact mode; group B, fixed position and noncontact mode; group C, nonfixed position and contact mode; and group D, nonfixed position and noncontact mode. There are six samples in each group. In each sample, the nonlased area served as control. The energy density was 160 J/cm², and water spray was kept at 20 mL/min.

Morphological examination

After laser irradiation, all samples were observed by stereoscope. Then, 12 samples, three for each group, were dehydrated with a graded series of ethanol, dried to a critical point with CO₂, and sputter-coated with platinum for scanning electron microscopy.

Histological examination

Twelve samples, three for each group, were decalcified with Plank and Rychlo solution and embedded in selloidin. The serial histological sections (30 μm) were stained with hematoxylin and eosin and then examined by light microscopy.

RESULTS

Morphological study

For each sample, compared with the control area, a well-defined defect with clear-cut, sharp margins was produced on the irradiated area. For fixed position (groups A and B), holes deep into bone tissues were formed (Fig. 1B). Then the holes were split longitudinally by diamond bur and chisel, so that inner surfaces could be observed and ablation depth could be measured. For nonfixed position (groups C and D), contour of defects depended on the way the sapphire tip moved. In the present study, grooves and rectangular cavities were made.

Macroscopic observation

In groups A and B, the holes made by Er:Cr:YSGG laser were cylinder-like with regular inner surfaces. Depth of the holes was greater in group A (4.43 ± 0.49 mm) than in group B (2.81 ± 0.33 mm; values expressed as mean ± SD). The difference was significant (p < 0.01, statistically analyzed by Student's t test). In group A, carbonization zones (black) were found on the bottom one-third of the hole (Fig. 1A). While in group B, no burning or carbonization (black or brown) was observed.

In groups C and D, the laser produced grooves with sharp edges and flat surfaces, as well as cavities with cratered floors (Fig. 2). In both groups, there was no sign of bone tissue carbonization on the walls and floors of the defects.
Er,Cr:YSGG Laser Irradiation on Bone

FIG. 2. Representative stereoscopic photographs of bovine mandibular bone samples. (A) In group C, rectangular cavity with sharp edge and cratered floor made by laser (original magnification, ×1.5). (B) In group D, groove with sharp edge and flat surface (original magnification, ×2).

SEM observation

Cortical bone irradiated by Er,Cr:YSGG laser showed a mass of prism structure on the flat bone matrix which, as a whole, appeared to have a scaly appearance, almost without debris. Haversian canal and osteocyte lacunae were observed (Fig. 3A). Spongy bone showed a reticulated frame structure (Fig. 3B). In group A, carbonization and melting bone matrix was noted. In groups B and C, melting and recrystallized bone matrix appeared (Fig. 4).

Histological study

On the laser-irradiated surface of each sample, there was a transparent necrotic layer with an irregular margin. Tiny clusters of cells or tissue were noted on the layer. However, histological examination showed there was a difference between group A and groups B, C, and D. In groups B, C, and D, neither carbonization nor a degeneration zone was observed. Osteocytes in adjacent bone tissues were morphologically intact. The thickness of the thermally damaged zone was less than 10 μm. In group A, beneath the necrosis and carbonization, there was an additional lightly stained necrotic layer that could not be clearly separated from normal adjacent tissue. The thickness of the thermally damaged zone was >100 μm (Fig. 5).

DISCUSSION

Some conventional surgical instruments, such as turbine burs, cause thermal necrosis, aerosolized particle release, access, and depth control limitation. The development of laser technique for bone surgery offers an alternative to conventional instruments and methods.14 However, before a type of laser is used to perform clinical bone ablation, careful research on the interaction of bone and laser must be completed.

In the present study, a new type of pulsed mid-infrared laser (Er,Cr:YSGG laser) was used. Among the currently used lasers that emit in the near- and mid-infrared spectral ranges, its absorbability in water is one of the greatest, approximately 10 times greater than that of CO₂ laser, 200 times greater than that of Ho:YSGG laser, and 20,000 times greater than that of Nd:YAG laser. It also shows fairly high absorbability in hydroxyapatite.15,16 There have been reports on successful ablation by Er,Cr:YSGG laser for other hard tissues, such as...
FIG. 4. Scanning electronic microscopy revealed thermal damage to lased bone matrix. (A) In group A, carbonization of bone matrix is visible (arrow). Intact prism structure was broken (original magnification, ×500; bar = 50 μm). (B) In group C, melting and recrystallized bone matrix was noted (original magnification, ×3,500; bar = 5 μm).

FIG. 5. Histological microscopy revealed thermal damage of bone tissue after laser irradiation. (A) In group C, there was a transparent necrotic layer with an irregular margin (T). Tiny cluster of cells was noted in the layer (arrow). Neither carbonization nor degeneration zone was observed. Osteocytes in adjacent bone tissues were morphologically intact. The thickness of thermally damaged zone was less than 10 μm (bar = 50 μm). (B) In group A, beneath carbonization (arrows) and transparent necrotic zone (T), there was an additional lightly stained necrotic layer without cell's appearance (L), which could not be clearly separated from normal adjacent tissue. The thickness of thermally damaged zone was >100 μm (bar = 100 μm; H&E stain).

enamel and dentin. However, unlike enamel or dentin, bone has different composition and structure. It is a biologic material with an inherent nonhomogeneity, consisting of compact (substantia compacta) and spongy (substantia spongiosa) forms. The interstitial substance of bone is composed of two major components, organic matrix and inorganic salts, each comprising about 50% of dry weight. The inorganic portion consists of a form of calcium phosphate that is very similar to the mineral hydroxyapatite. Water molecules are closely associated with the organic and inorganic portions. The specimens used in the present study were taken from adult bovine mandibular bone. Each of the specimens had both types of bone; the compact type forms a thick cortical layer on the surface, and under it there is a layer of spongy bone, which is quite similar to human mandibular bone.

Although laser irradiation was performed in different methods, each lased specimen in the study showed well-defined and clear-cut defects with sharp margins and regular surfaces. Moreover, the contour of the defect could be controlled by the movement of the sapphire tip, so that holes, grooves, and cavities were produced. Thus, the Er,Cr:YSGG laser could give precise bone ablation. Our results also showed that, although the same laser was used at the same parameters, irradiation in different methods could achieve different effects, including different ablation depth and different thermal damage to adjacent tissue. Irradiation in contact mode (group A) resulted in significantly greater ablation depth than in noncontact mode (group B), which meant that ablation in contact mode could have a greater ablation rate. Histological and morphological findings revealed that thermal damage was different among the four groups. In group A, carbonization and a lightly stained necrotic layer without cell's appearance were indicative of apparent thermal damage to adjacent tissue. Thickness of histologically damaged zone in this group was >100 μm. Eriksson and Albrektsson demonstrated that the threshold for bone sur-
Er,Cr:YSGG Laser Irradiation on Bone

vival was 47°C for 1 min, which represents an approximate 10°C increase in temperature and a duration time of 1 min. When the contact mode was used in fixed position, laser energy was concentrated to local bone tissue, causing efficient cutting as well as thermal damage. Besides, contact mode and fixed position prevented sufficient water spraying on laser bone surface to relieve thermal damage to adjacent tissue. This may explain the greater ablation rate as well as more severe thermal side effects in group A. Melted bone matrix was noted in groups C and D by SEM observation, which was not found in group D. However, histological examination could not find marked differences among groups B, C, and D. Thermal damage was minimal among the three groups: osteocytes were morphologically intact in adjacent tissue, and thickness of histologically damaged zone was <10 μm. Those facts suggest that ablation by Er,Cr:YSGG laser has minimal thermal damage if performed carefully.

The mechanism of bone ablation by this laser is not clear. Based on previous studies, two types of mechanisms have been proposed. First, the thermal effect of laser causes vaporization of bone tissue directly. Second, the Er,Cr:YSGG laser is known to have a great absorbability in water (absorption coefficient μ = 7,700 cm⁻¹) due to its wavelength, so absorption of laser irradiation and conversion into thermal energy causes a local concentration of heat, and when the water is heated to boiling point, an explosive vaporization will occur. Results of the present study suggest that both mechanisms may be at work. Carbonization and melting of bone tissue shows direct thermal effect, while the irregular margin of transparent necrotic layer spray according to laser energy density also acts as an important other factor in avoiding severe thermal damage to adjacent tissue.

Based on the present study and previous reports, Er,Cr:YSGG laser is expected to have a clinical use in dentistry, such as periodontal surgery and apical surgery. Yet laser surgery may cause the risk of emphysema by air and water pressure and the risk of infection by bacteria scattering, which restricts its wide application. Future investigation will study the fluctuating temperatures of bone tissue during ablation. In addition, studies on side effects and the healing process in vivo will be important to evaluate the tissue response to bone ablation.

CONCLUSION

The results of our studies in vitro lead us to conclude that Er,Cr:YSGG laser allows for precise surgical bone cutting and ablation with minimal thermal damage to adjacent tissue. Irradiation in different methods may achieve different ablation rates and thermal damage. It is helpful to choose the optimal method according to the tissue to be ablated.

REFERENCES


Address reprint requests to:
Koukichi Matsumoto, D.D.S., Ph.D.
Department of Endodontics
Showa University School of Dentistry
2-1-1 Kitasenzoku, Ohta-ku
Tokyo 145-8515, Japan

E-mail: Koukichi@senzohu.showa-u.ac.jp