

## Bactericidal Effect of Erbium YAG Laser on Periodontopathic Bacteria

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**Background and Objective:** Recently Erbium (Er) YAG laser has been developed for dentistry. It may be suitable for periodontal therapy. This study examined the bactericidal effect of the Er:YAG laser on periodontopathic bacteria *in vitro*.

**Study Design/Materials and Methods:** After spreading the bacterial suspension of *Porphyromonas gingivalis* or *Actinobacillus actinomycetemcomitans* on agar plates, a single pulse laser was applied to the agar plates at the energy density of 0.04–2.6 J/cm<sup>2</sup>. The growth of the bacterial colonies on the lased agar plates was examined after anaerobic culture. *P. gingivalis* colonies were also individually exposed to the single pulse laser at the energy of 1.8–10.6 J/cm<sup>2</sup>. The colony forming units of the irradiated colonies were counted.

**Results:** Growth inhibitory zones were found at the irradiated sites at the energy of about 0.3 J/cm<sup>2</sup> and higher. The survival ratios of the viable bacteria in the lased *P. gingivalis* colonies decreased significantly at the energy of 7.1 and 10.6 J/cm<sup>2</sup>, as compared with that of the control.

**Conclusion:** These findings suggest that the Er:YAG laser has a high bactericidal potential at a low energy level.

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Key words: antiseptic procedure, laser therapy, periodontal treatment

### INTRODUCTION

Since Maiman developed a ruby laser in 1960 [1], various kinds of lasers have been introduced and applied in dentistry [2–7]. Several studies on the application of lasers to the treatment of periodontal disease have been reported concerning with gingivectomy, gingivoplasty, and removal of dental deposits [8–11]. Recently, the application of the Nd:YAG laser to treat periodontal diseases has been increasingly reported, especially to treat periodontally exposed dental root surfaces [12–15]. However, the Nd:YAG laser application has some unsolved effects to the tissues below the irradiated area because it penetrates into the tissue and has a risk to cause heat generation.

Compared to the Nd:YAG laser, the recently developed Erbium YAG (Er:YAG) laser is anticipated to be useful in dental applications, because its wavelength emitting at 2.94  $\mu\text{m}$  is highly ab-

sorbed in water [16–18]. The Er:YAG laser has been studied so far mainly for cavity preparation and the tooth pulp reaction in the dental field [19–29]. There are few reports about the applications of Er:YAG laser to periodontal therapy. In the treatment of periodontal diseases, the debridement of the dental root surfaces including removal of bacterial plaque, subgingival dental calculus, and diseased root cementum is an important procedure. Previously, we found that the Er:YAG laser could remove subgingival calculus from extracted human teeth at a low energy level under water irrigation with less temperature rise and cementum loss [30]. This finding suggested

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that the Er:YAG laser could be applied to the treatment of periodontally exposed dental root surfaces.

If this laser irradiation could not only remove subgingival calculus but also kill periodontopathic bacteria, better healing of the periodontium might be expected by this procedure. In this study, therefore, we examined the bactericidal effect of the Er:YAG laser to periodontopathic bacteria *in vitro*.

## MATERIALS AND METHODS

### Laser Apparatus

The laser used in this study was the prototype Erbium YAG Laser; ML 12 (HOYA Corporation, Tokyo, Japan). The apparatus was composed of a power supply and a laser head. The features of the apparatus were wavelength 2.94  $\mu\text{m}$ , maximum energy 1 J, maximum pulse repetition rate 10 pulses per second (pps), and pulse duration 200  $\mu\text{sec}$ . The spot size diameter of the laser light produced by the laser head was 6.0 mm. He-Ne laser which was provided in the laser apparatus as an indicating light was not used during all experiments.

### Target Microorganisms and Culture Conditions

The microorganisms used in this study were *P. gingivalis* 381 and *A. actinomycetemcomitans* Y4. Periodontitis is classified into several types [31]. There might be specific bacteria that are involved in each type of periodontitis; *P. gingivalis*, which is a black pigmented bacterium, is associated with adult or rapidly progressive periodontitis, while *A. actinomycetemcomitans*, which is a non-black pigmented bacterium, is associated with localized juvenile periodontitis. Both bacteria are considered to be important periodontopathic bacteria.

*P. gingivalis* was cultured in trypticase-soy blood (TS) agar plates using the steel-wool-jar method [32] or in pepton yeast (PY) broth in a rubber stoppered tube at 37°C anaerobically (N<sub>2</sub> 80%, H<sub>2</sub> 10%, and CO<sub>2</sub> 10%). *A. actinomycetemcomitans* was cultured in brain heart infusion (BHI) agar plates at 37°C in a 95% air to carbon dioxide medium. Every experiment was performed under aerobic conditions.

Mitsuoka's diluting solution (30 ml of a 0.78% K<sub>2</sub>PO<sub>4</sub> solution, 30 ml of a 0.47% KH<sub>2</sub>PO<sub>4</sub>, 1.18% NaCl, 1.20% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.12% CaCl<sub>2</sub>, and 1.25% MgSO<sub>4</sub>NH<sub>2</sub>O solution, 0.4 g of L-cystein HCL · H<sub>2</sub>O, 0.4 g of ascorbic acid, and 740 ml of

distilled water, equilibrated with 100% CO<sub>2</sub>) was used to dilute a bacterial suspension [33,34].

### Experiment 1: Bactericidal Effect of the Er:YAG Laser Irradiation on *P. gingivalis* and *A. actinomycetemcomitans*

**Detection of the bactericidal effect of the Er:YAG laser (Exp. 1-1).** The purpose of this experiment was to examine the bactericidal effect of the Er:YAG laser when the laser was applied directly to *P. gingivalis* and *A. actinomycetemcomitans* on agar plates.

After 5 days of anaerobic culture, one *P. gingivalis* colony with a diameter of about 0.5 mm was harvested and diluted with 10 ml of PY broth. A bacterial suspension was prepared by subculturing the dilution for 3 days. The suspension (0.1 ml) was spread on the surface of a new TS agar plate. The surface of the agar plate was immediately exposed to a single pulse laser with a 6.0 mm spot size. Energy levels of 0.04, 0.07, 0.1, 0.3, 0.4, 0.7, 1.4, and 2.6 J/cm<sup>2</sup>/pulse were used. Single pulse laser irradiation was performed in duplicate for each energy level on the same plate. After anaerobic incubation for 5 days, the irradiated agar plate was visually estimated to determine whether growth inhibitory zones had been formed or not. This experiment was carried out in triplicate (Fig. 1).

The same experiment was performed on *A. actinomycetemcomitans* using BHI agar plates and the irradiated agar plates were incubated for 7 days.

**Detection of the effect of irradiated agar plate on the bacterial growth (Exp. 1-2).** The purpose of this experiment was to examine whether the Er:YAG laser irradiation caused degeneration of the agar medium and thereby affected the bacterial growth.

Two diagonal quarters of a new TS agar plate were irradiated uniformly at the energy of 1.4 and 2.6 J/cm<sup>2</sup>, making each 6.0 mm diameter spot of the pulsed laser overlap slightly. Then, the same *P. gingivalis* suspension as that used in experiment 1-1 was diluted 10<sup>4</sup>, 10<sup>5</sup>, and 10<sup>6</sup> times using Mitsuoka's diluting solution. Each dilution (0.1 ml) was spread on the surface of the previously irradiated TS agar plate uniformly and incubated for 5 days. Non-irradiated sites on the same plate were employed as controls. After anaerobic culture, the growth of the colonies on each quarter of the agar plate was visually estimated. This experiment was carried out in triplicate (Fig. 2).

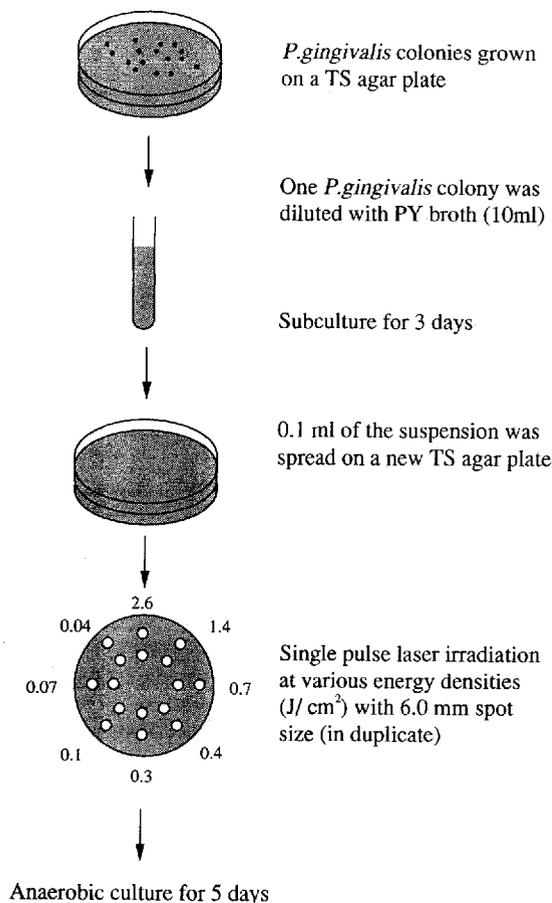


Fig. 1. Illustration of the experimental design of Exp. 1-1; detection of the bactericidal effect of the Er:YAG laser.

**Detection of the surviving bacteria in the splash produced by colony ablation following the Er:YAG laser irradiation (Exp. 1-3).** The purpose of this experiment was to detect whether the splash produced by laser irradiation to a bacterial colony still contained viable bacteria.

After 7 days of anaerobic culture of *P. gingivalis* on TS agar plates, three colonies with a diameter of 2.0 mm were selected. A sterilized spacer 1.5 mm in height was put around each colony, and a sterilized sapphire glass 20.0 mm in diameter and 2.0 mm in thickness, permitting 95% transmission of the Er:YAG laser light, was placed over the colony in contact with the spacer. Each *P. gingivalis* colony was exposed to the single pulse laser at the energy of  $2.6 J/cm^2/pulse$  through the sapphire glass. After the laser irradiation, the lower side of the glass was immediately attached closely on a new agar plate for 5 minutes so that the splash produced by the colony

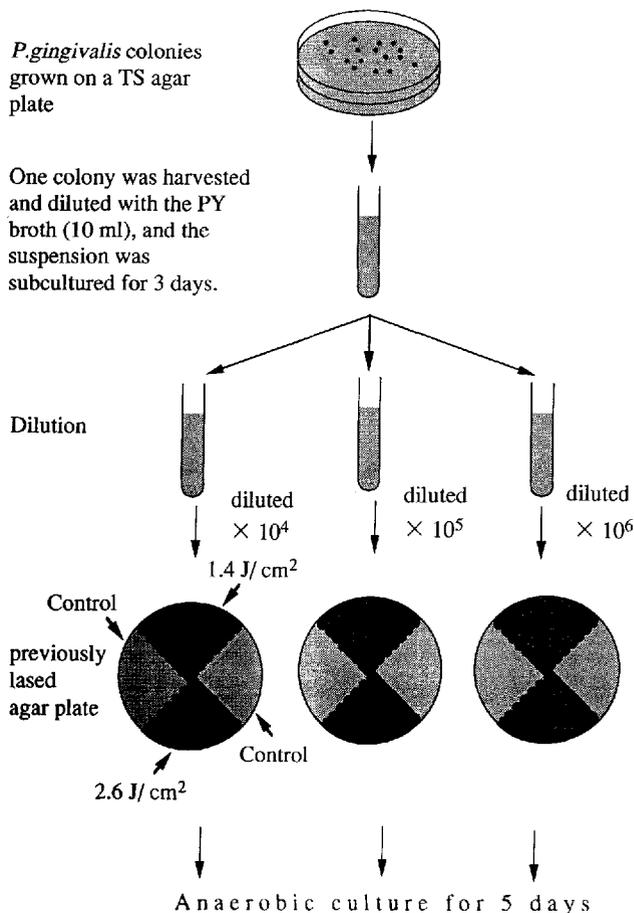


Fig. 2. Illustration of the experimental design of Exp. 1-2; detection of the effect of irradiated agar plate on the bacterial growth.

ablation on the glass was transferred to the agar medium. After removal of the sapphire glass from the agar plates, the plates were cultured for 5 days anaerobically, and the growth of the colonies was observed. As a positive control, the same culture was done using the sapphire glass that was brought in contact with a viable colony (Fig. 3).

### Experiment 2: Degree of the Bactericidal Effect of the Er:YAG laser on a *P. gingivalis* Colony

The degree of the bactericidal effect of the Er:YAG laser on a *P. gingivalis* colony was examined.

Following 5 days of anaerobic culture of the bacteria, 15 colonies with a diameter of 0.8 mm were selected. Twelve *P. gingivalis* colonies were exposed to a single pulse laser at a 2.0 mm spot size. Energy densities of 1.8, 3.5, 7.1, and  $10.6 J/cm^2$  pulse were used and three colonies were assigned for each energy. Three non-irradiated

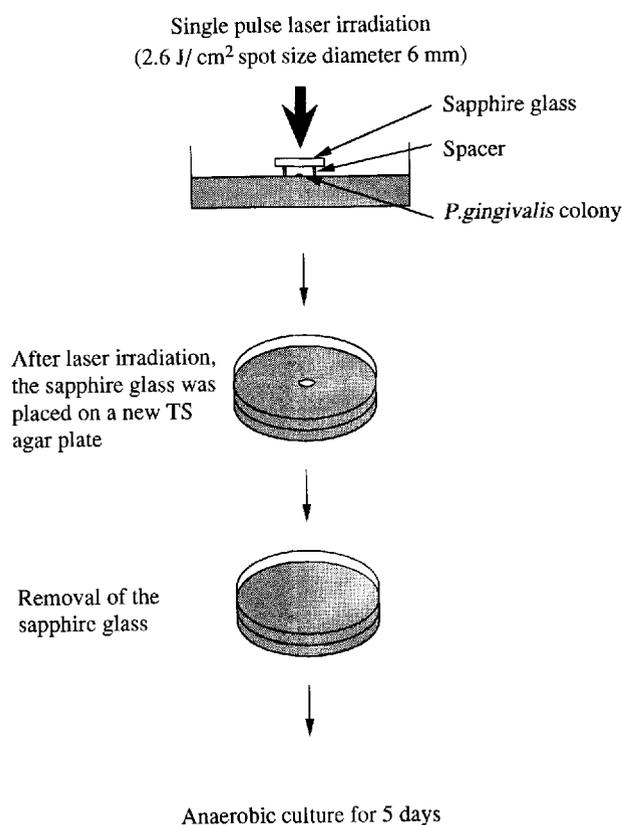


Fig. 3. Illustration of experimental design of Exp. 1-3; detection of the surviving bacteria in the splash produced by colony ablation following the Er:YAG laser irradiation.

colonies were used as controls. Each irradiated and control colony was harvested and put into 10 ml of Mitsuoka's diluting solution containing sterilized glass beads to provide a homogenous suspension. After vortexing for 30 seconds, the medium was diluted  $10^2$  times, and 0.05 ml of the dilution was plated on a new TS agar plate. After anaerobic incubation for 5 days, the colony forming unit (CFU) of the irradiated colony for each energy density was determined by counting the number of the colonies grown on the agar plate. The CFU data for three colonies for each energy density were averaged. Each experiment was repeated five times and the mean CFU was determined. The survival ratio of the viable bacteria in the irradiated colony for each energy density was calculated by dividing each CFU for each energy density by the mean CFU for non-lased control colony.

For statistical analysis, Mann-Whitney U test was used to detect the significant difference between the CFUs at each energy density and the

control, and between the survival ratios for each energy density and the control.

## RESULTS

### Experiment 1: Bactericidal Effect of the Er:YAG Laser Irradiation on *P. gingivalis* and *A. actinomycetemcomitans*

**Detection of the bactericidal effect of the Er:YAG laser.** When the single pulse laser was applied to the agar plate at the energy density of 0.04, 0.07, 0.1, 0.3, 0.7, 1.4, and 2.6 J/cm<sup>2</sup>, slight ablation marks were observed at the sites irradiated at 0.3 J/cm<sup>2</sup> and higher on the surface of the agar plates. After anaerobic culture, growth inhibitory zones were found on the agar plates in close coincidence with the range of the single pulse laser irradiation at the energy density of about 0.3 J/cm<sup>2</sup> and higher for both *P. gingivalis* and *A. actinomycetemcomitans* (Fig. 4).

**Detection of the effect of irradiated agar plate on the bacterial growth.** No obvious difference was detected among the bacterial growth on the areas previously irradiated at the energy density of 1.4 and 2.6 J/cm<sup>2</sup> and the non-irradiated control areas (Fig. 5). These findings indicated that the irradiated agar medium did not affect the bacterial growth.

**Detection of the surviving bacteria in the splash produced by colony ablation following the Er:YAG laser irradiation.** Part of the *P. gingivalis* colony was ablated by the single pulse laser irradiation at the energy density of 2.6 J/cm<sup>2</sup>. The splash produced by colony ablation was clearly attached to the sapphire glass over the irradiated colony (Fig. 6). After anaerobic culture of the new agar plate to which the splash was transferred, no colony formation was observed, while colony formation was clearly observed in the positive control. These findings indicated that the splash produced by the laser irradiation to a colony did not contain any surviving bacteria.

### Experiment 2: Degree of the Bactericidal Effect of the Er:YAG Laser on a *P. gingivalis* Colony

Each *P. gingivalis* colony with a diameter of about 0.8 mm was exposed to a single pulse laser at the energy density of 1.8, 3.5, 7.1, or 10.6 J/cm<sup>2</sup>. At the energy density of 1.8 and 3.5 J/cm<sup>2</sup>, only the surfaces of the colonies were ablated and the color of the colonies changed to white, while colony ablation occurred clearly from the inside at the energy density of 7.1 and 10.6 J/cm<sup>2</sup> (Fig. 7). Table 1 shows the results of the CFUs of the ir-

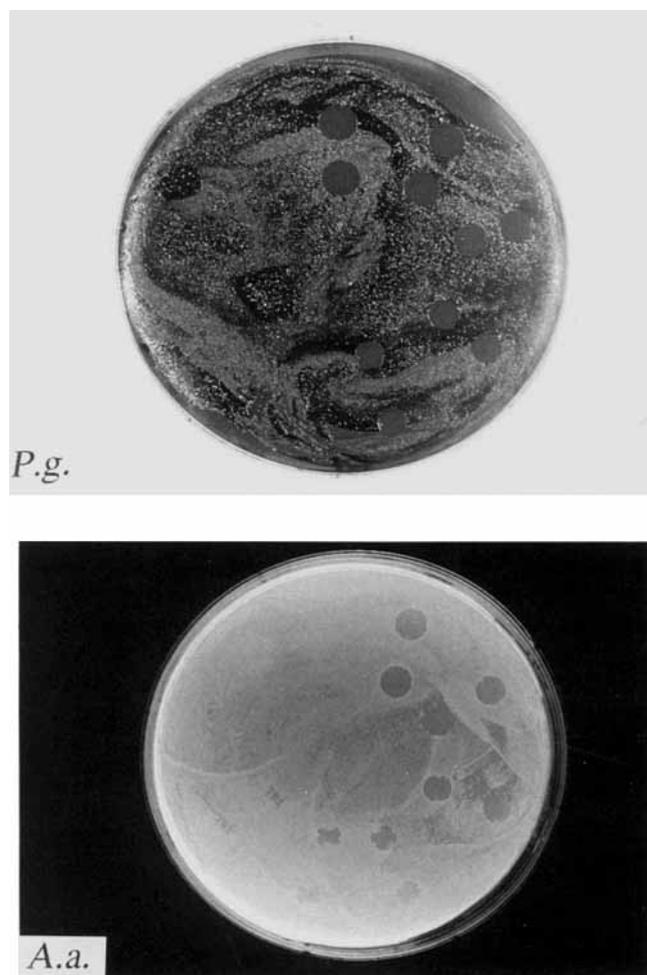


Fig. 4. The result of the Exp. 1-1: detection of the bactericidal effect of the Er:YAG laser. A single pulse laser in duplicate at a different energy was applied to *P. gingivalis* (upper), or *A. actinomycetemcomitans* (lower) on agar plates. The agar plates were cultured for five days (*P. gingivalis*) or seven days (*A. actinomycetemcomitans*). Clockwise starting from the top of the plates, the energy is 2.6, 1.4, 0.7, 0.4, 0.3, 0.07, and 0.04 J/cm<sup>2</sup>. With both *P. gingivalis* and *A. actinomycetemcomitans*, growth inhibitory zones were found at the energy of about 0.3 J/cm<sup>2</sup> and higher.

irradiated colonies. The mean CFU for the non-irradiated control colony was 28.9, from which the CFU of the original colony with a 0.8 mm diameter was calculated  $5.8 \times 10^5$  CFU/colony. The survival ratio of the viable bacteria in the lased colony was  $100.0 \pm 14.7$ ,  $125.4 \pm 30.5$ ,  $91.3 \pm 35.1$ ,  $31.9 \pm 18.2$ , and  $17.2 \pm 5.8\%$  (mean  $\pm$  S.E.,  $n = 5$ ) at the energy density of 0, 1.8, 3.5, 7.1, and 10.6 J/cm<sup>2</sup>, respectively. The CFU and the survival ratio of the irradiated *P. gingivalis* colonies were significantly reduced at the energy density of 7.1 and 10.6 J/cm<sup>2</sup>, compared with the control ( $P < 0.05$ ; Table 1, Fig. 8).

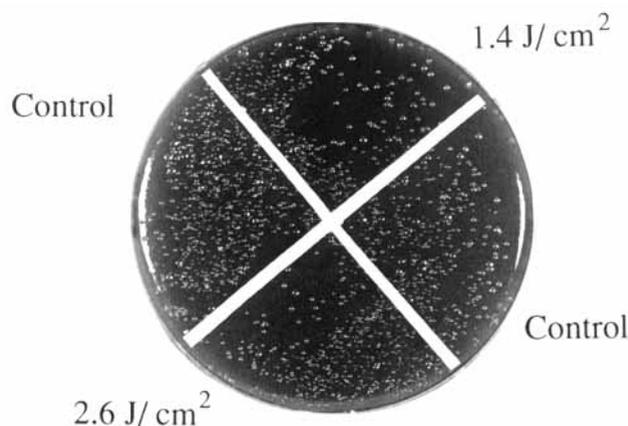


Fig. 5. A representative result of Exp. 1-2; detection of the effect of irradiated agar plate on the bacterial growth. No obvious differences were observed among the number of the bacterial colonies grown on the previously irradiated areas at the energy density of 1.4 and 2.6 J/cm<sup>2</sup> and the non-irradiated areas on the same agar plate. (The agar plate culturing the 10<sup>4</sup> times diluted bacterial suspension.)

Also, as another finding, the colonies produced by lased bacteria were smaller than those of non-lased bacteria.

## DISCUSSION

Lasers are considered to have several possible applications to periodontal treatment such as sterilization effect on bacteria in the periodontal pockets, debridement effect by the ablation of dental deposits, and root conditioning effect by the ablation of the diseased root surface. Moreover, if laser light is delivered by a flexible fiber, the laser might be able to reach the sites where conventional, mechanical instrumentation cannot access. Several researchers have reported studies on laser applications to periodontal therapy using the Nd:YAG laser. Cobb et al. reported that the Nd:YAG laser irradiation showed the potential for suppression or eradication of the subgingival microflora of the patients with generalized adult periodontitis [12]. Ito et al. reported that the Nd:YAG laser radiation effectively removed the smear layer, uncovered dentinal tubules, and exposed collagen fibers on the root surface [13]. However, Morlock et al. suggested that the use of the Nd:YAG laser during root preparation would result in physical changes of the root surface even at relatively low energy levels [14]. Trylovich et al. also indicated that the Nd:YAG laser altered the biocompatibility of the cementum surface to make it unfavorable for fibroblast

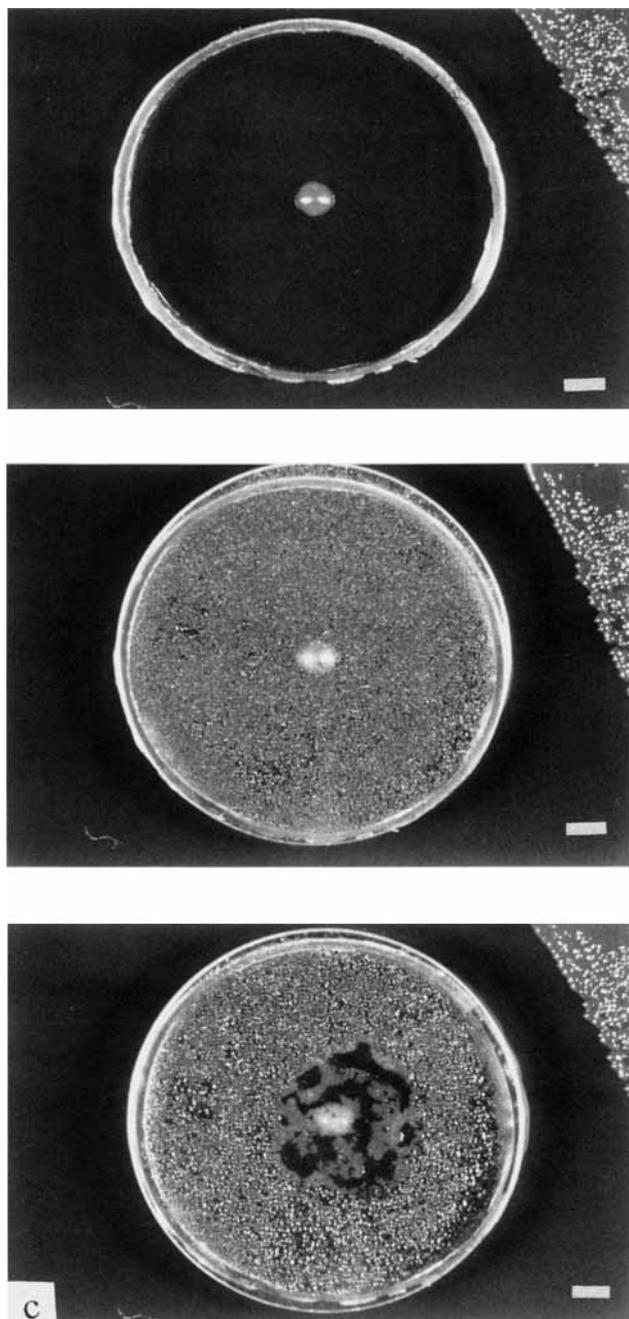


Fig. 6. The photographs show the procedure of Exp. 1-3; detection of the surviving bacteria in the splash produced by colony ablation after the Er:YAG Laser irradiation. a: A sterilized spacer was put around the colony. b: A sterilized sapphire glass was put over the colony in contacted with the spacer. (Drops of water on the glass were formed by water evaporation of the agar.) c: The *P. gingivalis* colony was exposed to the single pulse laser at the energy of  $2.6 \text{ J/cm}^2/\text{pulse}$  through the sapphire glass. The splash produced by colony ablation was attached to the lower side of the sapphire glass. Bar: 2 mm.

attachment [35]. In addition, Tseng and Liew showed that scaling with the Nd:YAG laser did not remove a significant amount of the subgingival dental calculus in vitro [36]. Thus, in spite of some merits, the use of the Nd:YAG laser for root surface preparation still seems not to have great promise clinically due to its thermal side effect.

On the other hand, the Er:YAG laser has been reported to have a high potential of ablating hard tissues such as tooth or bone with less heat effect because of its high absorption in both water and hydroxyapatite. The applications of the Er:YAG laser to the removal of dental caries, cavity preparation, and tooth pulp reaction have been studied. However, there are few reports about periodontal therapy with the Er:YAG laser. Our previous in vitro study indicated that the Er:YAG laser could remove subgingival calculus on the root surface at the energy density of about  $10.6 \text{ J/cm}^2/\text{pulse}$  and 10 pps under water irrigation with the probe tip in vertical contact with the calculus [30]. Also, Er:YAG laser would have a high potential for soft tissue and osseous surgery in the periodontal treatment. If this laser irradiation is accompanied by a sufficient bactericidal effect, it would help to improve the healing of the periodontium.

Since the Er:YAG laser is a high power laser, it would be expected to have bactericidal effect at a high energy level in the same way as Nd:YAG and  $\text{CO}_2$  lasers. However, a characteristic of the Er:YAG laser with the higher absorbability in water is that the threshold of the bactericidal effect of the laser would be much lower than those of other high power lasers. In this in vitro study, the bactericidal effect of the Er:YAG laser to periodontopathic bacteria was examined at a low energy level.

The present study showed that the Er:YAG laser had a bactericidal effect on *P. gingivalis* and *A. actinomycetemcomitans* at the same energy. The minimum lethal power was at the energy density of  $0.3\text{--}0.4 \text{ J/cm}^2$ . These findings were obtained from the appearance of the growth inhibitory zones after direct laser irradiation to *P. gingivalis* and *A. actinomycetemcomitans* on the agar plates (the colony inhibition assay of experiment 1-1). However, other possibilities were also considered to be associated with the formation of growth inhibitory zones; the laser irradiation might degenerate the agar medium resulting in the inhibition of bacterial growth or it might scatter viable bacteria in the irradiated area by causing the explosive ablation.

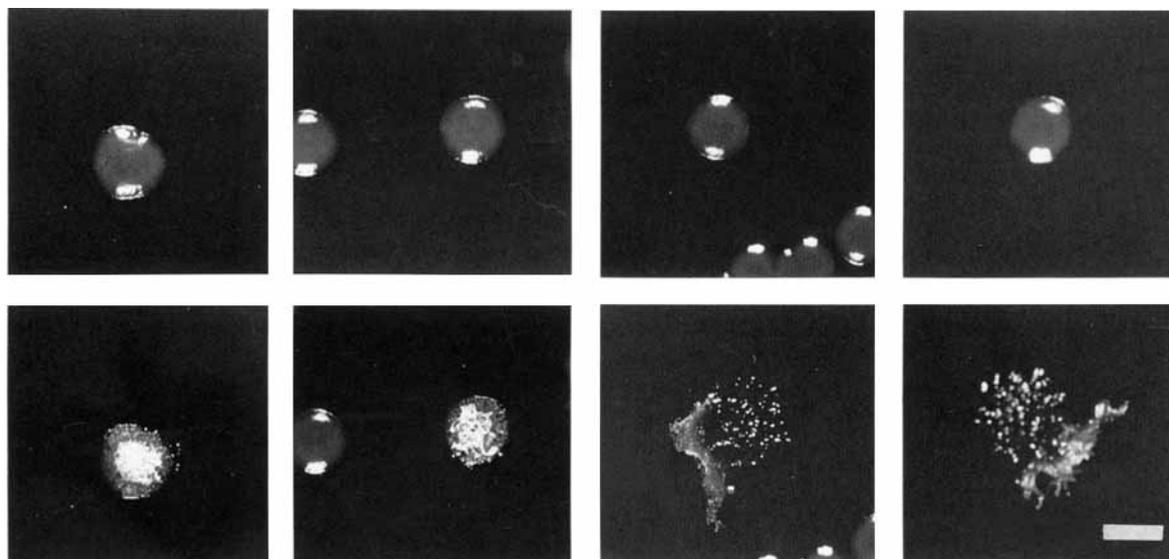


Fig. 7. Photographs before (upper row) and after (lower row) single pulse laser irradiation to one *P. gingivalis* colony (Exp. 2). The energy was 1.8, 3.5, 7.1, and 10.6 J/cm<sup>2</sup> from left. At 1.8 and 3.5 J/cm<sup>2</sup>, only the surface of the colonies ablated and colour of the colonies changed to white. At 7.1 and 10.6 J/cm<sup>2</sup> colony ablation occurred from the inside. Bar: 1 mm.

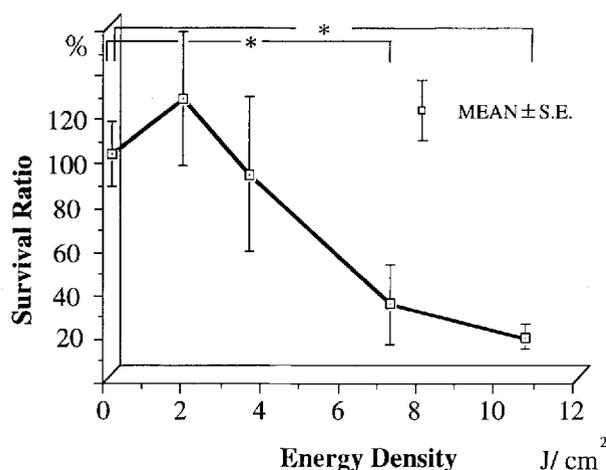


Fig. 8. Survival ratio of *P. gingivalis*. (Exp. 2). Comparison of the survival ratio of the colonies lasered at different energy level. The survival ratio of the lasered *P. gingivalis* colony was significantly reduced at 7.1 and 10.6 J/cm<sup>2</sup> compared with the control (\**P* < 0.05).

Therefore, experiments were conducted to eliminate the other possible causes for the formation of the growth inhibitory zones. Experiment 1-2 indicated that there was no obvious difference between the bacterial growth on the previously irradiated and the non-irradiated areas on the agar plate. Experiment 1-3 showed that the splash produced by pulsed laser irradiation to a bacterial colony contained no viable bacteria.

These findings suggest that the growth inhibitory zone is neither due to the effect of the irradiated agar medium to the bacterial growth nor to the scattering of viable bacteria from the irradiated area by the pulsed laser irradiation. These findings indicated that the growth inhibitory zones could be explained as a result of the bactericidal effect of the Er:YAG laser.

In periodontitis, the causative bacteria are mainly present in the form of a biofilm, that is dental plaque. Experiment 2 was performed to examine the degree of the Er:YAG laser's killing effect on a bacterial colony supposing the dental plaque, at the energy density of 10.6 J/cm<sup>2</sup>/pulse which is suitable for laser scaling. The CFU of the irradiated *P. gingivalis* colony was significantly reduced at the energy density of 7.1 and 10.6 J/cm<sup>2</sup>. The survival ratio of the viable bacteria in the irradiated *P. gingivalis* colony decreased to 17.2% at the energy density of 10.6 J/cm<sup>2</sup>. These findings indicate that the laser light can penetrate into and ablate the bacterial colony at the energy density of 7.1 J/cm<sup>2</sup> and higher. These also suggest that the Er:YAG laser would have enough bactericidal effect on the dental plaque at the energy level suitable for laser scaling.

There are some reports about the laser's bactericidal effect. Zakariassen et al. reported that the CO<sub>2</sub> laser could kill *Streptococcus sanguis*, *Streptococcus mutans*, or *Actinomyces viscosus* on glass

TABLE 1. CFUs of the Colonies Exposed to the Single Pulse Laser at Different Energy Levels (Bacterial Suspension: 100 Times Dilution)

Experiment	Energy density (J/cm <sup>2</sup> /pulse)									
	0		1.8		3.5		7.1		10.6	
	CFU	Ave.	CFU	Ave.	CFU	Ave.	CFU	Ave.	CFU	Ave.
1st	44		75		77		9		5	
	15	33.0	30	63.3	78	68.7	3	7.3	0	3.7
	40		85		51		10		6	
2nd	14		70		36		37		11	
	37	29.7	21	41.0	25	31.3	47	32.3	15	10.3
	38		32		33		13		5	
3rd	15		3		6		1		0	
	10	11.0	3	2.7	8	6.7	6	4.7	0	0.0
	8		2		6		7		0	
4th	55		47		10		1		3	
	30	31.7	10	31.0	5	14.3	2	1.0	2	2.7
	10		36		28		0		3	
5th	63		60		7		2		14	
	27	39.3	30	43.3	6	11.0	1	1.0	2	8.3
	28		40		20		0		9	
Mean		28.9		36.3		26.4		9.3		5.0
±S.E. <sup>a</sup>		±4.3		±8.8		±10.2		±5.3		±1.7
Statistic <sup>b</sup>					*			*		

<sup>a</sup>S.E., Standard error.

<sup>b</sup>Mann-Whitney Test was applied.

\* $P < 0.05$ .

slides at the energy density of 6–19 kJ/cm<sup>2</sup> [37]. Dedrich et al. performed CO<sub>2</sub> laser irradiation to a 1 µl droplet of bacterial suspension ( $9.8 \times 10^7$ – $1.3 \times 10^9$  cells/ml) 1–1.5 mm in diameter on a microscope coverslip. They reported that the energy density required to kill more than 99.5% of the bacteria was less than 200 J/cm<sup>2</sup> [38]. Schults et al. performed Nd:YAG laser irradiation to a 0.5 ml bacterial suspension ( $1.0 \times 10^8$  cells/ml) in the wells [39]. They reported that *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* exhibited decreased viability when exposed to energy density greater than 1667 J/cm<sup>2</sup> during a 10 second exposure. Cobb et al. studied the effects of Nd:YAG laser to periodontal pockets of adult periodontitis patients. They reported that microbial sampling of the irradiated pocket treated at energy settings of 1.75 W at 20 pps for 1 minute (energy level: 87.5 mJ/pulse, total delivered energy: 105 J) showed persistent low levels of *P. gingivalis* or *Prevotella intermedia*, compared with that before treatments [12]. Keates reported

that an argon fluoride excimer laser destroyed the viability of a single *Staphylococcus aureus* colony at the average power density of 315 mJ/cm<sup>2</sup>/pulse at 10 pps and the average irradiation time of 64 seconds (average power density: 202 J/cm<sup>2</sup>) [40].

It is difficult to compare the results of our study on the bactericidal effect with other high power lasers, because the experimental methods and irradiation conditions are quite different among the papers. That is, the differences in the spot diameter, exposure time and area, and operating mode in a continuous or a pulse, and the condition of the target organism in a bacterial colony or a suspension make it impossible to compare the lethal effect of the laser by only their total delivered energy. Comparison by total energy density is considered to be more preferable than that by total energy, even though it still may be inaccurate. Some studies are lacking in the description of energy density and minute irradiation conditions for calculating energy density. However, comparing roughly the results of our study with

those of other hard lasers described above, the Er:YAG laser is considered to exert a bactericidal effect at a much lower energy density.

Moreover, Powell and Whisenant reported that the argon laser was able to sterilize microorganisms on the dental instruments at the lowest energy level among argon, CO<sub>2</sub>, and Nd:YAG lasers [41]. As for the bactericidal effect of argon laser, Henry et al., using the colony inhibition assay which was similar and comparable to our experiment 1-1, reported that the argon laser was selectively toxic to black-pigmented bacteria, particularly *P. intermedia* and *P. gingivalis*, at 70 J/cm<sup>2</sup> under aerobic conditions [42]. These reports also support the killing effect of the Er:YAG laser at a lower energy level.

The mechanisms of bactericidal effects by lasers depend on their wavelength. In the case of He-Ne laser, bacteria can be killed by the use of the laser in combination with appropriate photosensitizing compounds. The photosensitizers absorb the He-Ne laser and cause a photochemically mediated bactericidal effect [43,44]. Using an excimer laser, Keates found that the most probable mechanism for the death of all the microorganisms was the breakage of molecular and chemical bounds [40]. The selective toxicity of the argon laser to the black-pigmented periodontopathic species would be due to the endogenous photosensitizers such as protohaemin in *P. gingivalis* and protoperiphrin in *P. intermedia* which absorb highly the light at this wavelength [42]. The mechanism of the lethal effect of a high power laser such as Nd:YAG and CO<sub>2</sub> are assumed to be the destruction of bacterial cells by a thermal effect produced from high energy irradiation. As for the CO<sub>2</sub> laser, the emission wavelength 10.6 μm, is highly absorbed by water. The energy is converted to heat when it is absorbed by the water molecules in the tissue. On the other hand, the Nd:YAG laser, which is not absorbed well in water, penetrates and scatters in the tissue resulting in heat accumulation within the target.

The wavelength of the Er:YAG laser emitting at 2.94 μm is more highly absorbed in water [16–18]. The absorption rate of Er:YAG laser in water is theoretically ten times higher than that of the CO<sub>2</sub> laser, and about 20,000 times higher than that of Nd:YAG laser [45,46]. As a mechanism of hard tissue ablation by the Er:YAG laser, the "micro-explosion" effect is supposed [20,23,44,47–49]. The Er:YAG laser light is mainly absorbed by water in the hard tissues and evaporates the water molecule. In consequence, evaporation of water

raises the inner pressure and causes micro-explosion that destroys the hard tissue. In the same way, when a bacterial cell containing much water is exposed to the Er:YAG laser, the light is mainly absorbed by water in the cell and water evaporation causes cell destruction resulting in the cell death. As another mechanism, the temperature rises momentarily extremely high on irradiation and the heat effect may also kill the bacteria at the same time.

In this study, the irradiated bacteria produced smaller colonies than the non-irradiated bacteria. Schultz et al. obtained the same findings using the Nd:YAG laser [39]. As Dworkin suggested, these findings might indicate that the surviving bacteria after exposure to laser irradiation have undergone sublethal damage [50]. The exact mechanism of cell damage due to laser irradiation is unknown at present. More evidence about the cell death and damage mechanism with the Er:YAG laser is required.

In conclusion, the present study indicated that the Er:YAG laser had a bactericidal effect at a low energy and was capable of killing both black-pigmented and non-black-pigmented bacteria at the same energy. Antiseptic procedure would be expected with the use of the Er:YAG laser.

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