Lasers Med Sci (2011) 26:193–204
DOI 10.1007/s10103-010-0763-3

ORIGINAL ARTICLE

In vitro studies of the ablation mechanism of periodontopathic bacteria and decontamination effect on periodontally diseased root surfaces by erbium:yttrium–aluminum–garnet laser

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Received: 20 September 2009 / Accepted: 21 January 2010 / Published online: 23 March 2010

Abstract The erbium:yttrium–aluminum–garnet (Er:YAG) laser is now increasingly used in periodontal therapy. The purpose of this study was to investigate the effect of Er:YAG laser irradiation on the morphology of periodontopathic bacteria and to compare the bacterial elimination effect of the laser and the ultrasonic scaler on diseased root surfaces in vitro. Colonies of *Porphyromonas gingivalis* were exposed to a single-pulse Er:YAG laser at 40 mJ and were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Also, 20 pairs of periodontally diseased root surfaces with subgingival calculi of freshly extracted teeth were treated by Er:YAG laser scaling at 40 mJ/pulse (14.2 J/cm^2 per pulse) and 10 Hz with water spray or ultrasonic scaling, or were not treated. The efficiency of each treatment was determined as the area treated per second, and the treated surfaces were examined by SEM. The material scraped from the treated root surfaces was cultured in aerobic and anaerobic conditions, and the numbers of colony forming units (CFUs) were compared. SEM and TEM showed that the Er:YAG laser had easily ablated the bacterial colony, leaving an ablation spot with a crater and the surrounding affected area showing melted branch-like structures. The laser irradiation was as equally effective and efficient as the ultrasonic scaler in performing root surface debridement. The CFUs after laser treatment were significantly fewer than those after ultrasonic scaling in aerobic and anaerobic culture conditions. Er:YAG laser ablates periodontopathic bacteria with thermal vaporization, and its bacterial elimination effect on the diseased root surfaces appears to be superior to that of the ultrasonic scaler.

Keywords Bactericidal effect · Er:YAG laser · Scaling · *Porphyromonas gingivalis* · Tooth root

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Introduction

Periodontitis is a chronic inflammatory disease caused by bacterial infection. It is predominantly associated with colonization of Gram-negative anaerobic microorganisms. In particular, *Porphyromonas gingivalis* is known to be one of the etiological agents of periodontitis in adults

[1]. Periodontal treatment involves the removal of calculus, together with bacterial biofilms, from the diseased root surface. Conventionally, hand curettes and power-driven devices such as ultrasonic scalers are used for mechanical root surface debridement. Although they have been proven to be effective therapeutic methods, they are laborious and technique-dependent, and the bacterial elimination is incomplete [2, 3]. Also, the access of conventional mechanical instruments to narrow spaces is limited. Therefore, numerous antibacterial agents and/or antibiotics have been used to further suppress the periodontal pathogens [4]. However, frequent application of such chemical agents has led to the development of resistant bacterial strains [5]. Therefore, attempts have been made to find other novel therapeutic adjuncts or alternatives to supplement the conventional mechanical methods.

The use of light in the medical and dental fields has been reported for many years. From ultraviolet radiation to visible light, various light sources were introduced and are still used for therapeutic purposes. The infrared and near-infrared high-power lasers such as carbon dioxide (CO_2), neodymium:yttrium–aluminum–garnet (Nd:YAG) and diode lasers were introduced earlier in dentistry, and, based on their effective tissue ablation, hemostasis and bactericidal effects, these lasers have been clinically applied as adjuncts or alternatives to mechanical tools in the field of periodontics since the early 1990s [6, 7]. Recently, the application of the erbium:yttrium–aluminum–garnet (Er:YAG) laser in periodontal and peri-implant therapy has received much attention [8–12], due to its ability to ablate both periodontal soft [13, 14] and hard [15–17] tissues effectively. Studies have demonstrated the effectiveness of the Er:YAG laser in dental caries removal [18, 19], root surface debridement [20–24], and periodontal and peri-implant surgery [25–28], thus indicating the antimicrobial effect of this laser. After we first reported on the bactericidal effect of the Er:YAG laser on periodontopathic bacteria in 1996 [29], many in vitro and in vivo studies have further evaluated the potential ability of the Er:YAG laser to eliminate bacteria from periodontal pockets [23, 30, 31], root canals [32–34] and implant surfaces [35, 36]. However, the detailed mechanism of bacterial ablation and the decontamination effect on the periodontally diseased root surfaces have not yet been sufficiently elucidated.

Therefore, the purpose of this study was to investigate the morphological changes in periodontopathic bacteria after Er:YAG laser irradiation in order to speculate on the bacterial ablation mechanism, and to compare the bacterial elimination effect of Er:YAG laser irradiation in debridement of periodontally diseased root surfaces with that of an ultrasonic scaler in vitro.

Materials and methods

Experiment 1 Effect of Er:YAG laser irradiation on the morphology of bacterial colonies (Fig. 1a).

Bacterial culture and laser irradiation

The bacterium employed was *Porphyromonas gingivalis* ATCC33277. *P. gingivalis* was cultured anaerobically on trypticase-soy (TS) blood agar plates using the steel-wool–jar method at 37°C [nitrogen (N_2) 80%, hydrogen (H_2) 10%, and CO_2 10%]. After cultivation, nine *P. gingivalis* colonies with a diameter of approximately 2 mm were selected, and each colony was put on a glass plate and exposed to a single pulse of Er:YAG laser at 40 mJ (panel setting 50 mJ, energy density 14.2 J/cm^2 at the tip end and approximately 8.0 J/cm^2 on the colony surface). The apparatus employed was an Er:YAG laser (2.94 μm), model ML22, Erwin™ (HOYA Photonics Corp., Tokyo, Japan, and J. Morita Mfg. Corp., Kyoto, Japan). Laser irradiation was performed perpendicularly to the colony from a 2 mm distance using a straight contact tip with a diameter of

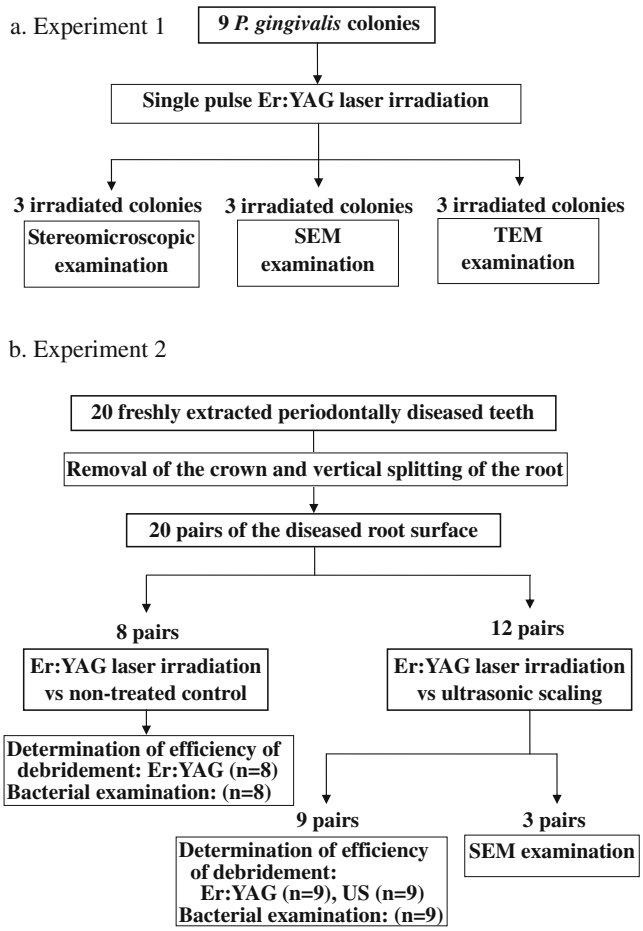

Fig. 1 Outline of experiments 1 and 2 (*US* ultrasound)

600 μm (transmission rate approximately 80%). This energy output was employed based on our previous study [20, 21]. We adjusted the energy setting by measuring the actual output power using a power meter (Field Master™, Coherent, USA). After irradiation, three of the nine colonies were observed through a stereomicroscope. The other six colonies were examined by scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

Specimen preparation for SEM observation

Three of the remaining six irradiated colonies were fixed immediately after the irradiation for 2 h with 2.5% glutaraldehyde fixative solution and then rinsed with 0.1 M phosphate buffer solution overnight. The specimens were postfixed with 1% osmium tetroxide for 2 h and dehydrated in ethyl alcohol solutions. After immersion in 3-methyl butyl acetate, critical-point dehydration in liquid CO_2 was performed. The specimens were then sputter coated with gold and observed by SEM (S-4500, Hitachi Co., Ibaragi, Japan) at magnifications of ×250, ×5,000, and ×35,000.

Specimen preparation for TEM observation

The remaining three colonies were fixed in glutaraldehyde solution, postfixed with osmium tetroxide, dehydrated and washed with propylene oxide. The specimens were then embedded in epoxy resin and polymerized for 1 week. Ultra-thin cross-sections approximately 60–70 nm thick were prepared with a diamond knife, stained with uranyl acetate and lead citrate, and examined under by TEM (H-600 electron microscope, Hitachi Co.) at magnifications of ×2,000 and ×20,000.

Experiment 2 Bacterial elimination effect of Er:YAG laser irradiation in debridement of periodontally diseased root surfaces (Fig. 1b)

Sample preparation

Twenty periodontally diseased teeth (four incisors, ten premolars and six molars) having bands of subgingival calculi with horizontally equal distribution on the proximal root surface were used. These teeth were extracted due to the diagnosis of severe periodontitis with deep periodontal pockets, as well as severe bone loss, from the patients attending the Dental Hospital of Tokyo Medical and Dental University and were collected after informed consent to their use for research purposes had been obtained. The experimental protocol was approved by the ethics committee of the Faculty of Dentistry, Tokyo Medical and Dental University (no.417). Immediately after extraction, the teeth were preserved in a sterilized liquid transport medium at 4° C and used for the experiment within 7 h. The crown was cut off from each tooth, and the root was axially divided into half with a sterile band saw. A few teeth which showed displacement of calculus during the splitting of the root were excluded from the experiment. By splitting the tooth, we divided the area with subgingival calculus equally between each half of the root. In each sample a treatment area having approximately the same square was determined macroscopically. The root halves were then individually fixed onto sterile plastic plates with an adhesive and self-cured acrylic resin to prevent contamination of the treated surface.

Eight of the 20 pairs were employed to investigate the bacterial elimination effect of Er:YAG laser on the diseased root surface; the left half of each pair was treated with an Er:YAG laser and the right half was left untreated as the control. The remaining 12 pairs were used to compare the decontamination effect of Er:YAG laser with that of the ultrasonic scaler; the left half was treated with the laser and the right half was treated with an ultrasonic scaler. All the laser and ultrasonic treatments were performed by the same operator (A. A.)

Er:YAG laser treatment

The laser irradiation was performed at 40 mJ/pulse (energy density of 14.2 J/cm^2 per pulse) and 10 Hz under sterile physiological saline water irrigation (20 ml/min) by syringe, in accordance with our previous experiment [20, 21]. A straight contact tip was applied in oblique contact mode at 30° angulations to the root surface and moved in a sweeping motion. Root-surface debridement, including calculus removal, was performed until no residual calculi were observed in the treated area on careful macroscopic inspection.

Ultrasonic treatment

For the ultrasonic treatment, an ultrasonic system (Solfy™, J. Morita Mfg. Corp.) and its universal tip with a sharp point (reference no. 107-051U) were used. A power setting of 4, which is the standard for clinical scaling, was selected from the 2–6 range. The contact tip was applied in oblique contact mode at 15° angulations and moved in a sweeping motion under sterile physiological saline irrigation (20 ml/min) by syringe.

Determination of efficiency of treatment

All the root specimens except for the non-treated control were included in this analysis. Before and after treatment, all the

specimens treated by Er:YAG laser or by ultrasonic scaler were photographed. The time required for root surface debridement was determined for each treatment, not including the time for the inspection of the residual calculi during operation. The treated areas were then measured by computer software designed to measure area (NIH image, National Institutes of Health, USA). The efficiency of the scaling was expressed as the area treated per second.

Examination of the bacterial elimination effects

Bacterial analysis was performed on all eight pairs of the laser versus non-treated control experiment and nine of the 12 pairs of the laser versus ultrasonic experiment. Immediately after laser or ultrasonic scaling, the superficial material on the treated root surfaces or on the non-treated surfaces, including microbial deposits, if any, was very carefully scraped with a sharp and sterile curette in three strokes with light pressure, avoiding contact with the peripheral surface.

The collected debris was suspended in 10 ml of Mitsuoka's diluting solution [37], vortexed for 2 min and diluted 10 times and 100 times. Aliquots of 0.05 ml of original solution (dilution rate ×1) and each dilution (×10 and ×100) were spread uniformly on the surface of two new TS agar plates. One plate was incubated aerobically for 1 week and the other was incubated anaerobically for 2 weeks.

After cultivation, the total numbers of colony forming units (CFUs) in both aerobic and anaerobic conditions for each treatment and non-treated control were determined. If the CFUs were countable at the plural dilution rates, the number of CFUs in the lowest dilution rate was adopted. Then, the logarithms of the numbers of CFUs were calculated and compared. If no colony was observed in any diluting rate, the CFU was counted as 0 and the logarithm was determined as 0. The CFU of black-pigmented anaerobic rods (BPARs) in the anaerobic culture, which is considered to be the major group of periodontopathic bacteria such as *P. gingivalis* and *Prevotella intermedia* [38, 39], were also counted in the same manner.

SEM observation

The other three pairs of the laser/ultrasonic treatment group were subjected to SEM observation, and the treated surfaces of the roots were examined at magnifications of ×100 to ×30,000.

Statistical analysis

A paired *t*-test was applied in order to examine the significance of the difference in treated areas, required times, efficiencies of debridement, and CFUs, between the Er:YAG laser and the ultrasonic scaler/untreated control. Results were considered to be significant at $P<0.05$. The analysis was conducted using Stat View 4.11 software (Abacus Concepts Inc., Berkeley, USA).

Results

Experiment 1 Effect of Er:YAG laser irradiation on the morphology of bacterial colonies

Stereomicroscopic observation

The laser irradiation produced an ablation spot with a diameter of approximately 750–900 μm on the *P. gingivalis* colony (Fig. 2a). The center of the spot was completely ablated, presenting a crater approximately 200–300 μm in diameter where no bacteria were observed. The affected area surrounding the ablation crater showed an approximately 300–400 μm wide, white-and-brownish colored zone with a bubbling appearance.

SEM observation

The ablation spot showed two distinctive areas: an ablation crater in the center and the surrounding affected area. This area consisted of many large pores surrounded by meandering, branch-like structures (Fig. 2b). At high magnification, the surface of the branch-like structures had a melted appearance. The surface on the inner side of the affected area had a smooth texture (Fig. 2c,d), whereas the surfaces of the structures on the outer side had a rugged appearance with numerous round protrusions (Fig. 2e,f). The size and the shape of each protrusion corresponded to those of the original individual bacterium, which was evident in the cleft of the colony surface in the non-irradiated site (Fig. 2h). Figure 2g shows a schematic illustration of the irradiated colony.

TEM observation

Figure 3a shows the cross-sectional TEM image of the ablation area. The laser beam was applied from the upper side of the picture, and the lower side was in contact with the glass plate. The upper oblique border shows the ablated surface accompanied by branch-like structures. In this examination, various levels of alterations to each bacterium caused by the Er:YAG laser irradiation could be clearly observed. In the less laser-affected portion of the lower side, near the glass plate, most bacteria had retained their original round form and were closely packed

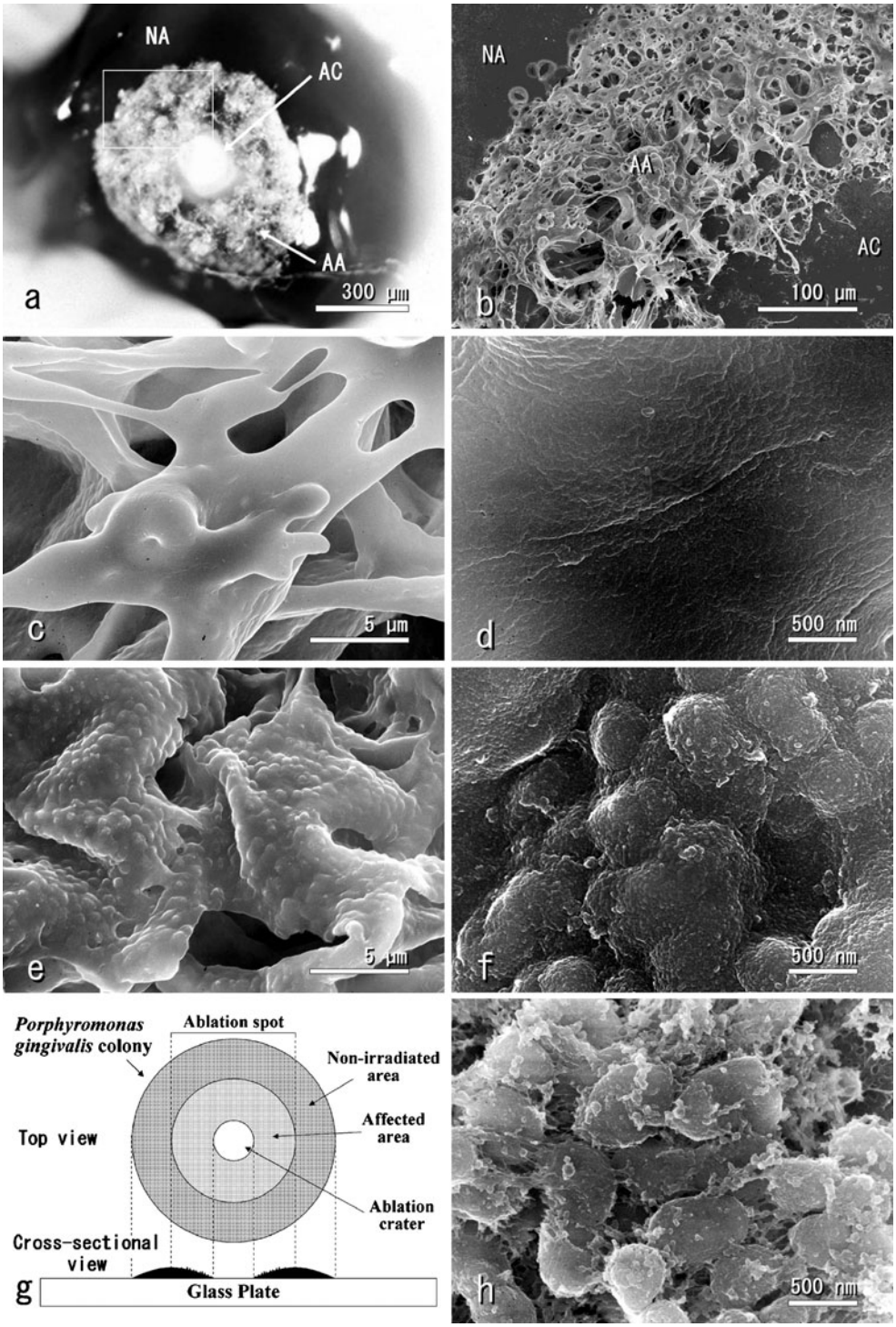

Fig. 2 Morphological changes in a *Porphyromonas gingivalis* colony following a single pulse of Er:YAG laser irradiation. **a** Stereomicroscopic photograph of the irradiated colony. No bacteria were observed in the center of the ablation spot, which was surrounded by a white-and-brownish changed area with a bubbling appearance. **b**–**f**, **h** Scanning electron micrographs. **b** The ablation spot (close-up of the square area **a**). Note the appearance of the ablation spot with the completely ablated crater in the lower right and the surrounding affected area. Many large pores surrounded by meandering, branch-like structures were observed. **c** The surface of these branch-like structures on the inner side of the affected area, close to the ablation crater, had a melted appearance. **d** The ultra-high magnification of figure **c** revealed a completely smooth texture of the surface. **e** The surface of branch-like structures at the outer side of the affected area, far from the center of the ablation crater, showed a rugged appearance with numerous round protrusions. **f** The ultra-high magnification of figure **e**. The size of these protrusions corresponded to those of individual round bacteria observed in the non-treated site (**h**). **g** A schematic illustration of the irradiated colony. **h** The original intact bacteria were evident in the cleft of the colony surface on the non-irradiated area. *AC* ablation crater, *AA* affected area, *NA* non-irradiated area. **b** ×250; **c**, **e** ×5,000; **d**, **f**, **h** ×35,000

(Fig. 3b). The branch-like structures existing on the outer side of the affected surface showed more changes, which consisted of denatured bacteria as a result of their fusion and/or shrinkage and the condensation of the cells and inter-cellular substances (Fig. 3c). Figure 3d illustrates the most severe alteration, which was found in the branch-like structures on the inner side of the affected surface close to the center of the ablation spot. The inside of the structures was completely amorphous and included very few shrunken cell components, possibly due to the almost complete water vaporization and the melting and resolidification of organic components of the cells and inter-cellular substances.

Experiment 2 Bacterial elimination effect of Er:YAG laser irradiation

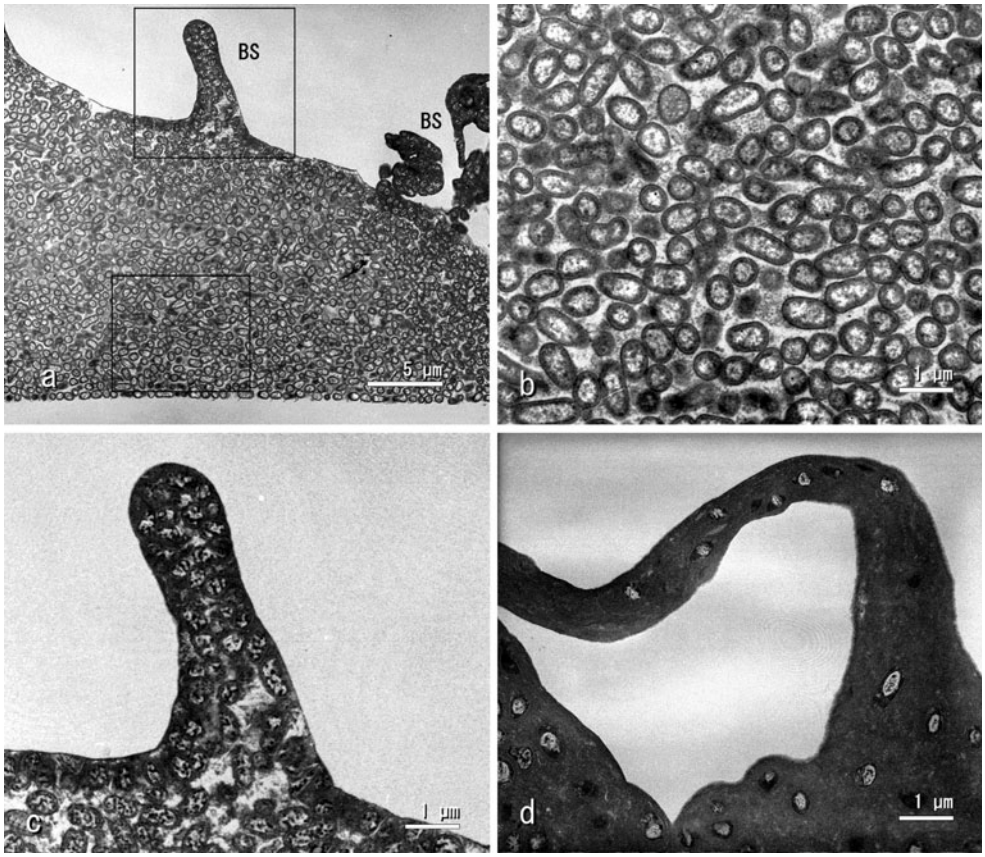

Fig. 3 Transmission electron micrographs of the affected area surrounding the ablation crater on the *Porphyromonas gingivalis* colony following a single pulse of Er:YAG laser irradiation. **a** The low magnification shows the ablated surface. Irradiation was applied from the upper side of the picture, and the lower side was in contact with a glass plate. The right side is the direction to the center of the ablation crater, and the upper side is the ablated surface, showing denaturation of bacteria and the formation of branch-like structures (*BS*). **b** Close-up of the lower square in **a**. The less-affected area at a lower portion near the glass plate reveals closely packed round bacteria. **c** Close-up of the upper square in **a**. The clearly affected area close to the ablated surface shows the denatured appearance of the bacteria, shrinkage of bacterial bodies and condensation of cells and extracellular substances, but the cells still retain their individual form. **d** The branch-like structures in the severely affected area closer to the center of the ablation crater are completely amorphous, with only a few bacteria remaining inside. **a** ×2,000; **b**–**d** ×20,000

The Er:YAG laser could ablate subgingival calculi and bacterial plaque effectively with the probe in light contact with the root surface and with no application of pressure to remove calculus.

Efficiency of root debridement

In the experiment of laser versus ultrasonic scaler, the treated area, required time and efficiency of debridement were 19.4±8.2 mm^2, 150.2±56.4 s and 0.14±0.05 mm^2/s [mean ± standard deviation (SD); n=9], respectively, for the laser treatment, and 17.8±7.3 mm^2, 142.4±52.8 s and 0.13±0.04 mm^2/s, respectively, for the ultrasonic scaling. There were no significant differences between the treated areas, required times and efficiencies of laser and ultrasonic root debridement under the conditions of the study. As for the experiment of laser versus non-treated control, the efficiency of the laser treatment was 0.16±0.01 mm^2/s (mean ± SD; n=8). During debridement with the laser or the ultrasonic scaler, easy detachment of calculi which occurred as a result of the root-splitting procedure was not noticed.

Bacterial elimination effects of the treatments

The CFUs and their logarithmic values for the treated root surfaces in the experiment comparing laser with the non-treated control are shown in Table 1. The mean logarithmic values of the CFUs of laser treatment and non-treated control were 0.08±0.21 and 1.89±0.79 (mean ± SD; n=8) in the aerobic condition, 0.38±0.43 and 3.71±0.65 in the anaerobic condition, and 0 and 2.83±0.93 in the BPAR count, respectively. The number of CFUs on the laser-treated root surfaces was significantly lower than that of non-treated root surfaces in all culture conditions (P<0.05). Er:YAG laser treatment could significantly reduce root surface contamination.

Table 1 CFUs after culture of the substances taken from the Er:YAG laser-treated root surfaces and the non-treated control root surfaces

Sample no.	CFU (logarithm)					
	Aerobic culturing		Anaerobic culturing		BPARs	
	Laser-treated surfaces	Control	Laser-treated surfaces	Control	Laser-treated surfaces	Control
1	4 (0.60)	281 (2.45)	3 (0.48)	10400 (4.02)	0 (0)	3100 (3.49)
2	0 (0)	34 (1.53)	3 (0.48)	1780 (3.25)	0 (0)	810 (2.91)
3	0 (0)	60 (1.78)	0 (0)	10800 (4.03)	0 (0)	2800 (3.45)
4	0 (0)	3620 (3.56)	3 (0.48)	62200 (4.79)	0 (0)	29000 (4.46)
5	1 (0)	18 (1.26)	2 (0.30)	620 (2.79)	0 (0)	140 (2.15)
6	0 (0)	84 (1.92)	20 (1.30)	2070 (3.32)	0 (0)	110 (2.04)
7	0 (0)	16 (1.20)	0 (0)	2050 (3.31)	0 (0)	290 (2.46)
8	0 (0)	25 (1.40)	0 (0)	14100 (4.15)	0 (0)	50 (1.70)
Logarithm (mean ± SD)	0.08±0.21	1.89±0.79	0.38±0.43	3.71±0.65	0±0	2.83±0.93
P	0.0003		<0.0001		<0.0001	

The CFUs and their logarithmic values in the experiment comparing laser versus ultrasonic scaler are shown in Table 2. The mean logarithmic values of the CFU after laser and ultrasonic treatment were 0.11±0.32 (mean ± SD, n=9) and 0.77±0.70 in the aerobic condition, 0.60±0.68 and 1.59±0.87 in the anaerobic condition, and 0.08±0.23 and 0.36±0.74 in the BPAR count, respectively. The number of CFUs on the laser-treated root surface was significantly lower than that of the ultrasonically debrided root surface in the aerobic and anaerobic conditions (P<0.05). The CFUs of the BPARs showed no significant difference between both treatment groups.

SEM observation

The root surface treated with the ultrasonic scaler was generally smooth, with an absence of bacterial biofilm as well as subgingival calculus (Fig. 4a), but shallow grooves

Table 2 CFUs after culture of the substances taken from the Er:YAG laser-treated root surfaces and the ultrasonically scaled root surfaces

Sample. No.	CFU (logarithm)					
	Aerobic culturing		Anaerobic culturing		BPARs	
	Laser-treated surfaces	Ultrasonically scaled surface	Laser-treated surfaces	Ultrasonically scaled surface	Laser-treated surfaces	Ultrasonically scaled surface
1	9 (0.95)	5 (0.70)	14 (1.15)	5 (0.70)	1 (0)	0 (0)
2	0 (0)	6 (0.78)	0 (0)	14 (1.15)	0 (0)	0 (0)
3	0 (0)	40 (1.60)	56 (1.75)	18 (1.26)	5 (0.70)	0 (0)
4	0 (0)	1 (0)	2 (0.30)	3 (0.48)	1 (0)	0 (0)
5	0 (0)	1 (0)	1 (0)	62 (1.79)	0 (0)	0 (0)
6	0 (0)	44 (1.64)	18 (1.26)	1010 (3.00)	0 (0)	20 (1.30)
7	0 (0)	0 (0)	1 (0)	13 (1.11)	0 (0)	0 (0)
8	0 (0)	41 (1.61)	0 (0)	396 (2.60)	0 (0)	96 (1.98)
9	1 (0)	4 (0.60)	8 (0.90)	165 (2.22)	0 (0)	1 (0)
Logarithm (mean ± SD)	0.11±0.32	0.77±0.70	0.60±0.68	1.59±0.87	0.08±0.23	0.36±0.74
P	0.03		0.02		0.32	

and defects resulting from the ultrasonic tip instrumentation were occasionally observed. At high magnification, the ultrasonically scaled surface had a smooth texture covered with a smear layer (Fig. 4c). However, micro-irregular structures were sometimes observed in the area of the micro-defects and micro-grooves. The root surface treated by Er:YAG laser was also free from biofilm as well as calculus and relatively flat at low magnification (Fig. 4b). Some shallow, crater-like defects and scratches caused by the contact tip were occasionally observed on the lased root surface. The features of the treated root surface were not remarkably different between the three lased samples which received irradiation of different durations. At high magnification, the laser-treated root surface revealed a characteristic, scaly or flaky microstructure with no thermal changes such as melting, resolidification or cracking (Fig. 4d). Also, the biofilm at the area bordering the treated and non-treated surfaces appeared different in both groups. Boundary biofilms close to the ultrasonically scaled areas retained their normal form (Fig. 4e), showing a grass-like appearance at high magnification (Fig. 4g). In contrast, the remaining biofilm at the border between the laser-treated and non-treated areas showed thermal denaturation due to en route vaporization, such as a shrunken, melted appearance with bubbles and branch-like structures affected by the irradiation (Fig. 4f,h).

Regarding the bacterial elimination effect, both treatments generally achieved almost all biofilm removal from the treated surface. However, in both treatments, some islets of the biofilm or bacteria still remained at the non-contact sites in the treated areas, such as between the marks of instrumentation or in the original micro-defects or the micro-grooves. In particular, a relatively large number of remaining bacteria was occasionally observed between the marks of instrumentation on the ultrasonically scaled surfaces (Fig. 5a–d). The frequency of detection of residual bacteria and the number of residual bacteria were much fewer in the laser-treated site. In addition, residual bacteria on the ultrasonically scaled surface retained their original form, while some residual bacteria on the laser-treated surfaces generally had a thermally denatured appearance, with a melted and fused, or shrunken, structure (Fig. 5f)e,f.

Discussion

Owing to the promising characteristics, the Er:YAG laser has been clinically applied to periodontal pocket treatment, including root surface debridement [8, 10–12, 23]. Previously, we reported that the Er:YAG laser can effectively ablate calculus [13, 20, 21], and we also demonstrated the bactericidal effect of the Er:YAG laser at a low energy level [29]. In the abovementioned study, when periodontal bacteria (*P. gingivalis* and *Aggregatibacter actinomycetemcomitans*) were irradiated with Er:YAG laser, growth-inhibitory zones were found in the sites irradiated at approximately 0.3 J/cm^2 and higher. Also, when *P. gingivalis* colonies were individually exposed to a single pulse laser, the survival ratio of the viable bacteria in the lased colonies decreased significantly at 7.1 J/cm^2 and 10.6 J/cm^2. However, in spite of the gradual increase in the clinical application of Er:YAG lasers for periodontal pocket treatment, including root debridement, detailed information on its decontamination ability is still lacking. The effect and mechanism of bacterial ablation by Er:YAG laser are not completely clear, and the degree of its bactericidal effect on periodontally diseased root surfaces compared with that of mechanical instruments has not been shown in detail.

In our study, the ablation process of the bacteria was clarified in the SEM and TEM examination by our observing morphological changes of the irradiated *P. gingivalis* colonies. Macroscopically, the Er:YAG laser easily produced an ablation spot on the *P. gingivalis* colony, surrounded by a band of affected area showing numerous specific branch-like structures. SEM and TEM observation indicated that the branch-like structures were the thermally fused or melted products of the bacterial colony after irradiation, and the structure was speculated to be the intermediate phase of the thermal evaporation of organic components in the bacterial body and extracellular substances following water vaporization. Thus, unlike the hypothesized mechanism of 'thermo-mechanical' or 'photo-mechanical' ablation in hard tissue ablation [8], 'thermal evaporation' of water and organic components would be the major process in the ablation of bacterial biofilms with the Er:YAG laser, as with other hard lasers [40].

On the bactericidal effect of the Er:YAG laser, Schoop et al. compared the bactericidal effects of the Nd:YAG, diode, Er:YAG, and erbium, chromium:yttrium–scandium–gallium–garnet (Er,Cr:YSGG) lasers under standardized conditions from an endodontic aspect and reported that the Er:YAG laser showed the best bactericidal effect [31]. Using microscopy, Schwarz et al. clinically demonstrated a decrease in the numbers of motile rods and spirochetes after Er:YAG laser treatment of periodontal pockets comparable to that of mechanical treatment [23]. Furthermore, the bactericidal potential of Er:YAG laser on micro-structured implant surfaces has also been recently analyzed with successful results [32, 33]. However, in a recent clinical and microbiological study, Derdilopoulou et al. reported that bacterial reduction in periodontal pockets following the Er:YAG laser treatment was not superior to that after ultrasonic scaling [41]. Furthermore, when Tomasi et al. evaluated the treatment of pockets with Er:YAG laser in a periodontal maintenance program, no differences were reported in the microbial profiles between Er:YAG laser treatment and

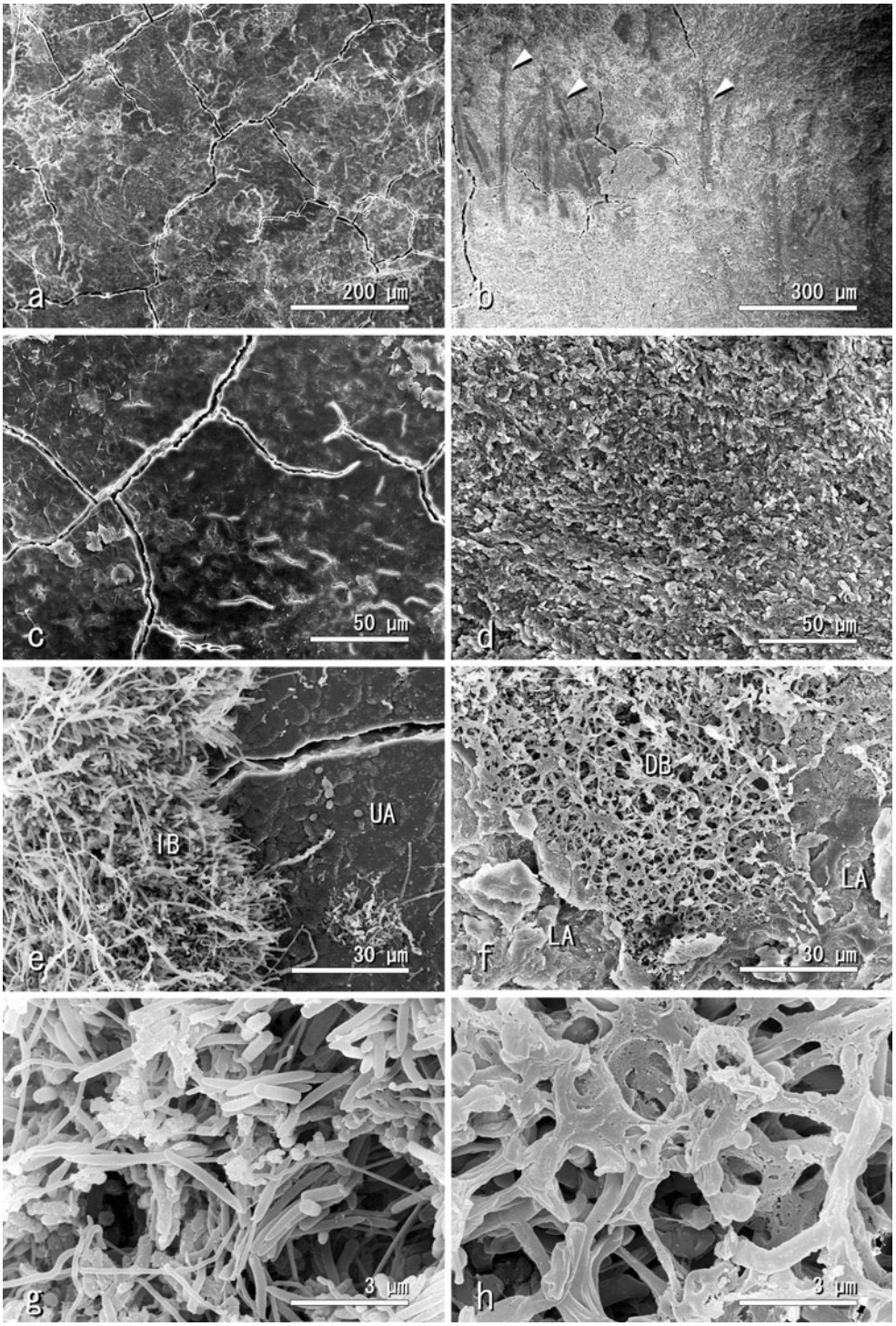

Fig. 4 Scanning electron micrographs of the root surface treated by ultrasonic scaler (**a**, **c**) or Er:YAG laser (**b**, **d**), and the biofilm at the boundary of the ultrasonically (**e**, **g**) or laser-treated (**f**, **h**) area. **a** The root treated with the ultrasonic scaler generally had a smooth surface. **b** The laser-treated area also had a relatively flat appearance at low magnification, but some shallow, crater-like defects and scratches caused by the contact tip (*arrowheads*) were occasionally observed. **c** At high magnification, the ultrasonically scaled surface generally exhibited a smooth texture covered with a smear layer. **d** At high magnification, a characteristic micro-roughness with numerous sharp or round, pointed projections resulting from cementum ablation were evident on the laser-treated root surface. Micro-cracks in these pictures are artifacts, but the lased surface generally had fewer cracks. **e** The right side of the image is the ultrasonically treated area (*UA*) and the left side is intact biofilm (*IB*) showing a grass-like appearance. **f** The lower right and left sides of the image show the laser-treated area (*LA*), and the upper middle shows the boundary denatured biofilms (*DB*). **g** At high magnification, the boundary biofilms close to the ultrasonically treated area retained their normal form. **h** At high magnification, in contrast to those in the ultrasonically treated area, the boundary biofilms close to the laser-treated area showed thermal denaturation due to en route vaporization, such as a shrunken and melted appearance with bubbled and branch-like structures. **a** ×150; **b** ×100; **c**, **d** ×500; **e**, **f** ×1,000; **g**, **h** ×10,000)

ultrasonic scaling [42]. Thus, at present, with respect to elimination of bacteria from periodontal pockets, the superiority of the Er:YAG laser over conventional mechanical treatment has not yet been demonstrated.

In this study we focused on the decontamination effect of the Er:YAG laser on the root surface and revealed that the Er:YAG laser had a significant decontamination effect on periodontally diseased root surfaces and also had a significantly better decontamination effect than that of the ultrasonic scaler with respect to the total numbers of CFUs of aerobic and anaerobic microbes, although the difference in the CFUs of BPARs between both treatment groups was not statistically significant. The frequency of incomplete elimination of BPARs was similar in both treatments, but the number of remaining BPARs was much fewer in the laser treatment. Regarding the BPAR count, the Er:YAG

laser showed at least an elimination effect equal to that of the ultrasonic scaler.

Although we did not measure temperature during our study, high temperatures would not have occurred during irradiation, as our group has previously reported minimal temperature elevation during Er:YAG laser scaling in an in vitro study [21] that used the same irradiation parameters as used in this study. Actually, the SEM images revealed a micro-structured root surface with no thermal changes following Er:YAG laser irradiation.

The improved decontamination effect of Er:YAG laser might be explained by the difference in characteristics of both devices. The main action of the ultrasonic scaler is achieved by direct contact with the bacterial biofilm, whereas the laser has the potential to kill bacteria and ablate bacterial biofilm even without direct contact. The features of the remaining plaque biofilm observed after Er:YAG laser irradiation were thus interesting. Although macroscopic inspection indicated complete removal of calculus and microbial deposits, SEM observation revealed that some islets of the biofilm or infiltrated bacteria still remained in the remaining micro-defects or micro-grooves of the treated areas in both treatments. The detection of residual bacteria was more frequent, and the number of bacteria was much larger, at the non-contact sites, such as between the marks of instrumentation after ultrasonic scaling, than those after Er:YAG laser scaling. This indicated that ultrasonic scaling was less effective in removing bacteria from the diseased root surface, possibly due to incomplete instrumentation of the entire surface. Furthermore, in the case of ultrasonic scaling, the residual bacteria after treatment generally retained their normal forms, while, in the laser-irradiated sites, such residual bacteria had a melted or shrunken appearance. These affected bacteria would be devitalized and detoxified by thermal denaturation of the bacterial substances. Likewise, remaining biofilms on the boundary of treated and non-treated areas of ultrasonically scaled root surface completely

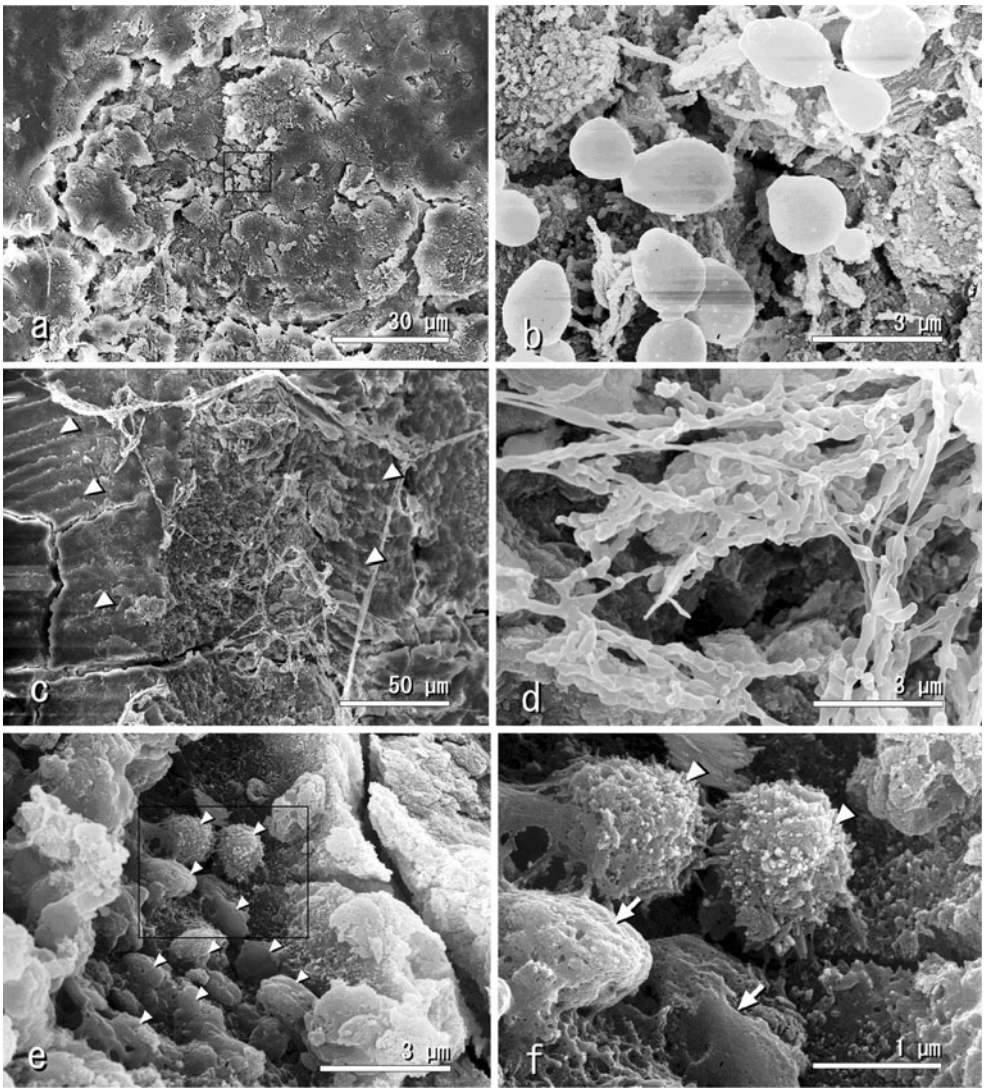

Fig. 5 Scanning electron micrographs of the residual bacteria on the root surface after ultrasonic scaling (**a**–**d**) or Er:YAG laser scaling (**e**, **f**). Some islets of the biofilm or bacteria still remained at the non-contact sites in the treated areas in both treatments. **a**, **c** The ultrasonically scaled surface. A relatively large number of remaining bacteria was observed in the original micro-defects and grooves or between the traces of ultrasonic tip instrumentation (*arrowheads*). **b** Close-up of the square in **a**. **d** Close-up of part of **c**. At ultra-high magnification, the residual bacteria on the ultrasonically treated surface retained their original form (**b**, **d**). **e** The laser-treated surface. Most of the residual bacteria on the lased root surface have a thermally denatured appearance, such as a melted or shrunken structure (*arrowheads*). **f** Close-up of the square in **e**. At ultra-high magnification, a few bacteria appeared partially or slightly affected (*arrowheads*) and the others showed moderate or severe thermal denaturation (*arrows*). **a** ×1,000; **b**, **d** ×10,000; **c** ×500; **e** ×10,000; **f** ×30,000)

retained their original form of bacterial flora, whereas the remaining biofilms at the marginal region on the laser-treated surface showed a zone with a denatured appearance in the SEM examination. The laser light delivered via optical fibers loses part of its collimation property and diverges after being released from the end of the contact tip. Therefore, it is considered that Er:YAG laser irradiation has the potential to kill bacteria and remove bacterial biofilms more efficiently, not only at the contact point but also in a larger area targeted by the divergent beam in non-contact mode, than does ultrasonic instrumentation that exerts its effects only at the point or small area where the tip end directly contacts the surface. This advantageous property may partly explain the relatively better bactericidal performance achieved by Er:YAG laser in our study.

Er:YAG laser irradiation was performed at 10 Hz in our study, as in most previous studies. In this condition, laser scaling provided a level of calculus removal similar to that provided by ultrasonic scaling, and the efficiency of debridement was almost equal in the two treatments. Thus, in this study, root debridement was performed under similar efficiency in both treatments, allowing a comparison of decontamination effect to be made between the two treatment modalities. Recently, the clinical performance of the Er:YAG laser has been further improved; higher pulse rates up to 50 Hz have become available, and a chisel-type contact chip that has a larger irradiation spot size than the conventional round-end tip has been developed in new laser systems [23, 27, 28]. With the use of the new chisel tip, in combination with such higher pulse rates, more efficient root debridement and more improved bactericidal effects can be clinically expected in Er:YAG laser treatment.

In conclusion, we demonstrated that Er:YAG laser irradiation caused thermal vaporization of periodontopathic bacteria and that, under the equal efficiency of root debridement, the decontamination effect of the Er:YAG laser on diseased root surfaces was superior to that of the ultrasonic scaler in vitro. These results suggest an advantageous aspect of the Er:YAG laser for periodontal pocket treatment, including root surface debridement. Further basic and clinical studies are necessary to clarify the effectiveness and the usefulness of the Er:YAG laser at higher pulse rates for root surface debridement and decontamination.

Acknowledgements This study was supported, in part, by a grant from the Global Center of Excellence Program for Frontier Research in Tooth and Bone Diseases at Tokyo Medical and Dental University, and a grant-in-aid for Scientific Research(c) (nos. 13672183, 16592064, and 19592382) to A.A., from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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