Effects of Irradiation of an Erbium: YAG Laser on Root Surfaces*

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THE APPLICATION OF ERBIUM:YAG LASER (Er:YAG) irradiation has been investigated for periodontal therapy. The purpose of this study was to evaluate the effects of Er: YAG laser irradiation on root surfaces using a scanning electron microscope (SEM) and to determine the laser's ability to remove lipopolysaccharides (LPS). Infrared spectrophotometry was used to investigate the effects of the laser on LPS applied to root dentin pellets. Premolars extracted for orthodontic reasons were prepared for this study. The crowns were resected below the cemento-enamel junction, longitudinally sectioned, and the contents of the pulp chamber were removed. Then 15 root tips (5×5×1 mm) were classified into 3 groups of 5 each as follows: group 1, tips without any treatment; group 2, planed tips with the cement layers left untouched; and group 3, planed until the dentin surface was disclosed. The center of each specimen was used as the experimental irradiated area and the peripheral area served as a control. The quantity of vapor delivered by Er:YAG laser was highly increased, and the irradiated areas displayed little morphogenetic changes. The lyophilized sample LPS 0111 B4 from *E. coli* was then mixed with potassium bromide and pressed into a tablet, which was examined at 4,000–650 Kayser. The lyophilized LPS had a peak at 2.94 μm. LPS on the root dentin pellets was cleared away as much as possible by 150 washings in pyrogen-free water using an ultrasonic cleaner. Five μl of 24 EU LPS solution was dropped on the root dentin pellets, which were then irradiated by the Er: YAG laser, and washed using the ultrasonic cleaner in pyrogen-free water. The amount of the extracted LPS solution was determined by spectrophotometer at 405nm. The Er: YAG laser could remove 83.1% of the LPS. This study suggests that Er:YAG laser irradiation might be useful for root conditioning in periodontal therapy. However, clinical testing is necessary to establish what, if any, utility the Er:YAG laser has as a part of periodontal therapy. *J Periodontol 1997;68:1151–1155.*

Key Words: Lasers; tooth root; periodontal diseases/therapy.

Recently, the application of laser irradiation to periodontal therapy has begun to be investigated. Tewfik et al.[1] reported the effects of a modified Nd:YAG laser on root cementum topography and fibroblastic attachment, and suggested that the modification of cementum surfaces depends on the energy level of the laser irradiation. Morlock et al.[2] evaluated the effects of the Nd:YAG laser treatment on root surfaces in vitro, alone or combined with conventional scaling and root planing. They suggested that the application of 1.5W Nd:YAG laser irradiation during the root preparation, even at relatively low energy levels, would result in physical changes on the root surfaces.

Ito et al.[3] reported a study on the effects of the Nd: YAG laser irradiation in the removal of a root surface smear layer after root planing, and compared it to treatment with citric acid, which led to a partial dentin denudation. Their study indicated that Nd:YAG radiation effectively removed the smear layer, uncovering dentinal tubules and exposing collagen fibers on the root surface without widening the orifices of the dentinal tubules after root planing with citric acid conditioning.

The lipopolysaccharides (LPS) located in the outer membrane of the Gram-negative bacterial cell wall are a common inflammatory factor in endodontic and periodontal lesions. Among the LPS biological activities, the toxicity and the immunogenicity of many higher organisms have been mentioned.[4–8] Trylovich et al. in an in vitro study on the effects of the Nd:YAG laser on fibroblast attachment to endotoxin-treated root surfaces, re-

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Figure 1. Group 1 (non-debridement). The surface is irregular and shows debris. The bottom appears amorphous (original magnification ×150).

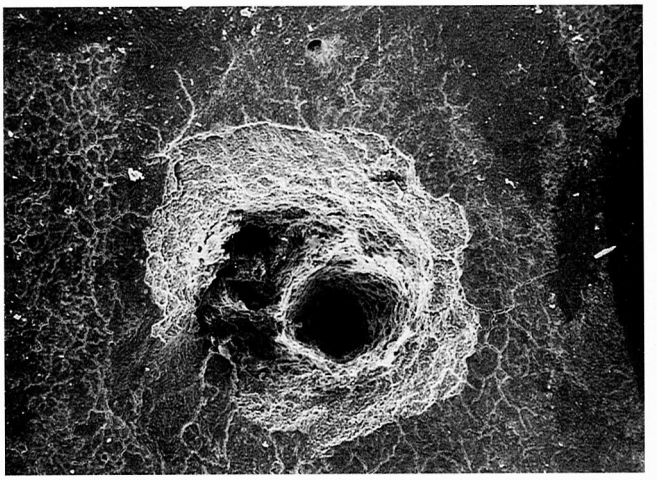

Figure 2. Group 2 (cementum group). The crater is a deep penetration showing a surface partially cracked and fissured (original magnification ×150).

ported that the growth of fibroblasts was inhibited on the laser-treated cementum surfaces. Their study suggested that the laser could alter the biocompatibility of the cementum surface, making it unfavorable for fibroblast attachment.

On the other hand, the Er:YAG laser, emitting at a wavelength of 2.94 μm, is highly absorbed by water.[10-12] Kellar and Hibst[13,14] reported that the pulsed Er:YAG laser, which promised to effectively remove enamel and dentin, produced minimal thermal damage to adjacent tissues.[15-17]

The purpose of this study was to evaluate the effects of Er:YAG laser irradiation on root surfaces.

MATERIALS AND METHODS

SEM Observation of Root Dentin Surfaces

Premolars extracted for orthodontic reasons were resected just below the cemento-enamel junction (CEJ), as well as longitudinally sectioned, and the pulp contents were removed. Fifteen root tips of 5×5×1 mm were classified into 3 groups of 5: group 1, no treatment (non-debridement); group 2, root planed using hand curets[†] in an attempt to remove remaining accretions and achieve a smooth, glass-like surface; the cement layers were left (cementum group); and group 3, cleaned until the appearance of dentin surfaces. Group 2 then received 150 repeated washings in pyrogen-free water[‡] using an ultrasonic cleaner.[§]

All specimens were irradiated with an Er:YAG laser at an energy density of 300mJ/cm^2 (100mJ,15pps).

The specimens were then dehydrated in a series of graded ethanol solutions, transferred into 2-methyl-2-propanol[‖] and freeze-dried. They were sputter-coated with palladium and examined using scanning electron microscopy.[¶]

Determination of the Infrared Spectrum of LPS

Three mg of a lyophilized LPS sample from *Escherichia coli* 0111 B4[#] was prepared and then irradiated with the Er: YAG laser at an energy density of 300mJ/cm^2 (100mJ, 15pps). The control was not irradiated.

Each sample of 2mg was then mixed with 200mg of potassium bromide and pressed for 4 minutes. The mixture and grinding were done in a multi-motor for 5 minutes. The specimens were placed in a cylindrical steel die which was then enclosed in a large cylinder connected to a vacuum pump. While the system was still being evacuated, the die was subjected to a pressure of 10 ton/cm^2 for 5 minutes. The pressed tablets were released from the die. The infrared spectrum of the tablet was determined at wave numbers from 4,000 Kayser to 650 Kayser.**

Evaluation of the Effectiveness of Laser Irradiation to Remove LPS

Dentin pellets, prepared using the same method as the experimental group 1, were cleaned and the LPS were removed as much as possible by 150 repeated washings in pyrogen-free water[‡] using an ultrasonic cleaner[§] to reach the minimum detectable LPS. The concentration of the extracted LPS was determined with a chromogenic limulus amoebocyte lysate assay kit[††] at a 405nm wavelength.[‡‡] All tests were performed using the same batch

[†]Gracey curets, Hu-Friedy Co., Chicago, IL.
[‡]Flow Laboratories, Irvine, Scotland.
[§]Model W-113, Hondadenshi, Toyohashi, Japan.
[‖]Wako Chemical Co., Osaka, Japan.
[¶]Model JSM T-300, Nihondenshi, Tokyo, Japan.
[#]DIFCO, Detroit, MI.
**Model JIR 100, Nihondenshi, Tokyo, Japan.
[††]QCL1000, Bio-Whittaker, Walkersville, MD.
[‡‡]Model U-1100, Hitachi, Tokyo, Japan.

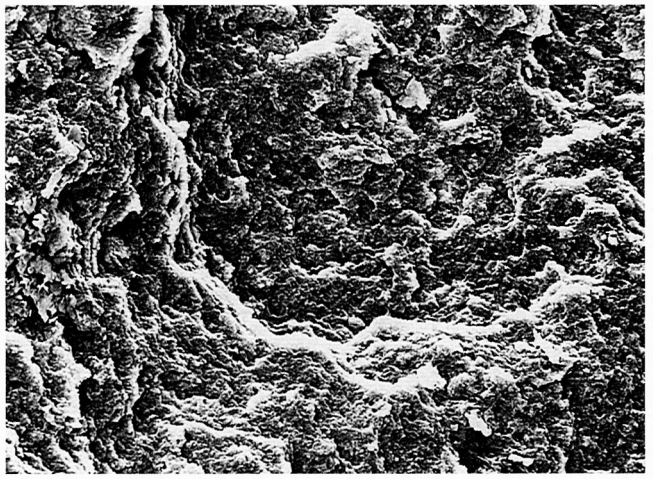

Figure 3. Group 2 (cementum group). Showing fusion of the crater surfaces. The bottom is uneven and irregular (original magnification ×1000).

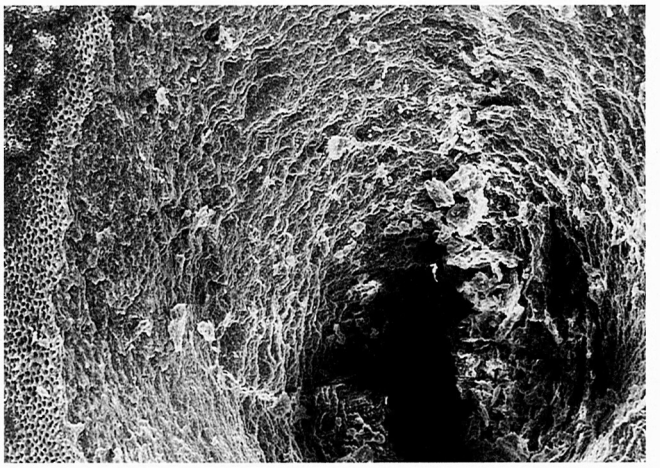

Figure 4. Group 3 (dentin group). Non-irradiated area (control) has no smear layer, and the margins are clear (original magnification ×150).

of reagents reconstituted according to the manufacturer's instructions. The LPS solution (5μl of 24 EU) was dropped on the tablets with a micropipette. They were then dried in a desiccator. The dentin pellets were irradiated with the Er:YAG laser[§§] at an energy density of 35.4mJ/cm^2 (100mJ, 1pps). The control was not irradiated. The LPS were removed from these irradiated dentin pellets in pyrogen-free water using an ultrasonic cleaner for 30 minutes, and the concentration of the extracted LPS was determined as described above with an assay kit[††] and a 405nm wavelength spectrophotometer.[‡‡] The concentration changes of removed LPS on the root surface were determined after laser irradiation.

RESULTS

SEM Observation

In group 1, on the laser irradiation area, the craters were small and deep, showing very irregular walls (Fig. 1) which were not amorphous; but the margins were clear. The fiber bundles impaired the overall performance of laser irradiation. The irradiated cementum surfaces in group 2 appeared uneven, irregular, and amorphous (Figs. 2 and 3). The irradiated areas in group 3 showed an absence of melted areas on the inner wall at low magnification (Fig. 4), and non-irradiated areas were not covered by a smear layer. In group 3, orifices of dentinal tubules appeared on the irradiated areas, as did a few melted surfaces at high magnification (Fig. 5).

Infrared Spectrum of LPS

The infrared spectrum of LPS is shown in Figure 6. The lyophilized LPS sample had a peak at 2.94μm which also corresponds to the wavelength of the Er:YAG laser. The peak of the irradiated sample changed to 2.92μm.

[§§]Model DL-ER2020, Osada Co., Tokyo, Japan.

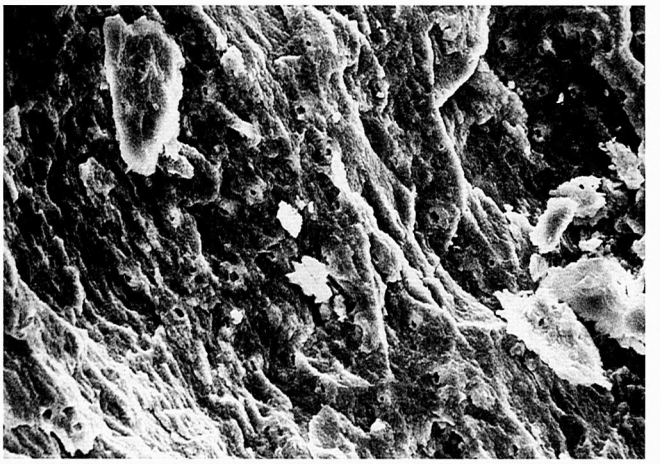

Figure 5. Group 3 (dentin group). Showing partial fusion of the dentin surfaces. Dentinal tubule orifices appeared on the irradiated area (original magnification ×1000).

Effectiveness of Laser Irradiation in Removing the LPS

The Er:YAG laser irradiation could remove 83.1% of LPS from the root surfaces (Table 1).

DISCUSSION

The LPS in the cell surface of Gram-negative bacteria have been acknowledged to play many biological roles, such as pyrogenicity, mitogenicity, immunogenicity, and macrophage activation of serum complement.[4–8] They have also been implicated in the initiation and progression of periodontitis.[18–30] In vitro studies on the reduction of the biological activities of LPS with mild alkali, sodium dodecylsulphate, and EDTA (ethylene diamine tetraacetic acid) had been previously carried out.[31–33] In this study, Er:YAG laser irradiation has been found to be effective for dental hard tissues with little damage to the surrounding structures, except the irradiated areas. The LPS on

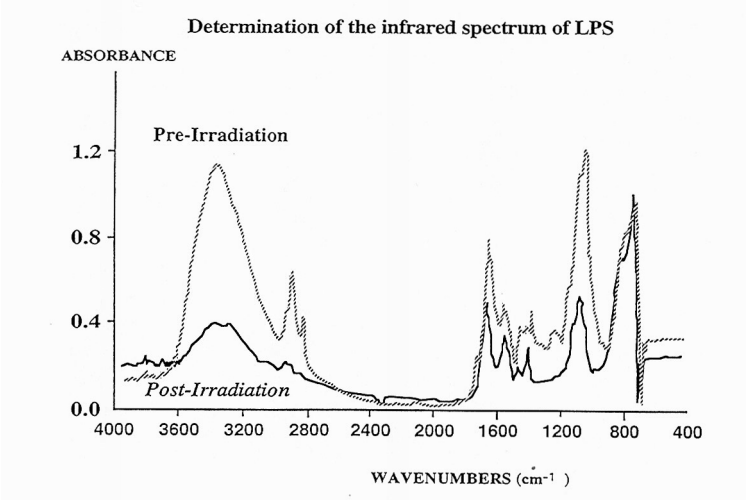

Figure 6. Determination of LPS infrared spectrum.

root surfaces can be completely removed by ultrasonic instrumentation, but this procedure is long lasting.[34]

The results of the present study suggest that Er:YAG laser irradiation can effectively and rapidly remove 83.1% of the LPS, supporting the theory that lasers might be able to remove LPS from root surfaces in periodontal treatment.

The potential use of lasers in treating periodontal disease has been advocated with hopes that it will be capable of sterilizing the affected root surfaces and periodontal pocket microflora, as well as permitting curettage of the adjacent pocket epithelium and enhancing the removal of calculus from surfaces.[35,36]

In addition, it will be necessary to study cell attachment on the root surfaces after removing LPS by Er:YAG laser irradiation and the restoration of periodontal tissues. Concerning the group 2 methodology, the cement layers, present at the pilot study, were not removed and could also be confirmed by SEM observations, as in groups 1 and 3.

The Er:YAG laser has a wavelength of 2.94μm, which corresponds to the wavelength of the LPS (2.92μm). The peak of the infrared spectrum of LPS in the irradiated samples changed to 2.92μm.

This suggests that the Er:YAG laser-irradiated LPS samples will undergo a chemical change, and that LPS removal may have been due to ablation on the root surfaces, mechanical elimination, and heat during irradiation.

The chemical compositions of the LPS preparations from *Escherichia coli* differed considerably according to the extraction method. The LPS are composed of the following 3 distinct structural regions: an O-specific polysaccharide, a common core, and a lipid component called lipid A. The lipid A region has been suggested to induce mitogen reaction and PBA (polyclonal: B cell activation).[37,38]

Although unresponsiveness to endotoxin was usually observed in C3H/HeJ spleen cells, LPS prepared by extraction with aqueous butanol had potent mitogenic activity. This extraction and the purification procedures resulted in an LPS-protein complex with at least two independent immunostimulatory components.[39–43] Biochemical analysis of this lipid A-associated protein was reported in several studies[44–46] which demonstrated a number of distinct components with various molecular weights ranging from 40,000 to 8,000.

Table 1. Effectiveness of Laser Irradiation in Removing LPS when Applied to Root Surfaces

Number	Percentage Removal
1	78.9
2	79.4
3	80.1
4	80.2
5	81.7
6	83.5
7	83.6
8	84.9
9	89.0
10	89.3
Average	83.1
SD	±3.77

The chemical structure of the changes in the Er:YAG laser-irradiated LPS was shown in this study, suggesting that Er:YAG laser irradiation may be useful for root conditioning in periodontal therapy.

In conclusion, this study indicates that Er:YAG laser irradiation provided a marked vaporization, and the resulting craters were deeper with a clear margin. The spectrum peak of the Er:YAG laser also has the same wavelength as the peak of the LPS spectrum. Moreover, the Er:YAG laser was found to remove 83.1% of LPS after its application to root surfaces. In future studies, we should examine the effects of laser irradiation on root conditioning in periodontal treatment. We will also continue to examine the structural change of LPS.

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