In Vivo and In Vitro Effects of an Er:YAG Laser, a GaAlAs Diode Laser, and Scaling and Root Planing on Periodontally Diseased Root Surfaces: A Comparative Histologic Study

Frank Schwarz, Anton Sculean, Mohammad Berakdar, Ludwig Szathmari, Thomas Georg, and Jürgen Becker

Background and Objectives: The aim of the present histologic study was to compare the in vivo and in vitro effects of an erbium: yttrium, aluminum, and garnet (Er:YAG) laser (ERL), combined with a fluorescent calculus detection system, a diode laser (DL) and scaling and root planing (SRP) on periodontally diseased root surfaces.

Study Design/Materials and Methods: Twenty-four single rooted teeth, considered for extraction due to severe periodontal destruction, were included in the study. Prior to extraction all mesial root surfaces were randomly assigned to the following treatment groups: (1) ERL combined with a calculus detection system with fluorescence induced by 655 nm InGaAsP DL radiation (160 mJ/pulse and 10 pulses/second under water irrigation) (ERL), or (2) GaAlAs DL (1.8 W, pulse/pause relation 1:10), or (3) SRP using hand instruments. Immediately after extraction, all distal root surfaces were treated with the same instruments under standardized conditions. For light microscopic investigation, a plastic embedding technique was used to cut the undecalcified roots into 30 μm thick crosssections. The following parameters were recorded by an blind examiner: remaining debris, root surface morphology, and thermal side effects.

Results: Root surfaces instrumented with both, ERL in vivo and DL in vitro exhibited no detectable surface alterations. In contrast, ERL scaling in vitro and SRP in vivo/in vitro produced superficial microchanges in root cementum. However, irradiation with DL in vivo caused severe damages to the root surface (i.e., crater formation). There were no signs of thermal side effects in all laser treated groups. ERL provided subgingival calculus removal on a level equivalent to that provided by SRP. DL was unsuitable for calculus removal, using this power output, was unsuitable for calculus removal and altered the root surface in an undesirable manner.

Conclusions: The present in vivo results showed that (i) ERL, combined with a fluorescent calculus detection system, provided a selective subgingival calculus removal on a level equivalent to that provided by SRP, and (ii) DL, using this power output, was unsuitable for calculus removal and altered the root surface in an undesirable manner. Lasers Surg. Med. 32:359–366, 2003. © 2003 Wiley-Liss, Inc.

Key words: fluorescence; histology; laser; morphology; periodontal disease; scaling

INTRODUCTION

The principal objective in the treatment of periodontitis is the complete removal of all calcified and bacterial deposits from the root surfaces in order to stop disease progression [1,2]. Several treatment modalities, such as scaling and root planing (SRP), sonic and ultrasonic devices, or air abrasives have been employed with varying degrees of success in order to accomplish this goal [3–8]. One of the most documented treatments is SRP, which is accomplished using hand instruments. Numerous studies have reported beneficial results from this treatment modality in both clinical and microbial parameters [3,6,7]. However, removal of calculus using conventional hand instruments has been reported to be incomplete [1] and rather time consuming [9]. In order to improve the effectiveness and efficiency of root surface debridement, both sonic and ultrasonic scalers [5,8], and recently lasers have been used [10–14]. Among all lasers used in the field of dentistry, which include CO₂ (carbon-dioxide), the Nd:YAG laser (neodymium-doped: yttrium, aluminum, and garnet), and diode lasers (DLs), the Er:YAG laser (ERL) has been reported to be the most promising laser for periodontal treatment.
treatment. Its excellent ability to effectively ablate hard tissue and dental calculus without producing major thermal side-effects to adjacent tissue has been demonstrated in numerous studies [11,12,15–17]. Furthermore, the results from controlled clinical trials and case report studies have also indicated that non-surgical periodontal treatment with an ERL leads to significant gain of clinical attachment level [14,18–22]. This minimally invasive device may allow instrumentation of very deep and narrow pockets without leading to major trauma of the hard and soft tissues; i.e., removal of tooth substance and increase in gingival recession [14,17,22,23]. In contrast, several studies have confirmed the negative effects of the Nd:YAG and CO₂ lasers when used directly on root surfaces. These negative effects include thermal damage, such as carbonization and melting, to the root surface [24–26] which has been shown to inhibit attachment of fibroblasts and delay wound healing [27,28]. So far, there is limited information about the effects of DL radiation on the surface properties of root surfaces [29,30]. The results from a recent study showed that this laser may cause damage to periodontal hard tissues if irradiation parameters are not adequate [29]. Although periodontal treatment with an ERL offers indeed some interesting perspectives to the clinician, some questions are still present and need to be solved. One of them is the extent of the root surface damage after laser application. Histological and SEM examination showed that under in vitro conditions the ERL ablated not only the calculus, but also the superficial portion of the underlying cementum. The surface was left with an acid-etched appearance microscopically [11,12,31]. In contrast, SEM observations from a recent study showed that the clinical use of an ERL resulted in a smooth root surface morphology, even at higher energy settings [17]. However, SEM analysis may not be sufficient in order to investigate the effects of laser radiation on the subsurface layers of root cementum and dentin. In order to avoid any damages to the root surface, a subgingival calculus detection system with fluorescence induced by 655 nm InGaAsP DL radiation has been recently included in an ERL device. Preliminary in vitro results have shown that 655 nm DL radiation induces significantly stronger fluorescence in subgingival calculus than in cementum [32]. So far, there are no histologic data evaluating the in vivo effects of an Er:YAG, used with this fluorescent calculus detection system, or a DL on the surface properties of root cementum. Therefore, the purpose of the present study was to histologically determine the in vivo and in vitro effects of an ERL combined with a calculus detection system with fluorescence induced by 655 nm InGaAsP DL radiation, a GaAlAs DL and SRP on periodontally diseased root surfaces using light microscopic examination.

**MATERIALS AND METHODS**

**Study Design**

This study was conducted on 24 single rooted teeth (n = 48 surfaces), considered for extraction due to severe periodontal destruction, based on informed consent of the patients. Each tooth satisfied the following criteria: (1) probing pocket depths (> 8 mm) on at least two aspects (mesio-vestibular/meso-oral and disto-vestibular/disto-oral) as measured from the gingival margin to the bottom of the pocket, (2) mobility in horizontal and vertical direction, (3) no signs of curvus or artificial damage on the root surface, (4) no periodontal root surface treatment within the last 12 months, and (5) no root fractures or anatomical abnormalities. Furthermore, patients suffering from systemic diseases, which could influence the outcome of the therapy, were excluded from the study. All teeth were randomly assigned to the following treatment groups: (1) ERL, (2) GaAlAs DL, or (3) SRP using hand instruments.

**Treatments**

An ERL (KEY3®, KaVo, Biberach, Germany) device emitting a pulsed infrared radiation at a wavelength of 2.94 µm was selected for laser treatment. Laser parameters were set at 160 mJ/pulse and 10 pulses/second, and pulse energy at the tip (size 0.5 × 1.65 mm) was approximately 120 md/pulse (19.4 J/cm²) [14,18,22]. The laser beam was guided onto the root surfaces under water irrigation with a specially designed periodontal handpiece and a chisel-shaped glass fiber tip (2061, KaVo). The treatment was performed from coronal to apical in parallel paths with an inclination of the fiber tip of 15–20° [33] to the root surface. The exciting laser radiation was delivered by an InGaAsP DL as red light at a wavelength of 655 nm. The DL beam was delivered onto the root surface with the above-mentioned periodontal handpiece and the prismatic cut glass fibre tip (Fig. 1). The mode of detection of fluorescence has been previously described [34]. In brief, the InGaAsP DL radiation induces fluorescence in the irradiated mineralized substance. The laser radiation is guided to the angulated chisel-shaped glass fiber tip within a central fiber. Additional surrounding fibers are arranged around this central fiber that collect the fluorescent light emitted from the irradiated tissue. The fluorescent light is guided together with the reflected radiation as well as the ambient light from the surrounding fibers [32]. When the receiver no longer gets any fluorescence signals, the ERL beam is switched off. For the treatment of test group 2 a GaAlAs DL
(Ora-Laser-Jet 20°, Oralia, Konstanz, Germany) with a wavelength of 810 nm and 10,000 Hz toppulse was used. In our study, the pulse/pause relation was set at 1:10 at a power output of 1.8 W according to the instructions given by the manufacturer. The laser light was delivered by a 600 μm contact optic fiber (0.63 mJ/mm²) (Fig. 2). The mechanical subgingival instrumentation of test group 3 was accomplished using hand instruments (Hu-Friedy Co., Chicago, IL). All treatments were performed under local anesthesia until the mesial root surfaces were adequately debrided and planed. Immediately after instrumentation, the teeth were extracted. All root surfaces appeared unaltered by the extraction procedure. After that, moistened teeth were fixed in an artificial root socket and treatment of all distal

![Image](image_url)

Fig. 2. The diode laser (DL) light (810 nm) was delivered by a 600 μm contact optic fiber on the root surface.

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Fig. 3. Histometric evaluation: crater depth (CD) was measured by drawing a perpendicular line connecting the margins of the crater at the point of maximum depth (original magnification ×250).

![Image](image_url)

Fig. 4. Boxplots with outliers for the medians and Q1–Q3 quartiles of CD (μm) on root surfaces after instrumentation in vivo (ERL, DL, SRP). Lines below and above box plots: min, max.

### TABLE 1. Histometric Results (ERL, GaAlAs DL, SRP)

<table>
<thead>
<tr>
<th></th>
<th>Samples with craters (N)</th>
<th>Mean number of craters/sample</th>
<th>Mean CD (μm)</th>
<th>Samples with exposed dentin (N)</th>
<th>Samples with thermal side effects (N)</th>
<th>Samples with remaining debris (N)</th>
<th>Remaining debris as percentage of the mesial and distal root surface (N, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo group</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>ERL (n = 8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>DL (n = 8)</td>
<td>8</td>
<td>14.6</td>
<td>62.5</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>85</td>
</tr>
<tr>
<td>SRP (n = 8)</td>
<td>8</td>
<td>3</td>
<td>26.4</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td><strong>In vitro group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERL (n = 8)</td>
<td>8</td>
<td>6</td>
<td>36.8</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>DL (n = 8)</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>79</td>
</tr>
<tr>
<td>SRP (n = 8)</td>
<td>8</td>
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<td>24.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

ERL, erbium: yttrium, aluminum, and garnet (Er:YAG) laser; DL, diode laser; SRP, scaling and root planing; CD, crater depth.
surfaces was performed under near-clinical conditions. The end point of debridement in vitro was judged as the inability to detect subgingival calculus visually. The amount of time needed in all groups was, on average, 2 minutes per surface.

**Histological Examination**

All teeth were dehydrated in a graded alcohol series and embedded in metacrylate (Technovit®, Kulzer Gmbh, Wehrheim Its, Germany) using a standard method [35]. After that, crosssections were cut with a diamond saw at 100–150 μm. The slices were ground automatically by a special machine to a thickness of 30 μm. All historical sections were stained with toluidine blue and evaluated under a light microscope (Leitz Orthoplan, Leitz, Wetzlar, Germany) by one blind and calibrated examiner. The following parameters were recorded: remaining debris (yes/no) as a percentage of the observed root surface, craters (yes/no), mean number of craters per sample, mean depth of craters, exposed dentin (yes/no), and thermal side effects (carbonization, melting, cracking) (yes/no). Each surface was viewed with a 10 × 10 mm ocular grid subdivided into one hundred 0.1 mm squares to assess remaining debris (original magnification ×25). The total number of squares, which covered each surface from the connective tissue attachment to the cemento-enamel junction were counted. Only surface areas covering more than one-half of a square were counted. Then the number of squares with calculus were counted for each surface. A percentage of squares with calculus was then calculated [36]. Intra-observer reliability was evaluated by assessing remaining debris in two specimens of each group (n = 12) 10-fold. Crater depth (CD) was measured on photomicro-

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Fig. 5. Photomicrographs of in vivo treated root surfaces: C, cementum; CDJ, cemento-dentinal junction; D, dentin. a: ERL; b, hand instruments; c, d, GaAlAs DL (original magnifications: a, b: ×250; c, ×100; d, ×400).
graphs by drawing a perpendicular line connecting the margins of the crater at the point of maximum depth (original magnification ×250) (Fig. 3) [37]. Intra-observer reliability was evaluated by measuring mean CD in two specimens of each group (n = 12) 10-fold. The results were expressed as coefficient of variation.

**Statistical Analysis**

A software package (SPSS 11.0, SPSS, Inc., Chicago, IL) was used for the statistical analysis. Mean values and standard deviations for the histometric parameters were calculated. For the comparisons between groups, the unpaired t-test was used. Values of \( P < 0.05 \) were accepted as statistically significant.

**RESULTS**

**Instrumentation In Vivo**

Intra-observer variability (measurement of CD) was between 1.9 and 3.2%. The ERL produced homogenous and nearly smooth root surfaces without visible traces of the used fiber tip. Loss of cementum was generally non-existent or minimal (Table 1 and Fig. 4). Treated areas could not have been demarcated from untreated areas in respect of the surface morphology. Only the presence of calculus has given signs of the treatment borders (Fig. 5a). The histologic inspection of the root surfaces indicated that subgingival calculus was selectively removed (Fig. 5a). Histologically, no thermal damage, such as carbonization, melting, or cracking, was observed. The root surfaces treated using hand instruments generally had a smooth appearance. Some scratches, but also shallow craters caused by the curettes were occasionally observed in all specimens (Fig. 5b). Mean CD was 26.4 μm (Table 1 and Fig. 4). In contrast, DL irradiation caused severe damages to the root surface. Calculus-free areas examined histologically showed grooves and crater-like defects in all cross-sections (Fig. 5c,d). Mean CD was 62.5 μm. In six of eight samples, cementum was ablated through to the dentin (Table 1 and Fig. 5c,d). Histologically, no major thermal damage, such as carbonization, melting, or cracking, was observed. The maximum number of craters was observed in the DL group (\( P < 0.001 \)), and these craters were significantly deeper than those following treatment with hand instruments (\( P < 0.001 \)) (Table 1).

**Instrumentation In Vitro**

Intra-observer variability (measurement of CD) was between 1.9 and 3.2%. Histologically, no major thermal damage, such as carbonization, melting, or cracking, was observed for all treatment modalities (Table 1). Root surface instrumentation with ERL resulted in an increased removal of cementum. In one of eight samples, cementum was ablated through to the dentin. All ERL irradiated specimens exhibited numerous shallow ablation cavities with notched-edged borders and linear tracking lines caused by the fiber tip (Figs. 6 and 7a,b). Mean CD was 36.8 μm (Table 1 and Fig. 6). Comparable to instrumentation in vivo, root surfaces treated using hand instruments generally had a smooth appearance (Fig. 7c). Some scratches, but also shallow craters caused by the curettes were occasionally observed in all specimens. Mean CD was 24.3 μm (Table 1 and Fig. 6). In contrast, DL irradiation resulted in no detectable alterations such as crater formation, exposure of dentin, charring, or cracking on the root surface (Table 1 and Fig. 7d). The maximum number of craters was observed in the ERL group (\( P < 0.01 \)), and these craters were significantly deeper than those following treatment with hand instruments (\( P < 0.01 \)) (Table 1).

**Calculus Ablation**

Intra-observer variability (assessment of remaining debris) was between 2.4 and 3.7%. Clinically, the ERL provided a level of subgingival calculus removal that was similar to that provided by hand instruments (Table 1). In both groups, the histological examination occasionally revealed very tiny residual calculus on the treated root surfaces of all specimens. Calculus-free areas examined macroscopically and histologically looked fairly well cleaned. However, in vitro instrumentation was more effective than in vivo instrumentation (Table 1). In contrast, all specimens treated with the DL exhibited large amounts of subgingival calculus. These light to dark brown areas of calculus covered large areas of the root surfaces (Fig. 7c). There was no difference between in vivo and in vitro instrumentation.

**DISCUSSION**

The results of the present study have demonstrated that under in vivo conditions subgingival calculus can be
removed almost completely from periodontally diseased root surfaces using both, an ERL in combination with a fluorescent calculus detection system or hand instruments. These findings are in accordance with the results from previous in vitro studies, which have shown that a pulsed ERL used with water irrigation was able to effectively remove subgingival calculus from the root surface [11,38]. This laser provided calculus removal on a level equivalent to that provided by an ultrasonic scaler [38]. The results of the present study have also shown that the subgingival calculus detection system with fluorescence induced by 655 nm InGaAsP DL radiation appears to be a valuable tool for the clinical detection of calculus, since instrumentation in vivo provided a level of subgingival calculus removal that was similar to that provided under in vitro conditions. However, in vitro instrumentation was more effective than in vivo instrumentation. This discrepancy might be explained by the fact that the intensity of fluorescence on cementum seems to be influenced by the surrounding media. The results from a recent in vitro study showed that the presence of blood or electrolytic solution considerably influenced the amount of fluorescence radiation. Nevertheless, the differential fluorescence between subgingival calculus and cementum was highly significant in air, blood, and electrolytic solution [32]. As mentioned above, elimination of all calcified deposits from periodontally involv-

Fig. 7. Photomicrographs of in vitro treated root surfaces: C, cementum; CDJ, cemento-dental junction; D, dentin; SC, subgingival calculus. a, b: ERL; c, hand instruments; d, GaAlAs DL (original magnifications: a–d: ×250).
ed root surfaces is a desired goal of SRP [2]. In this context, it is important to point to the results of previous studies which have shown that complete removal of subgingival calculus may not be predictably attainable following SRP with hand instruments [1,39]. Molar teeth seemed to be more difficult to clean than single-rooted teeth [39]. In this respect, it must be pointed out that one limitation of the present study was the lack of a group consisting of multi-rooted teeth, since both, hand instruments and ultrasonic devices were relatively ineffective in removing calculus from the furcal walls [40]. Further research is needed in order to investigate the relative effectiveness of the ERL for calculus removal in molar furcations. The present results have also shown that the DL was unsuitable for calculus removal, since macroscopic inspection of the root surfaces revealed the presence of large amounts of subgingival calculus. Because no previous work on DL for calculus removal in vivo appears to have been reported, the present results cannot readily be compared with those of other studies. Furthermore, the results of the present study have also indicated that in vivo application of DL radiation for nonsurgical periodontal treatment caused severe damages to the root surface, since grooves and crater-like defects were found in all cross-sections. However, there were no signs of thermal side effects, such as carbonization or melting. In contrast, lasing moistened specimens in vitro resulted in no detectable alterations on the root surface. These findings are in accordance with the results from a previous in vitro study, which have shown that DL radiation caused severe damages to the root surface when segments were covered by a thin blood film [29]. Irradiation at a power output of 1 W and below had barely any negative effect on the root surface whereas lasing at 1.5, 2.0, and 2.5 W resulted in partial or total carbonizations of the root samples. In contrast, lasing dry specimens and specimens moistened with saline resulted in no detectable alterations, irrespective of irradiation time and power output applied. This phenomenon might be explained by the high absorption values of the laser light at 810 nm in hemoglobin and other pigments. However, in vitro instrumentation with the ERL also resulted in an increased removal of cementum. All irradiated specimens exhibited numerous shallow ablation cavities with notch-edged borders and linear tracking lines caused by the fiber tip. In contrast, in vivo instrumentation with the ERL produced homogeneous and nearly smooth root surfaces without visible traces of the used fiber tip. A loss of cementum was generally non-existent or minimal. The in vitro results are in accordance with the results of previous studies, which have shown that an ERL ablated not only the calculus, but also the superficial portion of the underlying cementum, and that the treated surface had characteristic microstructures resulting from cementum ablation [11,17,31,37,38]. However, this microstructured root surface showed no cracks or thermal effects like melting after CO2- and Nd:YAG-laser irradiation [31]. The results of the present study support a previous SEM investigation that reported a selective calculus removal with the ERL under in vivo conditions [17]. All root surfaces treated in vitro showed visible crater-like defects with notch-edged borders. The depth of the surface damages varied with the power applied and was localized into cementum at energy settings of 120–160 mJ but also reached dentine at 180 mJ. Compared to that, all in vivo treated surfaces showed a homogeneous and smooth root surface morphology. The surface alterations were not related to the used energy setting. These observations, coupled with the findings that the ERL has also high antimicrobial effects against periodontopathic bacteria [10,13], probably explain, at least in part, the successful healing following non-surgical periodontal treatment as described in numerous clinical studies [14,18–22]. The achieved results also appeared to be stable over a 2-year period [22]. Although the combined treatment ERL and SRP did not seem to additionally improve the outcome of the therapy compared to laser treatment alone [18], it remains unclear to what extent this laser treated root surface is capable of supporting the new attachment of the periodontal tissues.

In conclusion, within the limits of this study, the in vivo results indicate that (i) ERL, used with a new fluorescent calculus detection system, provided a selective subgingival calculus removal on a level equivalent to that provided by SRP, and (ii) DL, using this power output, was unsuitable for calculus removal and altered the root surface in an undesirable manner.

REFERENCES


