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Evaluation of selective calculus removal by a fluorescence feedback-controlled Er:YAG laser in vitro

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Abstract

Objectives: To evaluate the removal of subgingival calculus and dental hard tissues depending on the threshold level of a fluorescence feedback-controlled Er:YAG laser.

Material and Methods: Twenty teeth with calculus on the root surface were treated with an Er:YAG laser. Laser settings were 140 mJ and 10 Hz. The initial fluorescence threshold level of 5 [U] was reduced at intervals of 1 [U] for every laser treatment. Areas of residual calculus (RC) were evaluated using a surface analysis software. Loss of dental hard tissues was assessed by histomorphometric analysis of undecalcified ground sections.

Results: Using a threshold value of 5 [U], the median amount of RC was 11% (0–78%). By lowering the threshold levels, the amount of RC decreased [level 1 [U]: 0% (0–26%)]. The laser-treated root surfaces revealed a statistically significant reduction of the cementum thickness [median: 80 μm (0–250)] compared with the non-treated opposite side [median: 90 μm (30–250)] ($p < 0.05$).

Conclusion: The amount of RC following laser irradiation depends on the fluorescence threshold level for a feedback-controlled Er:YAG laser. It might be suggested that this laser system may be used with a threshold level even lower than 5 [U] without removing a clinically relevant amount of root cementum.

Key words: Er:YAG laser; fluorescence; periodontal therapy; root debridement; subgingival calculus

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A primary goal in the treatment of periodontitis is the removal of bacterial deposits and the arrest of disease progression (American Academy of Periodontology, 2001). Scaling and root planning is the traditional method of controlling subgingival microflora for management of periodontitis. Numerous studies have reported beneficial results in both clinical and microbial parameters (Badersten et al. 1987, Ramfjord et al. 1987, Sato et al. 1993, Petersilka et al. 2002, Van der Weijden & Timmermann 2002). However, complete removal of calculus might be difficult because the morphology of the root (i.e. furcations, irregularities of surface) often complicates the achievement of the desired biologically compatible root surface (Sherman et al. 1990). Furthermore, removal of calculus using conventional hand instruments has been reported to be incomplete and rather time consuming (Kepic et al. 1990, Yukna et al. 1997). Since the introduction of lasers in dentistry, their clinical use in periodontics has gained in importance. Despite the suggestion that lasers are a desirable alternative to conventional root instrumentation, several studies could also demonstrate an irreparable damage of the root surface by laser energy (Frentzen & Körner 1999, Liu et al. 1999, Cobb 2006). The thermomechanical ablation mechanism and the high absorption of its wavelength by water may qualify the Er:YAG laser in particular as an effective tool in periodontal and also in general dental applications (Keller & Hibst 1989, Aoki et al. 1994, Ando et al. 1996, Watanabe et al. 1996, Schwarz et al. 2001, 2003, Eberhard et al. 2003).

In order to improve the outcome of non-surgical periodontal treatment, the principle of laser fluorescence measurements might be useful. It has been suggested that red light fluorescence is correlated with the presence of microorganisms (Koenig & Schneckenburger 1994). Enhancement of fluorescence radiation probably seems to result from porphyrins and other chromatophores synthesized by oral microorganisms

(Koenig et al. 1998). Previous in vitro results have shown that 655 nm diode laser radiation induces significantly stronger fluorescence in subgingival calculus than in the cementum (Folwaczny et al. 2002, 2004, Krause et al. 2003). The use of this laser-induced fluorescence to control selective calculus removal has been technically realized in an Er:YAG laser: treatment is controlled by the fluorescence signal from the root surface induced by a red-infrared diagnostic diode laser. Therefore, the laser is activated only if a certain threshold level for the fluorescence of the root surface is exceeded. A recently published study indicates that this device might be a valuable tool to improve selective calculus removal preserving root cementum during non-surgical periodontal treatment (Schwarz et al. 2006). Until now, no data about the influence of the fluorescence threshold level of the Er:YAG laser are available. Furthermore, the amount of cementum removal has not yet been systematically evaluated.

Hence, the aim of the present in vitro study was to evaluate the selective removal of subgingival calculus depending on different threshold levels of the fluorescence feedback-controlled Er:YAG laser. Additionally, the amount of root cementum removal should be assessed depending on the feedback-controlled endpoint of calculus removal.

Material and Methods

Teeth selection and preparation

To assess the hypothesis that (i) the efficiency of fluorescence-controlled Er:YAG laser debridement depends on the fluorescence threshold level and (ii) fluorescence-controlled Er:YAG irradiation has an impact on cementum removal, a total of 20 single-rooted freshly extracted human teeth were evaluated. Teeth were collected from different patients and stored in a physiological saline solution. The root surfaces examined in the present study were partially covered with calculus and located subgingivally before extraction. All teeth had been extracted for periodontal reasons. The time span between tooth extraction and the following treatment did not exceed 1 week. Teeth were fixed on a translation stage (Melles Griot, Rochester, NY, USA) using a silicone impression material (Contrast™, Voco, Cuxhaven, Germany) to facilitate a reproducible tooth position (Fig. 1) and exposing the root surface. The area of interest of the treated root surface was determined both by the diameter of the used application tip and by small artificial notches prepared in the coronal and apical part of the root substance on a distance of about 10 mm containing mineralized deposits.

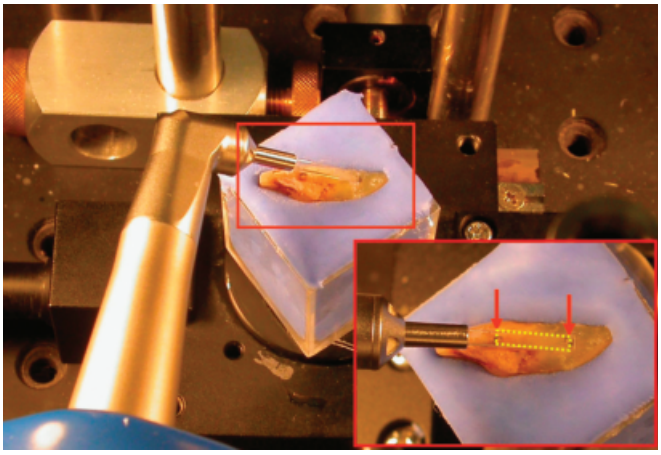

Fig. 1. Experimental set-up. Teeth and handpiece of the Er:YAG laser fixed on a translation stage. The angulation between the chisel-shaped application tip and the root surface was 15°. Detail: the area of interest of the treated root surface was determined both by the diameter of the used application tip (yellow marking) and by small notches prepared in the coronal and apical part of the root surface (red arrows).

Laser treatment

For laser treatment, an Er:YAG laser (Key Laser III™, KaVo, Biberach, Germany) emitting a pulsed infrared radiation at a wavelength of 2.940 µm was selected. The periodontal handpiece (No. 2061, KaVo, Biberach, Germany) and a novel designed chisel-shaped glass fibre application tip (size 0.4×1.65 mm, transmission factor: 0.81) were used to guide the laser beam onto the root surface under water irrigation (1 ml/min). Laser parameters were set at 140 mJ/pulse with a repetition rate of 10 Hz according to the manufacturer's recommendations. The respective energy density at the fibre tip was 17.2 mJ/cm^2.

The laser was equipped with a laser fluorescence feedback system. The laser light of an InGaAsP diode laser with a wavelength of 655 nm (red light) is transported through a fibre bundle to the tip of the handpiece within a central fibre. Additional surrounding fibres are arranged around this central fibre that collect the fluorescent light emitted from the irradiated tissue. The laser-induced fluorescence of the root surface is given in relative units from 0 to 99 and used to control the therapeutic irradiation by turning on the Er:YAG laser if the fluorescence value is above a pre-selected threshold level. If the fluorescence is below this value, the laser does not emit. For the present study, the evaluated threshold levels of the fluorescence feedback system were 5, 4, 3, 2 and 1[U]. The treatment was performed from coronal to apical in contact irradiation mode with an angulation of 15° between the fibre tip and the root surface. Teeth were covered with a layer of water before the actual treatment to ensure a proper cooling during laser irradiation. Before each measurement, a calibration of the feedback system of the Key Laser III™ was performed: an appropriate ceramic touchstone was used according to the manufacturer's instructions. For each tooth, an initial fluorescence threshold value of 5 [U], as recommended by the manufacturer for standard periodontal treatment, was reduced at intervals of 1 [U] for every laser treatment until the fluorescence feedback system did not indicate any emitted fluorescence from the root surface above the pre-selected threshold level or a value of 1 [U] was reached. All root surfaces were documented by digital photographs with respect to the different laser fluorescence threshold levels. Using the xy-table, the reproducible positioning of the handpiece was ensured with an accuracy of 10 µm.

Planimetric root surface evaluation

Before changing a threshold value, standardized digital photographs of the

teeth were taken with a magnification of 1:1. The digitized photographs were assessed with a surface analysis software (MegaCAD 4.8b, Megatech Software GmbH, Berlin, Germany) measuring the amount of remaining calculus with an accuracy of 0.01 mm^2. Areas of residual calculus (RC) were measured as a percentage of the total area of calculus before laser treatment using the notches as coronal and apical extensions and the diameter of the fibre tip as marginal extensions of root surface instrumentation.

Histological examination

For histomorphometric analysis, teeth were fixed in formalin and embedded in a light-activated polymethylmethacrylate medium (Technovit 7200, Haraeus Kulzer, Wehrheim, Germany). Undecalcified ground sections of 50 μm were cut and stained with toluidine blue. Root surfaces were examined at $\times$ 10 magnification with a stereomicroscope (Dialux EB, Leitz, Wetzlar, Germany). Digital images were evaluated using a software program (MegaCAD 4.8b, Megatech Software GmbH, Berlin, Germany) assessing the following parameters: loss of cementum layer and exposures of dentin. The thickness of the cementum layer was assessed on the lased root surface and the non-treated surface on the opposite side as control. Surfaces were divided into 10 sections each between the prepared notches, and the minimum thickness of the cementum of each section was measured (Fig. 2).

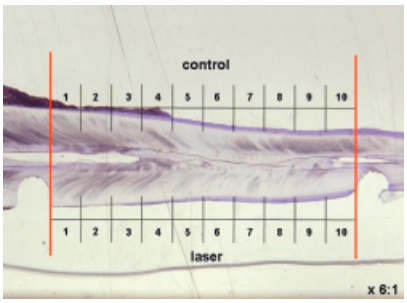

Fig. 2. Photomicrograph of an undecalcified ground section. Assessment of the cementum thickness on the lased root surface and the non-treated surface on the opposite side serving as control. The surfaces were divided into 10 corresponding sections between the prepared notches (red line). The minimum thickness of each section was measured. 6 $\times$, toluidine blue staining

Statistical analysis

For statistical analysis, normal distribution of all values was analysed by means of the Shapiro–Wilk test. As not all values were normally distributed, the amount of RC depending on the threshold level was analysed with a non-parametric test (Wilcoxon). Analysing the cementum thickness, values of the laser treated and the control side were also compared with the Wilcoxon test. Differences were considered statistically significant at $p < 0.05$.

Results

RC and fluorescence threshold level

The amount of RC depended on the laser fluorescence threshold level. A representative example of the specimens is given in Fig. 3. Using a fluorescence threshold level of 5 [U], the median residual amount of calculus was 11% (min: 0, max: 78%) related to the baseline amount of calculus (Fig. 4). By lowering the threshold levels, the amount of RC decreased [level 4 [U]: 0% (0–60%); level 3 [U]: 0% (0–60%); level 2 [U]: 0% (0–60%); level 1 [U]: 0% (0–26%)] (Fig. 4). Statistically significant differences could be shown for reduction from level 5 [U] to 4 [U] and level 2 [U] to 1 [U] ($p < 0.05$, Wilcoxon's).

Histomorphometric analysis of root surface

The histological observation of the root surface showed that tooth surfaces exhibited little dentin exposition but rather a reduction of cementum following fluorescence-controlled laser treatment. In 16 out of 200 evaluated sections, the cementum was ablated completely. The laser-treated root surfaces revealed a statistically significant reduction in the thickness of the cementum layer compared with the non-treated opposite side serving as control ($p < 0.05$; Fig. 5). After laser scaling, the median thickness of the cementum layer was 80 μm (min: 0 μm, max: 250 μm) compared with 90 μm (min: 30 μm, max: 250 μm) on the untreated sides. Neither cracking or carbonization indicating thermal damages nor crater

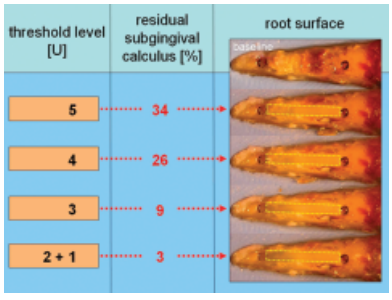

Fig. 3. Representative example for removal of calculus using the fluorescence-controlled Er:YAG laser using different threshold levels. Lowering of the laser fluorescence threshold values led to a reduction of the amount of residual calculus

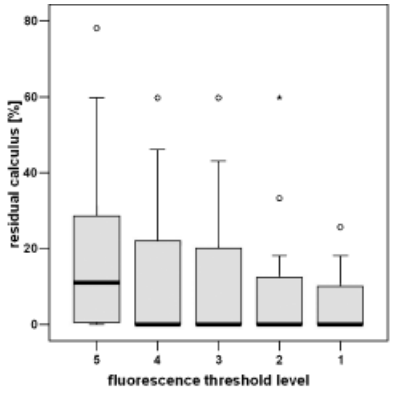

Fig. 4. Box plots of residual calculus (%) of total area related to the fluorescence threshold level ($n = 20$). The amount of residual calculus decreased with lower threshold levels. Statistically significant differences between threshold level 5 and 4 and 2 and 1 ($p < 0.05$, Wilcoxon's). Box plots show median, first and third quartiles and minimum and maximum values (whiskers). Outliers are marked as data points and asterisks.

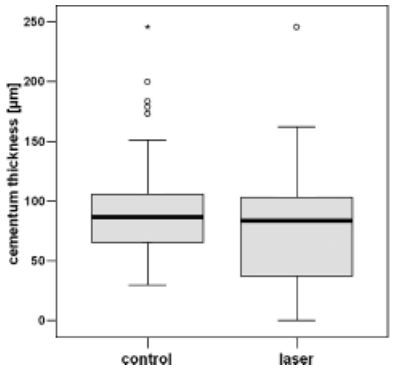

Fig. 5. Box plots of the cementum thickness (μm) of the untreated (control) and the Er:YAG lased root surfaces ($n = 20$). Statistically significant lower thickness of the cementum layer after laser scaling compared with the non-treated opposite site of the root surfaces ($p < 0.05$, Wilcoxon's). Box plots show median, first and third quartiles, and minimum and maximum values (whiskers). Outliers are marked as data points and asterisks.

walls were observed along irradiated root surfaces.

Discussion

The objectives of subgingival debridement are to remove not only the adherent bacterial plaque but also mineralized deposits. However, the removal of calculus using conventional hand instruments has been reported to be often incomplete and rather time consuming (Yukna et al. 1997). To improve the efficacy of root surface debridement, devices such as sonic and ultrasonic scalers have been used. However, sometimes the complex root anatomy makes it difficult to achieve a biologically compatible root surface (Kepic et al. 1990). Considering these difficulties in performing successful periodontal treatment, laser scaling was introduced as an alternative to conventional scaling procedures. Comparing different laser types, Er:YAG laser devices show characteristics making them a promising tool for periodontal treatment (Ishikawa et al. 2004). Therefore, the Er:YAG laser is most commonly studied for use in periodontal debridement procedures (Aoki et al. 1994, 2000, Schwarz et al. 2001, Eberhard et al. 2003). The present in vitro study confirmed the use of the Er:YAG laser as an alternative to conventional scaling procedures. It could be shown that the laser seemed to be suitable to achieve an almost complete removal of calculus during non-surgical periodontal treatment. However, a major problem in non-surgical periodontal therapy is that the present diagnostic methods used to indicate the endpoint of calculus removal are quite subjective and may thus lead to either under- or overinstrumentation. Traditionally, the presence or absence of subgingival calculus is explored using a probe. However, it has been demonstrated that it might be difficult to recognize the endpoint of root surface instrumentation (Sherman et al. 1990). Thus, the main failure in periodontal treatment may depend on calculus remaining after therapy. Ideally, a diagnostic tool should objectively indicate the presence or absence of subgingival calculus. Recently, a novel calculus detection system using spectro-optical technology has been shown to be both specific and sensitive (Krause et al. 2005). Laser fluorescence measurements primarily developed for caries diagnosis were also evaluated for detection of calculus (Folwaczny et al. 2002, 2004, Krause et al. 2003). By combining the possibility of the detection of calculus by laser fluorescence with an Er:YAG laser it is expected that the laser can be guided to complete calculus removal. The results of this study could demonstrate the capacity of selective calculus removal for an Er:YAG laser that is controlled by the fluorescence signal induced by a red-infrared diagnostic laser. This is in accordance with a recently published study evaluating the influence of fluorescence-controlled Er:YAG laser radiation on periodontally diseased root surfaces in vivo using different laser parameters (Schwarz et al. 2006). The authors of this study concluded that the Er:YAG laser with a fluorescence feedback system may be used clinically at a panel setting of 140 mJ and 10 Hz in order to optimize calculus ablation but also to prevent undesirable root surface alterations. Therefore, in our study these parameters were used as the basic panel setting. Furthermore, it was reported that the mean percentages of residual subgingival calculus were $6.2 \pm 3.9\%$. In the present study, the median value for RC ranged from 0% to 11%, depending on the respective threshold value. However, in the in vivo study the mean percentages of RC could only be related to the overall instrumented root surface and not to the baseline amount of calculus. Thus, these values are not contradicting to the higher values of the present study, assessing RC in relation to the baseline amount of deposits. Unfortunately, no information was given whether or how the calibration procedure of the fluorescence feedback system was performed and which fluorescence threshold level was used. In contrast, following Er:YAG laser radiation without a feedback system, a RC of 32% was observed (Eberhard et al. 2003). Regarding hand instrumentation, 4–13% of the surfaces appeared free of mineralized deposits (Eberhard et al. 2003, Braun et al. 2006, Schwarz et al. 2006). However, comparisons between studies dealing with calculus removal are difficult, as there is no consistency between study designs and methodologies.

Several studies described the morphological changes of root surface after Er:YAG laser treatment (Aoki et al. 1994, Fujii et al. 1998, Gaspirc & Skaleric 2001, Frentzen et al. 2002, Eberhard et al. 2003, Schwarz et al. 2006, Crespi et al. 2006). Many of these studies relied on scanning electron microscopy (SEM) observations. Until now, little information is reported about the effects induced by lasers on the cementum layer situated below the lased surfaces by analysis of undecalcified histologic sections. In an in vivo study on laser treatment without feedback system, it was reported that the Er:YAG laser treatment induced only a minimal reduction of the cementum (Eberhard et al. 2003). This is in contrast to in vitro studies showing influences on the cementum layer and dentin (Frentzen et al. 2002, Crespi et al. 2006). In an in vivo study employing the feedback-controlled Er:YAG laser, loss of cementum was generally non-existent, indicating that subgingival calculus was almost selectively removed (Schwarz et al. 2006). In the present study, the possibility of selective calculus removal could also be demonstrated. However, a partial loss of cementum was found in comparison with the untreated opposite site of the root surface. In very few instances, a denudation of dentin surface could also be observed. In this in vitro study, root surfaces of extracted teeth were covered with water during laser treatment. No signs of cracking or carbonization could be observed, indicating no thermal damages. Clinically, gingival tissue covering the root surface might prevent the irradiated area from sufficient water cooling and could therefore lead to thermal alterations of the cementum as observed in an in situ study on human corpses (Folwaczny et al. 2003). However, such alterations of the root surface have not been reported in studies that evaluated extracted teeth after laser irradiation in vivo (Eberhard et al. 2003, Schwarz et al. 2006). Concerning histomorphometric evaluation of cementum thickness, referring to the opposite side of the instrumented root surface as control and by examining an overall number of 200 corresponding surface sections, intra-individual differences of the cementum thickness occurring along the root of one tooth (Schroeder 2000) could be neglected. Regarding root surfaces after scaling and root planing using conventional hand instruments, the root cementum layer exhibited conspicuous root surface alterations or was completely removed including many deep scratches on the dentin layer (Eberhard et al. 2003, Crespi et al. 2006, Schwarz et al. 2006). This may result in dentin hypersensiblity and caries lesions (Adriaens et al. 1988, Haugen & Johansen

1988, Pashley et al. 1996). Although there exists some controversy about the amount of cementum removal, necessary for the creation of biocompatible root surface conditions (Nyman et al. 1988, Smart et al. 1990, Chiew et al. 1991), the root substance removal obtained in the present study appears to lie under the range as achieved with hand instruments. The substance removal was reported to range from 34 μm to 343 μm, depending on the number of strokes and the working force (Zappa et al. 1991, Ritz et al. 1991). Compared with root substance removal by sonic and ultrasonic devices, the present study lies within the range as that achieved with piezoelectric ultrasonic scalers (26–107 μm) and magnetostrictive ultrasonic scalers (14–411 μm), depending on the working parameters (Flemmig et al. 1998a, b). Assessing cementum removal laserprofilometrically, a defect depth of up to 24 μm could be demonstrated *in vitro* using magnetostrictive and piezoelectric ultrasonic devices with different scaler tips (Jepsen et al. 2004). Evaluating root surface removal with diamond-coated ultrasonic inserts, the mean depth of the root structure removed lies between 46.2 and 142.0 μm, depending on the number of instrumentation strokes (Vastardis et al, 2005). In a study evaluating the amount of root substance removal using Er:YAG laser radiation without the fluorescence feedback system, the defect depth ranged from 41 μm to 640 µm depending on the working tip angulation and energy setting (Folwaczny et al. 2001). In the present study, a lower removal of root substance could be shown. By repeating the application of the Er:YAG laser irradiation using different threshold levels within the same demarcated area on the root surface, cementum removal might be influenced. Normally, the laser device should not be activated once calculus is removed completely. However, in case of laser irradiation caused by lowering the threshold level and without any calculus present, the values for root substance removal measured in the present study can be taken as the worst case. Consequently, using only one single threshold level, one has to expect less root substance removal.

In conclusion, the present in vitro study indicates that the amount of RC on the root surface depends on the fluorescence threshold level for a feedback-controlled Er:YAG laser. Because ablation by this laser is controlled by the induced fluorescence of bacteria or bacterial products of the mineralized deposits, this method may be superior to current laser applications for periodontal treatment, determining the endpoint of debridement by subjective means. By lowering the fluorescence threshold level, a more complete removal of subgingival calculus could be achieved. One might conclude that calculus removal would be most effective without a feedback system. However, using the Er:YAG laser without a feedback system would result in a continuous emission of laser energy, even if there is no calculus present. As a result, laser energy might lead to root substance removal and thus overinstrumentation could occur. Based on these results, it might be suggested that the Er:YAG laser may be used at a panel setting of 140 mJ and 10 Hz with a threshold level even lower than the 5 [U], recommended by the manufacturer, and undesirable root surface alterations during non-surgical periodontal treatment might be prevented.

On the basis of these results, future studies are warranted to validate the present data under in vivo conditions, before a specific fluorescence threshold level can be recommended for clinical use.

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Conflict of Interests: The authors declare that they have no conflict of interests.

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Clinical Relevance

Scientific rationale for the study: Laser fluorescence-controlled calculus removal using an Er:YAG laser might facilitate periodontal debridement procedures. However, there are no data about the appropriate fluorescence threshold level.

Principal findings: The amount of residual subgingival calculus depended on the laser fluorescence threshold level. Even with a low threshold level, the amount of root cementum reduction after Er:YAG laser debridement was only minimal.

Practical implications: Using a fluorescence-controlled Er:YAG laser, a more complete calculus removal might be achieved by lowering the fluorescence threshold level. Clinically, undesirable major root surface alterations during non surgical periodontal treatment can be prevented.