Evaluation of Selective Caries Removal by a Fluorescence Feedback-Controlled Er:YAG Laser in vitro

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Key Words
Dental caries • Fluorescence • Laser • Treatment

Abstract
Aim: To establish a fluorescence threshold level that could guide a therapeutic Er:YAG laser through a caries lesion to determine a therapeutic endpoint of caries removal. Materials and Methods: A total of 65 extracted human teeth, 35 with dentine caries and 30 healthy, were used for this study. An Er:YAG laser system that emitted at a wavelength of 2.94 µm was used. The laser was equipped with a laser fluorescence feedback system, excitation wavelength 655 nm, to control the irradiation by the Er:YAG laser. The evaluated threshold levels of the fluorescence feedback system were 3, 7, 8, 10, 12, 15 and 20. After treatment the teeth were prepared for histological staining according to the method of Brown and Brenn for the identification of bacteria. The specimens were subjected to a quantitative evaluation of residual bacteria on the treated dentine surface. In addition, the internal fluorescence of dentine and potential fluorescence changes of dentine after laser irradiation were evaluated. Results: About 80% of the irradiated dentine surface showed residual bacteria with threshold levels of 20, 15, 12, and 10. Residual bacteria were not found with threshold levels of 7 and 3. The study revealed a significant increase in dentine fluorescence after laser irradiation. Conclusion: The results of the present in vitro study indicate that a fluorescence threshold level of 7 or 8 units can guide an Er:YAG laser to a complete removal of carious dentine.

By the time a caries process can be detected the lesion is no longer incipient [Gwinnett, 1971] and is generally destined to be restored because of the irreversible nature of the lesion [Angmar-Månsson and ten Bosch, 1987]. The most common procedures for removing infected dental hard tissues are by excavators and burs [Banerjee et al., 2000]. When removing demineralized dentine it is not always easy to know at what point excavation is completed because there is an apparent lack of objective clinical markers for the differentiation between infected and healthy dentine. The clinician tends to rely on the consistency of the tissue, but no rigorous correlation between the consistency of the dentine and the area of infection could be established and changes in hardness and colour are continuous and not abrupt. Therefore, the operative treatment of carious lesions by rotary instruments depends to a significant degree upon the clinical skills of the operator and has often resulted in considerable removal of healthy tooth structure [Banerjee et al., 2000]. Conse-
sequently, a clinical method for the distinction between the outer infected dentine and the inner non-infected dentine is required for the rational treatment of dental caries with minimal destruction of healthy tissues.

Several approaches for the selective removal of infected dentine have been introduced during the last decades including the use of caries detector dyes and the chemomechanical caries removal technique. The staining of different carious layers was introduced in 1975 by Ogushi and Fusayama [1975]. However the susceptibility to dye staining by acid red appears to be a feature of reduced mineral content rather than being specific for a carious lesion. Sound circumpulpal dentine and the dentino-enamel junction could also be stained, which may result in the unnecessary removal of sound dentine [Kidd et al., 1993; Yip et al., 1994]. The chemomechanical caries removal approach is basically a gel-assisted hand excavation that is also aimed to determine the endpoint of caries excavation. Chemomechanical caries removal may be associated with a risk of leaving caries in the dentino-enamel junction [Cederlund et al., 1999] and is much more time-consuming than conventional excavation [Tavares et al., 1988], even though other study groups reported successful caries removal with this system [Ericson et al., 1999].

There has been growing interest in using lasers as diagnostic devices that capitalize on measurements that discriminate between benign and malignant cells [Bohorouch, 2000]. These methods are based on laser-induced fluorescence that is used for a variety of applications like electrophoresis [Paez and Hernandez, 2001] or photodynamic therapy [Sternberg and Dolphin, 1993]. Fluorescence is a phenomenon by which the wavelength of the emitted light is changed into a longer wavelength upon absorption and subsequent re-emission of light. The fluorescence of dental hard tissues has been known for a very long time and more recently laser light was used to induce fluorescence of enamel in a sensitive, non-destructive diagnostic method for the detection of enamel demineralization and dental caries [Bjelkagen et al., 1982; Sundström et al., 1985; Lussi et al., 2004]. Laser fluorescence was introduced to aid detection of occlusal caries as an adjunct to visual inspection and radiographic examination [Hibst and Gall, 1998]. Red laser diode light is directed to the tooth surface by a specially designed probe tip, and the fluorescent signal is filtered from the incident light and fed back to the detector through the same device. The excitation wavelength of 655 nm (red light) induces a fluorescence signal that has been assigned to a protoporphyrin, which is present as a bacterial breakdown product [König et al., 1998].

The use of laser-induced fluorescence to control a device that is capable of removing dental hard tissues would be a very promising approach for the selective removal of carious dentine. It has been technically realized by the combination of an Er:YAG laser and a modification of laser fluorescence in one dental instrument. Within this system the removal of dental hard tissues by the Er:YAG laser is controlled by the fluorescence signal from the tooth surface induced by the red-infrared diagnostic laser. However, at present there are no data available on the feasibility of this system for selective caries removal.

Therefore, it was the aim of this in vitro study to establish a fluorescence threshold level that could guide the therapeutic laser through a caries lesion until no bacteria were detectable by histological staining and hence a therapeutic endpoint of caries removal was reached. Moreover, for the safe use of this system additional information about the internal fluorescence of dentine and on changes of the dentine fluorescence induced by Er:YAG laser irradiation were gathered.

**Materials and Methods**

**Specimens**

Several private dental offices provided a total of 65 extracted human teeth, 35 with dentine caries and 30 healthy, that were used for this study. In each office, the teeth were extracted for periodontal, orthodontic or prosthodontic reasons and were obtained from patients who consented to their use for research. The teeth were stored immediately after extraction in buffered saline at 8°C until further processing.

**Laser Device**

An Er:YAG laser system (Key III Laser: Kavo, Biberach, Germany) that emitted at a wavelength of 2.94 μm with a spot size of 0.63 mm was used. The output settings were 250 mJ/pulse and the pulse repetition rate was 4 pulses/s. These parameters were chosen on the basis of preliminary experiments. Irradiation of a focused beam was performed for the present experiments with the non-contact handpiece 2060 (Kavo, Biberach, Germany). The irradiated area was continuously cooled by a water spray system (1 ml/min). The laser was equipped with a laser fluorescence feedback system (fig. 1). The emitted light with a wavelength of 655 nm (red light) is transported through a fibre bundle to the tip of the handpiece and the same tip, but different fibres, samples the fluorescent light. The laser-induced fluorescence of the dentine is measured and used to control the therapeutic irradiation by turning on the Er:YAG laser if the fluorescence value is above a preselected threshold level. If the dentine fluorescence is below this value the Er:YAG laser does not emit. For the present study the evaluated threshold levels of the fluorescence feedback system were 3, 7, 8, 10, 12, 15 and 20.

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Fluorescence Threshold Level

For all experimental procedures the enamel was removed by high-speed burs and water cooling until access to the carious lesion was achieved. On the surface of the carious lesion small incisions were cut with a sharp scalpel blade to divide the lesion into three areas that were treated as follows: (A) no treatment, (B) caries removal with a bur at a speed of 700 rpm without water cooling and (C) laser irradiation. The specimens were assigned randomly to the different groups.

Before the experiments the operator received a full training in caries removal using the laser. After calibration of the fluorescence feedback system following the instructions of the manufacturer the handpiece was manually adjusted perpendicular to the treated surface. The handpiece was used in the non-contact irradiation mode with a distance from the tip to the cavity floor of approximately 12–15 mm. The pilot laser beam was used to adjust the correct distance and as caries removal progressed and the distance of the treated cavity floor to the tip of the handpiece changed, irradiation...
was paused and the distance was readjusted. The laser treatment was terminated if the fluorescence feedback system did not indicate any reflective fluorescence from the dentine above the pre-selected threshold level. No caries disclosing agent or dental probe was used to explore the laser-treated dentine surface after laser irradiation. For every pre-selected threshold level 5 specimens were treated.

Conventional bur treatment was performed by an experienced dentist in a dry field by means of steel burs of the appropriate size mounted on a micromotor at low speed. The consistency of the tissue was examined with a dental explorer to confirm the completeness of caries removal.

Sample Preparation

After treatment the teeth were cut through the incisions along the tooth axis in three specimens with a cutting saw (WoCo Apparatebau, Clausthal-Zellerfeld, Germany). The specimens were fixed in phosphate-buffered formalin for 24 h and then washed in Sörensen phosphate-buffered saline for another 24 h. After dehydration by alcohol the specimens were embedded in methacrylate (Sigma-Aldrich, Dreieich, Germany). The polymerized specimens were placed onto microscopic slides and ground to approximately 30- to 60-μm-thin slices. Two slices were fabricated from each specimen (n = 240). The slices were stained by the method of Brown and Brenn [1931] for the identification of gram-positive (blue) and gram-negative (red) bacteria.

Quantitative Evaluation

The samples were investigated under a microscope (Axiohot 2, Carl Zeiss, Jena, Germany) at a total magnification of ×100.8 (Lens Plan-Neofluar ×63 and ocular E-P1 ×10; Zeiss). A test grid was mounted on the focal plane of the eyepiece of the microscope and consisted of a square frame of a definitive size enclosing an area of 4,681 μm². Five test grids were randomly located on the surface of treated dentine and the number of test squares positive for bacteria within the dentinal tubules was expressed as percentage of the total number of squares covering the treated dentine surface. For each threshold level 5 teeth were used and two individual specimens were investigated quantitatively.

Internal Fluorescence of the Dentine

Twenty non-carious third molars that had been removed during orthodontic treatments were selected for this experiment. The teeth were divided into halves along the long axis by a cutting saw with water cooling.

In 10 teeth, beginning from the occlusal surface to the roof of the pulp chamber the fluorescence of the tooth hard substance was measured with the laser handpiece 2060 (Key III Laser, Kavo) and with the DIAGNOdent device (Kavo). After each measurement about 0.5 mm of dentine was removed with a low-speed bur until the pulp chamber became exposed. A micrometer measuring system under a stereomicroscope at ×16 magnification was used to measure the distance between the prepared tooth surface and the pulp chamber. In a second group (n = 10) this procedure was repeated to measure the internal fluorescence of the dentine in the opposite direction from the pulp chamber to the enamel-dentine border.

Fluorescence of Irradiated Dentine

Ten non-carious third molars were selected and cut along the tooth long axis as described to determine the fluorescence changes in dentine induced by therapeutic laser irradiation. The dentine was irradiated with the therapeutic Er:YAG laser – without the fluorescence-feedback system – under a stereomicroscope at ×16 magnification until approximately 0.5 mm of dentine had been removed. The fluorescence of the resulting dentine surface was recorded by both the therapeutic laser system (Key III laser handpiece 2060) and by the diagnostic device (DIAGNOdent) and subsequently 0.5 mm of dentine was removed with a rotary bur. Again, the fluorescence values were recorded and the dentine was irradiated by the Er:YAG laser until another 0.5 mm of dentine was removed. This procedure was continued until the pulp chamber became exposed.

Statistical Analysis

For each experimental group the fluorescence values were described by means ± standard deviations (SD). Obvious extreme values or outliers were not observed. Nevertheless, normal distribution was not assumed in the data for statistical testing, because for 10 teeth in each experimental group any test for normal distribution has only small power. Differences between reading points at different steps of the experimental procedure were evaluated with Friedman analysis of variances by ranks and multiple comparisons. Differences between the fluorescence readings of the DIAGNOdent and the laser and between different treatment regimes were compared at each step by Wilcoxon matched-pairs signed-rank test. All calculations were performed with the statistical program SPSS 11. Effects were regarded as statistically significant for p < 0.05.

Results

Fluorescent Threshold Level

The dentine surface of an untreated carious lesion showed that 100% of the dentinal tubules located in the carious lesion contained bacteria that completely filled the dentine tubules (fig. 2A). Following treatment of the carious lesions with a rotary bur and confirmation of caries removal with a dental explorer no bacteria were found in the residual dentine of the cavity floor (fig. 2E).

The results for the histological evaluation following caries removal by Er:YAG laser are presented in figure 2A–D. Employing the Er:YAG laser with fluorescence threshold levels of 20, 15, 12 and 10, respectively, revealed only a minor reduction of residual bacteria within the dentinal tubules from 94.0 to 88.0% (table 1). In contrast, the fluorescence threshold level of 8 resulted in a significant decrease in residual bacteria within dentinal tubules of the cavity floor to a mean percentage of 6.7%. Laser ablation of carious dentine with the guidance of the fluorescence threshold levels of 7 and 3 resulted in no residual bacteria within the dentine tubules located at the cavity floor (table 1).
Caries Treatment by Fluorescence-Guided Er:YAG Laser

Fluorescence of Dentine

The measurements of the dentine fluorescence by the laser handpiece and the DIAGNODent device from the enamel-dentine surface to the roof of the pulp chamber are illustrated in figure 3. The mean fluorescence values of healthy dentine measured by the DIAGNODent device were statistically significantly higher than those measured with the laser handpiece for every reading point \((p = 0.005)\). The statistical analysis demonstrated no significant differences between the mean fluorescence values for different measuring points from the dentinoenamel junction to the roof of the pulp chamber for the DIAGNODent device or for the laser handpiece, even though the values had a tendency to increase 2.0 mm away from the roof of the pulp chamber. In consequence, a mean fluorescence recording for dentine of \(6.2 \pm 2.4\) (mean ± SD) was measured with the DIAGNODent device and of \(1.1 \pm 0.8\) with the laser handpiece.

In a similar fashion fluorescence values of healthy dentine from the roof of the pulp chamber to the dentinoenamel junction are presented in figure 4. Again, the mean fluorescence values measured with the DIAGNODent device were statistically significantly higher than those measured with the laser handpiece. The experiment was reproduced 10 times on different samples.

Table 1. Means and SD of the residual amounts of bacteria (%) estimated by a quantitative approach on the laser-treated dentine surface with different threshold levels of the fluorescence feedback system

<table>
<thead>
<tr>
<th>Laser threshold value</th>
<th>n</th>
<th>Residual bacteria, % of tubules</th>
<th>mean</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated caries</td>
<td>5</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bur treatment</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>94.0</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>98.0</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>90.0</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>93.3</td>
<td>11.6</td>
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<tr>
<td>8</td>
<td>5</td>
<td>6.7</td>
<td>11.6</td>
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<td>7</td>
<td>5</td>
<td>0.0</td>
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<td>3</td>
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For control purposes untreated carious lesions and carious lesions treated by a bur were used. For each threshold level 5 teeth were investigated and the quantitative evaluation was performed on two separate specimens.
dent device were statistically significantly higher than those obtained with the laser handpiece for every reading point (p = 0.005). There were no statistically significant differences between the mean fluorescence values for individual measuring points from the roof of the pulp chamber to the dentinoenamel junction for both fluorescence devices. Thus, by scanning the dentine from the roof of the pulp chamber to the dentinoenamel junction – opposite to the direction of the first series of measurements – a mean fluorescence recording for dentine of $5.3 \pm 2.8$ was measured with the DIAGNOdent device and of $1.3 \pm 0.9$ with the laser handpiece.

**Fluorescence of Laser-Irradiated Dentine**

The Er:YAG laser irradiation significantly increased the fluorescence signal of sound dentine measured by DIAGNOdent (fig. 5) or the laser handpiece (fig. 6) compared to the dentine treated with a bur, irrespective of the reading point within the dentine (p = 0.005). The mean fluorescence of dentine increased from $5.4 \pm 2.5$ to $18.2 \pm 11.4$ after laser irradiation measured by the DIAGNOdent device, and from $1.4 \pm 1.1$ to $3.3 \pm 1.9$ measured with the laser handpiece.

**Discussion**

Present diagnostic methods used to indicate the endpoint of caries removal in a clinical situation are quite subjective and may thus lead to either under- or overinstrumentation. Ideally, a diagnostic tool should indicate the presence or absence of bacteria because of the infectious nature of the disease. A defined endpoint of caries removal would therefore coincide with the complete removal of all bacteria-infected dentine. The results of the present in vitro study could demonstrate the capacity of selective caries removal for an Er:YAG laser that is controlled by the fluorescence signal from bacterial breakdown products induced by a red-infrared diagnostic laser.

Hibst and Keller [1989] first demonstrated the effective ablation of dental hard tissues by means of the Er:YAG laser. The application of the Er:YAG laser has been used for caries treatment in vitro [Aoki et al., 1998; Armengol et al., 1999] and in clinical situations as well [Keller and Hibst, 1997; Keller et al., 1998]. No differences in the effectiveness of dentine removal by an Er:YAG laser compared to a conventional drill have been
reported [Hibst and Keller, 1989; Keller and Hibst, 1989; Shigetani et al., 2002]. In general, it is accepted that under adequate water spray and with a careful irradiation technique, cavities without any sign of thermal damage to the surrounding tissues as well as to the dental pulp could be produced with the Er:YAG laser [Hossain et al., 1999; Yamada et al., 2001]. Animal histological studies also showed that the pulp response to the Er:YAG laser appears to be similar to the response from high-speed handpiece application [Sonntag et al., 1996]. In an in situ study no pathological changes were observed 7 days after Er:YAG laser treatment by light transmission electron microscopy and 3 months postoperatively the apposition of tertiary dentine has been demonstrated [Nair et al., 2000].

The experiments aimed at determining a threshold level for the fluorescence-aided caries removal by an Er:YAG laser demonstrated almost no caries removal with threshold levels between 10 and 20, a marked reduction of residual bacteria below 10% with a threshold level of 8, and no detectable residual bacteria for threshold levels of 7 and smaller. It is noticeable that no linear correlation between the bacterial reduction and the pre-selected threshold level could be observed, but rather an abrupt decrease in a narrow range between 7 and 9 units. Thus, the fluorescence feedback system appears to operate by a true ‘yes’ or ‘no’ decision with respect to the detection of even small numbers of bacteria within the dentine. In contrast to other methods like explorers aimed to determine the endpoint of caries removal the induced fluorescence of 655 nm is likely related to bacterial components as proposed by König et al. [1998]. The relation between microorganisms or bacterial products and the fluorescence signal has been confirmed in the present study and in a recently published study by Iwami et al. [2003].

In contrast to other fluorescence-aided caries excavation techniques like the FACE method [Lennon et al., 2002; Lennon, 2003], that combines caries detection by fluorescence with caries removal by a rotary instrument, the fluorescence directly regulates the activity of the device used for dentine treatment, which might be an advantage during clinical use.

For the safe use of this system, additional information about the internal fluorescence of dentine and on changes of the dentine fluorescence induced by Er:YAG laser irradiation was required. With respect to the determination of a threshold level, substantial variations of the fluorescence in different regions of sound dentin would rule out the possibility of determining a single threshold level or even the use of this system for clinical caries removal.

In fact, previous studies showed that the transmission and scattering properties of dentine differ according to the direction of the tubules and this affected the fluorescence measurements [Vaarkamp et al., 1995; Iwami et al., 2003]. Therefore, in the first part of the present study we evaluated the fluorescence characteristics of sound dentine with the laser handpiece and with a laser fluorescence device to investigate the fluorescence readings from the dentinoenamel junction to the roof of the pulp. It could be demonstrated that the fluorescence readings showed only minor variations at different locations of the dentine that were not statistically significantly different. Increased fluorescence values of the dentine approaching the roof of the pulp chamber were also reported by Lussi et al. [2000]. Irrespective of the direction of the readings, towards the pulp or away from the roof of the pulp chamber, no differences in fluorescence signals were observed and it was therefore possible to determine mean fluorescence values for non-carious dentine.

Laser fluorescence has been proven as a useful tool for the detection of early lesions on the occlusal surface [Lussi et al., 1999, 2001; Francescut and Lussi, 2003], although the agreement between validated caries and the fluorescence signal is still unsatisfactory when using the device to detect carious dentine under enamel [Heinrich-Weltzien et al., 2003]. However, the carious lesion is not covered by enamel in the current approach and this might improve detection. When comparing the fluorescence readings by the laser handpiece and by laser fluorescence, the latter measurements were about 4–6 times greater, but the two sets closely correlated with each other. This observation was most likely due to different light transmission systems in the laser handpiece and in the DIAGNOdent device. The light transmission window of the laser handpiece has a larger diameter than that of the cone-shaped tip of the DIAGNOdent and the former picked up light from outside the target surface as well. Therefore, the manufacturer adjusted the sensitivity of the laser handpiece.

Another safety issue that had to be addressed before any clinical use was the possible impact on the dentine fluorescence by the Er:YAG laser irradiation itself. Instead of perpendicular measurements of sectioned carious teeth we selected an experimental protocol that closely resembled the clinical direction of caries removal towards the pulp chamber. Interestingly, the results of the present study could demonstrate that the ablation of dentine by the Er:YAG laser significantly increased the fluorescence values of the resulting dentine surface as measured by the laser and the laser fluorescence device. Again,
the laser fluorescence readings were greater by a factor of 5–6 than the laser readings. The observed increase of the fluorescence induced by the laser irradiation was by a factor of 2–3. It could be assumed that laser treatment of the dentine was followed by a dehydration of the dentine that could induce the observed increase in fluorescence. In contrast to the present findings in vitro studies demonstrated that dehydration of enamel was accompanied by decreased fluorescence values [Al-Khateeb et al., 2002; Pretty et al., 2004]. Whether structural changes of the dentine after laser ablation, dehydration or any other thermal effects were the origin of the changes in fluorescence values could not be evaluated in the present study; this question should be addressed by future research.

The increase in fluorescence values by the Er:YAG laser itself is of potential concern, because sound dentine could emit fluorescence signals exceeding the established threshold level leading to its removal. However, from the present data it could be calculated that 68 and 98% of fluorescence values read by the laser handpiece from irradiated sound dentine were in the range of 1.4–5.2 and 0–7.1, respectively. From these data it could be concluded that a threshold level of 7 or greater will not have any impact on changes in dentinal fluorescence induced by the laser irradiation itself for most of the cases under the present in vitro situation.

In conclusion, the results of the present in vitro study suggest that a fluorescence threshold level of 7 or 8 units could guide an Er:YAG laser to a complete removal of carious dentine. Because ablation by this laser is controlled by the induced fluorescence of bacteria or bacterial products this method may be superior to current methods for caries removal where the endpoint of the treatment is determined by subjective means. Thus, on the basis of these promising results future studies are warranted to validate the present findings under in vivo conditions, before fluorescence-controlled selective caries removal by Er:YAG laser can be recommended for clinical use.

References


Caries Treatment by Fluorescence-Guided Er:YAG Laser

Abstract
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