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ORIGINAL ARTICLE

Cavity size difference after caries removal by a fluorescence-controlled Er:YAG laser and by conventional bur treatment

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Abstract To determine the extensions of cavities prepared conventionally by bur or by a fluorescence-controlled Er: YAG laser. Sixty-five human teeth with dentine caries were bisected through the caries lesion and were treated by a fluorescence-controlled Er:YAG laser in a non-contact or a contact mode or by a rotary bur. The specimens were subjected to histological staining and a quantitative evaluation of cavity area (mm^2) by computer-assisted alignment. Data were tested for statistical significant differences by the Wilcoxon test ($p<0.05$). Twenty-three out of 29 cavities were smaller after caries removal with the non-contact laser compared to the bur. For a threshold level of seven, a cavity size difference of 1.63 (1.86) mm^2 was calculated compared to a cavity size difference of 5.35 (5.05) mm^2 after bur excavation. The differences were statistically significant ($p=0.029$). No significant differences were observed between the cavity size differences after excavation with the non-contact or the contact laser handpiece. Residual bacteria within the cavity floor were found only in low numbers after all treatments. The present in vitro study indicates that caries removal by a fluorescence-controlled Er:YAG laser using a threshold level of seven resulted in less dentine loss than preparations by a bur.

Keywords Dental caries · Laser fluorescence · Er:YAG laser · Feedback

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Introduction

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 chemo-mechanical caries removal technique. While staining may result in the unnecessary removal of sound dentine, the chemo-mechanical approach may be associated with the risk of leaving caries at the dentinal–enamel junction [3, 10, 22]. However, the marginal adaptation of restorative materials and primary molar teeth was rated better after chemo-mechanical removal compared to hand excavation [12].

Laser fluorescence was introduced to aid detection of occlusal caries as an adjunct to visual inspection and radiographic examination. The excitation wavelength of 655 nm (red light) induces a fluorescence signal that has been assigned to protoporphyrins and to the presence of bacteria in caries lesions [4, 9, 13]. Fluorescence to aid caries removal by conventional bur treatment has been used in vitro [15]. Recently, the suitability of a fluorescence-controlled Er:YAG laser for the selective removal of carious dentine in vitro has been demonstrated [4]. 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 a red-infrared diagnostic laser. The experimental set up was suitable to demonstrate the complete removal of bacterial infected dentine with threshold levels smaller than seven. However, the experiments could not exclude the removal of healthy dentine with the fluorescence-controlled laser beyond the outer infected layer. The aim of the following in vitro study was to compare the extensions of cavities following caries removal either prepared conventionally by a bur or by the fluorescence-controlled Er:YAG laser equipped with two different laser tips.

Materials and methods

Specimens

Several private dental offices provided a total of 65 extracted human permanent molar teeth with dentine caries 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 and with the contact handpiece 2061 (Kavo, Biberach, Germany). A sapphire fibre-optic with a cylindrical diameter of 1.1 mm was mounted to the handpiece 2061 for ablation of dentin in the contact mode. The irradiated area was continuously cooled by a water spray system (1 ml/min).

The laser was equipped with a laser fluorescence feedback system. 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 sample 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 pre-selected 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 six, seven and eight.

Experimental protocol for caries removal

The experimental protocol and the quantitative determination of the cavity size after excavation are illustrated in Fig. 1. For all experimental procedures access to the caries lesion was prepared by high-speed diamante burs under sufficient water cooling. The caries lesions were cut in two halves along the tooth long axis with a diamond saw of 300 µm width. The bisected caries lesions were treated either by the non-contact handpiece 2060, the contact handpiece 2061 or by a conventional bur. Randomisation of tooth halves was obtained by pre-numbered containers, which are allocated serially to the different treatment regimes. Before the experiments, one 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 non-contact handpiece was manually adjusted perpendicular to the cavity floor. For the non-contact irradiation mode, the handpiece was used in 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. For hard substance irradiation in the contact mode, the glass tip of the handpiece was placed on the dentine surface. The laser treatment was terminated if the fluorescence feedback system did not indicate any emitted fluorescence from the dentine above the pre-selected threshold level. For every pre-selected threshold level, ten specimens were treated.

Conventional bur treatment was performed by two experienced dentists in a dry field by means of steel burs of the appropriate size mounted on a micro-motor at low speed. The endpoint of caries removal was determined by hard to probing cavity walls. Two calibrated dentists that have not done the caries removal independently examined the consistency of the tissues with a dental explorer to confirm the completeness of caries removal by the rotary bur and the laser treated dentine surface.

Sample preparation

After treatment, the specimens were fixed in phosphate-buffered formalin for 24 h and 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 polymerised specimens were placed with the machine-cut surfaces onto microscopic slides and ground to approximately 30-µm-thin sections (Fig. 1). One section was fabricated from every treated tooth half. The sections were stained by the method of Brown and Brenn for the identification of Gram+ (blue) and Gram− (red) bacteria [2].

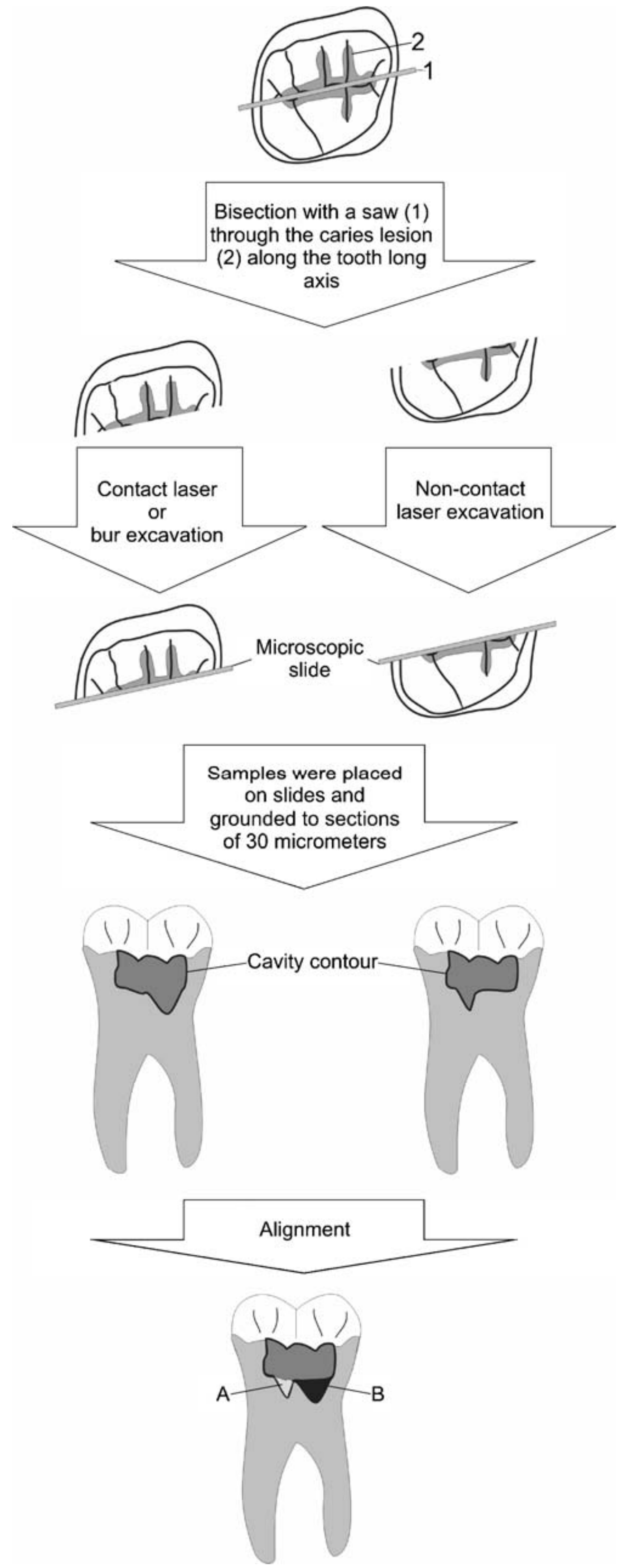


Fig. 1 The teeth were bisected with a saw (1) through the caries lesion (2) along the tooth long axis and the halves were randomly assigned to the experimental procedures. Either the non-contact laser application was compared to the rotary bur treatment or the non-contact laser was compared with the contact laser application. After caries removal, the tooth halves were fixated and the polymerised specimens were placed with the machine-cut surfaces onto microscopic slides and ground to approximately 30-μm-thin slices. The contours of the cavities were identified and computer assisted aligned. The cavity area (mm^2) for one of the three excavation techniques was determined by the area that was exclusively removed by one excavation technique (*A*=cavity size after excavation with the non-contact laser and *B*=cavity size after excavation with e.g. the bur). The area of the cavity that was removed by both treatment procedures was not taken into account

Quantitative evaluation of cavity dimensions

For the quantitative evaluation, corresponding tooth halves of one caries lesion were compared. Comparisons were computed between the non-contact laser and the bur treatment and the non-contact laser and the contact laser treatment. The stained sections were photographed under a microscope (Axiophot 2, Carl Zeiss, Jena, Germany) at a total magnification of ×2.5 (Lens Plan-Neofluar ×2.5 and occular E-P1 ×10; Fa. Zeiss, Jena, Germany) and afterwards, images were digitised. For the two corresponding sections from each dissected tooth, well-defined landmarks like cusps and the cemento-enamel junction were chosen for the following alignment of the cavities. The contours of the two different cavities were constituted and after appropriate calibration in mm^2, the area was calculated that was exclusively removed by one of the used excavation techniques (Fig. 1) using the Image J software (http://rsb.info.nih.gov/ij/). Because the exact contour of the coronally infected dental hard tissues could not be determined, the calculated area was not identical to the absolute dimensions of the cavity after excavation. In consequence, the primary outcome variable was the cavity size difference between two treatment regimes. Areas were measured with an accuracy of 0.01 mm^2 and every measurement was performed in duplicate.

Quantitative evaluation of residual bacteria

The sections were investigated at a total magnification of ×100.8 (Axiophot 2, Lens Plan-Neofluar ×63 and occular E-P1 ×10; Fa. Zeiss, Jena, Germany). A test grid was mounted on the focal plane of the eyepiece of the microscope that consisted of a square frame of a defined area of 4.9 μm^2. Five test grids were randomly located on the dentine profile of the prepared cavity and a number of test squares positive for bacteria within the dentinal tubules were expressed as percentage of the total number of squares covering the treated dentine surface.

Statistical analysis

For individual teeth, the cavity area (mm^2) according to the quantitative evaluation of the cavity dimensions was calculated. Differences in cavity size after the bur and the non-contact laser and the non-contact laser and the contact laser treatment were calculated for corresponding tooth halves. The distribution of the data was evaluated by the Kolmogorow–Smirnow test. Medians and inter-quartile ranges were computed for the three excavation techniques within one threshold level and differences were statistically tested for significance by the Wilcoxon test. Effects were regarded as statistically significant for $p<0.05$. All calculations were performed with the statistical program Statistical Package for the Social Sciences (SPSS) 11 (SPSS Software GmbH, München, Germany). The descriptive analysis of the residual bacterial content of the cavity floor was performed by calculating the percentage of positive test squares in relation to all investigated test squares in one experimental group.

Results

Evaluation of cavity dimensions after caries removal

One specimen was lost during sample preparation; thus, a total of 128 sections could be included for the calculation of the cavity size difference after caries removal with the Er:YAG laser and with the bur. All laser and bur treated cavities were rated caries-free by the two independent examiners with a dental explorer.

For individual teeth, the differences between the cavity size after non-contact laser and conventional bur treatment are presented in Fig. 2a. It was observed that the conventional bur removed less dentine than the non-contact laser handpiece in six out of 29 cavities with a range from 0.09 to 3.26 mm^2. The remaining 23 cavities were smaller after caries removal with the non-contact laser handpiece compared to the bur treatment with a range from 0.16 to 22.70 mm^2. Medians and inter-quartile ranges of the cavity size difference after treatment with the non-contact laser handpiece and the bur for the threshold levels of six, seven and eight were calculated and are presented in Fig. 3a. For a threshold level of six, two out of nine cavities were smaller after bur treatment compared to the laser treatment and seven cavities were smaller after treatment with the non-contact laser (Fig. 2a). A median cavity size difference of 2.60 (3.63) mm^2 was observed after treatment with the non-contact laser and of 7.25 (10.28) mm^2 after bur treatment. The differences between the cavity size difference after bur and after non-contact laser treatment using a threshold level of six were statistically not significant. For a threshold level

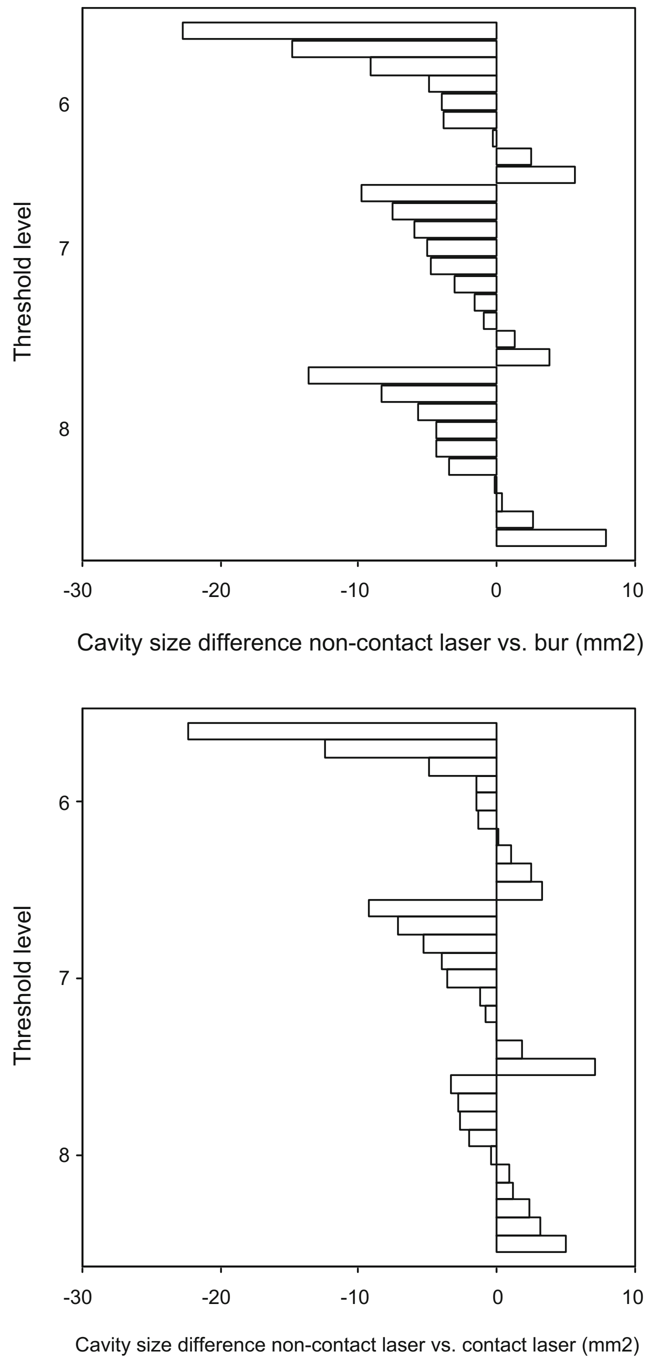
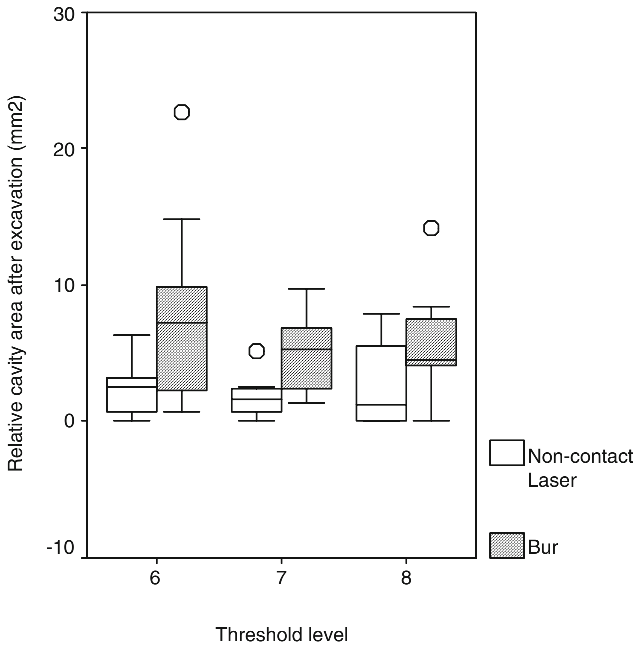


Fig. 2 Each *bar* represents the difference in cavity size between two corresponding cavities of one bisected caries lesion. **a** *Bars to the left* represent a larger substance removal and cavity size after treatment with the bur, *bars to the right* represent a larger cavity size after treatment with the non-contact laser. **b** *Bars to the left* represent a larger cavity size after the contact laser treatment; *bars to the right* represent a larger cavity size with the non-contact laser treatment

of seven, two cavities were smaller after bur treatment compared to eight cavities that were smaller after laser treatment (Fig. 2a). A median cavity size difference of 1.63 (1.86) mm^2 was calculated after non-contact laser treatment compared to 5.35 (5.05) mm^2 after bur excavation. The



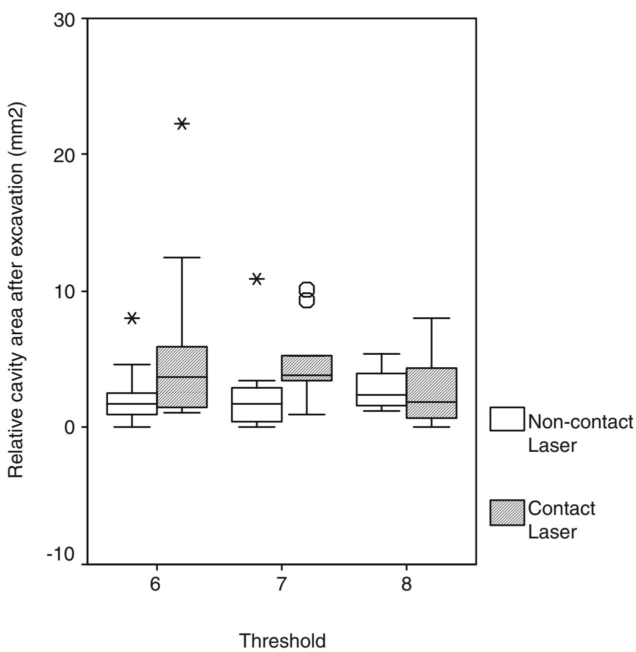


Fig. 3 *Box plots* presenting of the cavity size (mm^2) after treatment by the three caries excavation techniques used in the present study separated for every investigated threshold level. Differences of the cavity size after excavation with corresponding excavation methods were tested for statistical significance with the Wilcoxon test ($p < 0.05$). **a** *Box plots* of the cavity areas prepared by the non-contact laser or the rotary bur. **b** *Box plots* of the cavity areas prepared by the non-contact laser or the contact laser

differences between these areas were statistically significant (p=0.029). For a threshold level of eight, two out of ten cavities were smaller after bur treatment, in contrast to eight cavities that were smaller after non-contact laser treatment (Fig. 2a). A median cavity size difference of 1.20 (5.79) mm^2 was measured after non-contact laser preparation and an area of 4.49 (3.81) mm^2 after bur excavation. The differences were statistically not significant.

For individual cavities, the results for the differences in cavity dimensions after caries removal with the non-contact laser handpiece 2060 and the contact handpiece 2061 are illustrated in Fig. 2b. Thirty specimens could be evaluated in this experimental group. For 20 out of the 30 cases, caries treatment with the non-contact laser handpiece removed fewer dentines than caries treatment with the laser used in the contact mode with a range of cavity size from 0.00 to 22.31 mm^2. The remaining ten cavities were smaller after caries removal with the contact laser handpiece compared to the non-contact laser treatment with a range of cavity size from 0.00 to 10.95 mm^2. With respect to the different threshold levels, the results are as follows: For a threshold level of six, four out of ten cavities were smaller after contact laser excavation and six cavities were smaller after treatment with the non-contact laser (Fig. 2b). A cavity size difference of 1.72 (2.36) mm^2 was observed after treatment with the laser in the non-contact mode and of 3.74 (6.12) mm^2 after laser treatment in the contact mode. The differences between both treatment regimes were statistically not significant. For a threshold level of seven, two cavities were smaller after excavation with the contact laser handpiece compared to eight cavities that were smaller after excavation with the non-contact laser handpiece. A median cavity size difference of 1.72 (2.74) mm^2 was recorded for the non-contact laser treatment compared to 3.91 (3.30) mm^2 by the contact laser treatment. The differences between the areas were statistically not significant. For a threshold level of eight, five out of ten cavities were smaller after excavation with the contact laser and five were smaller after non-contact laser treatment. A median cavity size difference of 2.37 (2.66) mm^2 was calculated after non-contact laser excavation and a difference of 1.86 (3.83) mm^2 after contact laser excavation. The differences were statistically not significant.

Bacterial counts in the residual dentine surface

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. The Brown–Brenn staining following treatment of the carious lesions with a rotary bur and confirmation of caries removal with a dental explorer revealed that on average 1.33% of the test squares were positive for bacteria in the residual dentine of the cavity floor (n=30 teeth, 150 squares). The results for the histological evaluation following caries removal by the non-contact laser and by the contact laser for different threshold levels are presented in Table 1.

Table 1 Bacterial counts within the residual dentine surface evaluated by the enumeration of test grids following histological staining (*n*=stained sections)

	Number	Residual bacteria (%)
Untreated caries	10	100
Bur treatment	29	1.3
Non-contact laser		
Threshold level		
6	19	2
7	19	1
8	19	9
Contact laser		
Threshold level		
6	10	0
7	10	0
8	10	14

Discussion

The presently used diagnostic methods to indicate the endpoint of caries removal in a clinical situation are quite subjective and may thus lead to either under- or over-instrumentation. 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 a previous in vitro study demonstrated the capacity of a fluorescence-controlled Er:YAG laser for the selective removal of bacterial infected dentine. The laser is controlled by the fluorescence signal from bacterial breakdown products induced by a red-infrared diagnostic laser [4]. This study demonstrated that threshold levels of seven and lower could guide the laser to an endpoint of cavity preparation that coincided with the absence of histologically detectable residual bacteria within the cavity floor. However, the paradigm underlining the present study that all infection should be removed during caries therapy is actually under discussion [11, 19]. Recently, our group was able to demonstrate that the fluorescence-guided removal of infected dentine in close proximity to the dental pulp is not affected by interactions resulting from fluorescence emitted by the pulp tissues [14].

The main purpose of the present study was to compare the extensions of cavities prepared by the fluorescence-controlled Er:YAG laser and by a rotary bur. The comparison was focused on the cavity size, because previous experiments could not exclude that the laser removed not only bacterial contaminated but also healthy dentine. Because it is very difficult to gather absolute values for “over-excavation”, we compared the new laser procedure with the most common procedure for caries removal, the rotary dental bur [1]. The experiment used bisected caries lesions that were treated with the three different methods for caries removal and the analysis of the cavity size was performed by computer-assisted alignments. While computer-assisted alignment is a very accurate method for the calculation of areas, sectioning of the carious lesions has the handicap that the saw removed a small amount of the caries lesion and the two fragments were not necessarily of the same size. However, the randomised allocation of the bisected lesions to the experimental procedures ensured that no bias rose from this experimental detail.

Laser fluorescence has been proven as a useful tool for the detection of early lesions on the occlusal surface [5, 16, 17], although the agreement between validated caries and the fluorescence signal is still unsatisfactory when using the device to detect carious dentine under enamel [6]. Interestingly, a recent study failed to demonstrate the capability of the Diagnodent® device to guide an Er:YAG laser to remove the outer layer of infected caries dentine in vitro [23]. In contrast, the present study showed that the fluorescence controlled Er:YAG laser is well qualified for the removal of carious dentine. The present study showed that compared to the conventional bur, the fluorescence-controlled Er:YAG laser removed in 23 out of 30 cases, or in 75% of the cases less dentine regardless of the threshold level. For a threshold level of seven, the cavities after excavation with the non-contact laser were significantly smaller than after conventional bur treatment with equal amounts of bacterial counts after both treatments. A threshold level of six led to more hard substance removal than a threshold level of seven and, in consequence, the cavity areas were not statistically significantly different after both treatments. Excavation with a threshold level of eight is supposed to remove fewer dentines than lower levels; however, we could not observe this effect in our data. Few specimens showed less removal of dentine with the rotary bur compared to the non-contact laser treatment. Since the treatment procedures were all the same, the reason for this irregular observation might be related to structural irregularities of the lesion or the tooth hard substances that were not evaluated in the present study. In 66% of the observed cases, the non-contact laser removed less dentine than the contact laser treatment; however, we could not find any significant differences between the two treatment modalities. Different light transmission in the contact and non-contact laser handpiece may account for this variation in dentine removal. In particular, the beam profile of the non-contact handpiece is smaller than that of the contact handpiece and, in consequence, less tooth substance is removed during laser ablation. Based on these data, more research is needed to determine which laser device is more suited for the clinical use of caries removal.

When removing demineralised 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. 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 the considerable removal of healthy tooth structure [1]. The dentine area that has been removed less with the non-contact laser handpiece compared to the rotary bur amounted to a median value of approximately 3.95 mm^2, irrespective of the threshold level. However, what is the clinical significance of this value? In light of importance of the preservation of sound tooth substances, the following example may clarify the significance of these dimensions. One may translate an area of 3.95 mm^2 into a square with a side length of 2.0 mm. In relation to the mean dentine distance from the roof of the pulp to the dentine–enamel junction in first permanent molars of about 3.6 mm, this value is of considerable clinical significance [24].

Hibst and Keller first demonstrated the effective ablation of dental hard tissues by means of the Er:YAG laser. 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 effectively with the Er:YAG laser [7, 8, 18, 20, 21]. For the threshold levels of six and seven, the histological evaluation demonstrated that no or minimal amounts of bacteria were left within the residual dentine of the cavity floor. If bacteria were found within the cavity floor, the values were nearly identical to the values collected for the bur treatment. These data confirmed the results of our previous study that showed an almost identical reduction of bacteria with threshold levels of seven and lower [4]. Again, these results demonstrated the correlation between the fluorescence of carious dentine and the presence of bacteria that has been described also by others [9, 13].

Conclusions

In conclusion, the present study showed that the fluorescence-controlled Er:YAG laser required less dentine removal using a threshold level of seven compared to a rotary bur during caries treatment. The endpoint of the laser treatment was determined by the detection of no bacteria or bacterial counts comparable to the bur treatment within the residual dentine of the cavity floor. No differences were found between the non-contact laser handpiece and the contact laser handpiece. Excavation of a tooth half where the extension of the lesion is assessable is much easier than in a 3-dimensional cavity in a clinical situation. Thus, the function of the laser has to be further evaluated in clinical studies.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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