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ORIGINAL ARTICLE

Evaluation of selective caries removal in deciduous teeth by a fluorescence feedback-controlled Er:YAG laser in vivo

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Abstract This study investigated the ability and efficacy of an Er:YAG laser with a fluorescence feedback system for caries removal in deciduous teeth. Seventy-nine carious lesions were excavated using a fluorescence-controlled Er:YAG laser. Endpoint of treatment was defined by emission of fluorescence from the dentine surface below the preselected threshold level of 7 units and the subsequent termination of Er:YAG laser radiation. Dentine samples were obtained from the cavity floor, and viable counts of both *Streptococcus mutans* and *Lactobacilli*, expressed as colony forming units (log CFU), were evaluated. Preparation time was recorded to assess efficacy of the treatment procedure. *S. mutans* and/or *Lactobacilli* were found in 25 out of 79 lesions. Regarding the counts for *S. mutans* and *Lactobacilli*, the median log CFU was 0 (min, 0; max, 5.5) and 0 (min, 0; max, 6), respectively, with 2.4% of all samples yielding more than 100 CFU *S. mutans* and 4.8% yielding more than 100 CFU *Lactobacilli*. In 8 out of 79 cases, laser excavated cavities were not judged being caries-free using the conventional tactile criterion for assessing caries tissue. Focussing on these teeth, the median log CFU was 0 (min, 0; max, 0.5) for *S. mutans* and 0 (min, 0; max, 1.6) for *Lactobacilli*. The mean time for treatment was 2.3±1.2 min. Of the children, 93.8% rated the laser treatment to be comfortable. The study indicates that the fluorescence feedback-controlled Er:YAG laser might be an appropriate device for caries removal in children using the suggested threshold level of 7 units.

Keywords Caries removal · Deciduous teeth · Dentine caries · Er.YAG laser · Laser fluorescence

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Introduction

Various techniques for the removal of carious dentine have been described. In operative dentistry, the common concept for caries therapy is to remove decayed tissue mechanically during the excavation procedure using rotary instruments like burs or by hand instruments [6]. However, burs are associated with side effects such as noise, pain, overheating, vibration and discomfort [2, 7, 26]. For non-cooperative children, dental practitioners often prefer hand excavators because these cause less discomfort than a bur. Since the introduction of lasers in dentistry, their clinical use has become important. Previous studies have demonstrated both efficacy and patient acceptance of caries removal and cavity preparation using an Er:YAG laser [3, 5, 16, 20, 21]. However, during carious dentine removal, it is nearly impossible to know exactly when to stop excavation because of an apparent lack of objective clinical markers. As microbiological assessment of carious dentine during cavity preparation is not feasible in practice, clinical parameters like hardness, wetness and colour of the dentine are used to distinguish between active and inactive lesions [23, 36, 46]. Whereas a significant correlation has been

found between dentine hardness and the level of bacterial infection, the same correlation could not be found for tissue colour [24, 25]. Caries detector dyes were suggested to help differentiate between infected and sound dentine during caries excavation [40]. However, more recent studies have shown that these dyes are non-specific protein dyes that stain the collagen in the organic matrix of less-mineralised dentine whether it is infected or not [23, 36].

Another attempt to differentiate between carious and non-carious tissues is the use of laser fluorescence light [10, 33, 34, 35, 42, 43]. The phenomenon of dental hard tissue fluorescence was first described more than 90 years ago [45]. Since then, spectrographic studies have shown that carious dentine fluoresces more intensely in the red portion of the visible spectrum than sound dentine [1, 11]. Red fluorescence is thought to be emitted by porphyrins compounds synthesised by oral microorganisms in the carious lesion [28]. Thus, this kind of visible fluorescence may be used as a marker for infected dentine.

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. A caries excavation method (fluorescence-aided caries excavation, FACE) using orange-red auto-fluorescence as a marker for infected dentine during rotary caries excavation has recently been developed [30, 31]. Furthermore, the technology of caries detection by laser fluorescence has been combined with an Er:YAG laser for caries treatment. The emission of the Er:YAG laser is controlled by the fluorescence signal from carious tissue induced by a red-infrared diagnostic diode laser [14]. Therefore, the laser is activated only if the fluorescence emitted from the tooth surface exceeds a certain threshold level. A recently published in vitro study suggests that a fluorescence threshold level of 7 units could guide an Er:YAG laser to a complete removal of carious dentine [14]. This threshold level was confirmed for assessing the endpoint of caries removal without jeopardizing vital structures [29]. However, at present, there are no data published which validate these findings under in vivo conditions for both permanent and deciduous teeth. Additionally, this treatment procedure seems to be very effective for child patient [20]. It was suggested that the application of the Er:YAG laser system is a more comfortable alternative or adjunctive method to conventional mechanical cavity preparation especially in the treatment of children [20].

Hence, the aim of the present study was to evaluate the clinical performance of a fluorescence-controlled Er:YAG laser for caries removal in deciduous teeth. The hypothesis of a low level of bacterial infection after laser treatment in vivo was tested. Additionally, the aim of the study was to assess the clinical efficacy of a feedback-controlled Er:YAG laser treatment procedure and to evaluate sensations of pain and vibration.

Materials and methods

Study population

A total of 79 carious lesions in 42 children (19 female, 23 male) were included in this study. Only lesions with caries extending in the dentine layer were included. The subjects mean age was 7.7 years with a minimum of 3 and a maximum of 12 years. Patients were in good general health and with no complicating medical history. Teeth with signs of irreversible pulpitis were excluded.

All parents of the patients were informed about the aim of this trial in advance and signed a consent form according to the Helsinki Declaration II. The study had been approved by an ethics committee (dossier No. 05/1490, Independent Ethics Committee Freiburg, Germany).

Laser device

An Er:YAG laser device (Key III™, KaVo, Biberach, Germany), emitting a pulsed infrared radiation at a wavelength of 2.940 μm, was selected for laser treatment. The laser beam was guided onto the dentine surfaces under water spray irrigation (1 ml/min) with a non-contact handpiece (No. 2060, KaVo). The device was equipped with a laser fluorescence feedback system: The laser light of an InGaAsP diode laser with a wavelength of 655 nm 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 and collect the fluorescent light emitted from the irradiated tissue. The laser-induced fluorescence of the dentine surface is given in relative units [U] from 0–99 and controls the therapeutic irradiation by activating the Er:YAG laser if the fluorescence value is higher than a pre-selected threshold level. If the fluorescence is lower than this specific value, the laser does not emit.

Treatment procedures

Immediately before treatment, the absence of clinical signs of pulpal inflammation and the positive response to a thermal pulp test were confirmed. Local anaesthesia was used only if required by the patient. Enamel margins were removed with diamond burs in a high-speed handpiece to enable a straight access to the carious lesion. Before each laser treatment, a calibration of the feedback system of the Er:YAG laser was performed using an appropriate ceramic touchstone according to the manufacturer's instructions.

Laser parameters were set at 250 mJ per pulse with a repetition rate of 4 Hz and a pulse length of 400 μs. The used threshold level of the fluorescence feedback system was adjusted at 7 [U]. The working distance of the handpiece to the cavity floor was approximately 12 mm according to the manufacturer's instructions. For this distance, the corresponding focus diameter was 1.4 mm. During laser treatment, the patient and the dental personnel wore protective eyewear. The treatment followed all common guidelines for the safe use of lasers. Laser application was performed while moving the handpiece continuously above the carious lesion until the fluorescence feedback system could no longer detect any emitted fluorescence from the dentine surface above the pre-selected threshold level. The teeth were air-dried, cotton rolls were placed and, subsequently, dentine surface samples were obtained from the central cavity floor with a slow sterile rotating bur (Nr. 12,Komet, Lemgo, Germany) damped in saline until a 'burful' of dentine was collected [23, 24]. To obtain reproducible amounts of dentine, the operator had previously calibrated the sampling procedure. The bur was placed in an Eppendorf tube (Eppendorf, Wesseling, Germany) filled with 1 ml saline solution and shaken for 2 min to dislodge the adherent dentine sample. The completeness of caries removal was confirmed independently by two experienced investigators after laser excavation by tactile (sharp explorer) and auditory means of the air-dried cavities: Cavity preparation was considered complete when dentine was hard to a dental probe and the probe was capable to induce a sharp sound. Cavity preparation was continued with a conventional bur in cases when the investigators judged the caries removal to be incomplete. For every laser treatment, the time required for caries removal was measured. The preparation time was recorded using a stopwatch, starting with the first laser pulse until the laser did not emit any longer. To assess the patient's perception of pain and vibration during laser treatment, immediately after excavation with the laser, each child was asked to give a score according the following rating: 'comfortable', 'uncomfortable' or 'very uncomfortable'.

Microbiological procedure

CRT Bacteria™ (IvoclarVivadent, Ellwangen, Germany) was chosen as a commercial bacterial colony counting test kit to evaluate both *Streptococcus mutans* and *Lactobacillus* on the same two-sided dip slide containing mitis-salivarius and rogosa agar, respectively [4, 27]. Mitis-salivarius agar contains bacitracin, a polypeptide antibiotic which inhibits the growth of other oral microorganisms. Before the present study, the test was modified to assess bacteria in hard tissues of the cavity floor: Both agars were entirely covered with the dentine suspension described using a pipette. A $NaHCO_3$ tablet placed in the test vial released CO_2 when it came into contact with moisture, ensuring anaerobic conditions for bacterial growth. Dip slides were inoculated, incubated at 37°C for 3 days and evaluated according to the manufacturer's guidelines. Before the study, the investigator was trained by the developer of the microbiological diagnostic system in evaluating the respective test results.

Statistical analysis

For statistical analysis, patient means for bacterial counts were calculated so that the patient was the statistical unit. Results were expressed as total colony forming units per dentine sample and transformed to $\log_{10}$ (CFU per dentine sample). If the colony count on any media was zero, this value was used in the subsequent analyses. Median, maximum and minimum of viable bacterial counts, expressed at $\log_{10}$ (CFU+1), and the percentage of positive samples were calculated. In accordance with a previously published study [23], a bacterial load of higher than 1.0×10^2 CFU per sample was considered clinically significant. Therefore, the percentage of positive dentine samples were analysed with respect to this CFU value. All calculations were performed with the statistical program SPSS 12.0 (SPSS, Chicago IL, USA). Contingency table for the overall detection of *S. mutans* and *Lactobacillus* was calculated using the chi-square test. Differences were considered as statistically significant at $p<0.05$.

Results

S. mutans and/or *Lactobacilli* were found in 25 out of 79 lesions. The presence of *S. mutans* was observed in 14 and *Lactobacilli* in 15 out of 79 lesions, respectively. Regarding the patient as the statistical unit, *S. mutans* and/or *Lactobacilli* were found in 18 out of 42 samples, with 12 samples revealing *S. mutans* and 11 samples showing *Lactobacilli.* The median log CFU per sample was 0 (min, 0; max, 5, 5) for *S. mutans* and also 0 (min, 0; max, 6, 0) for *Lactobacillus* (Fig. 1). In most of the cases, the bacterial count was lower than 10 CFU per sample.

Regarding overall bacterial counts, 42.9% of the samples revealed bacterial infection. Values for *S. mutans* and *Lactobacilli* were 28.6 and 26.2%, respectively (Fig. 2). Including only those samples with a bacterial load higher than 100 CFU, the frequency of infected dentine specimens decreased to a value lower than 5% for the specific bacteria under study. No significant dependency could be found between the overall occurrence of *S. mutans* and *Lactoba-*

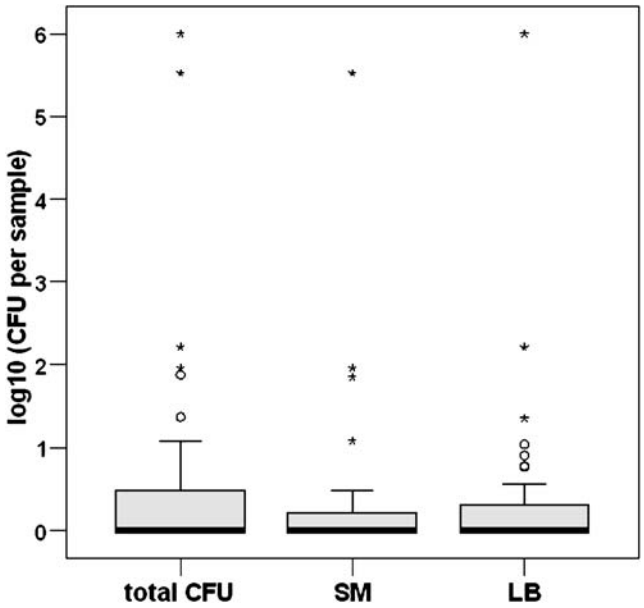

Fig. 1 Box plot shows the total number of bacteria (total CFU), *S. mutans* (*SM*) and *Lactobacilli* (*LB*) recovered from standardised dentine samples taken after Er:YAG laser caries removal. Main number of all bacteria was lower than 10 CFU per sample. No statistically significant differences between the number of *S. mutans* and *Lactobacilli* ($p<0.05$, Wilcoxon). Box plots show median, first and third quartiles, and minimum and maximum values (*whiskers*). Three times the box width marks the boundary between "mild" (*open circle*) and "extreme" (*asterisk*) outliers

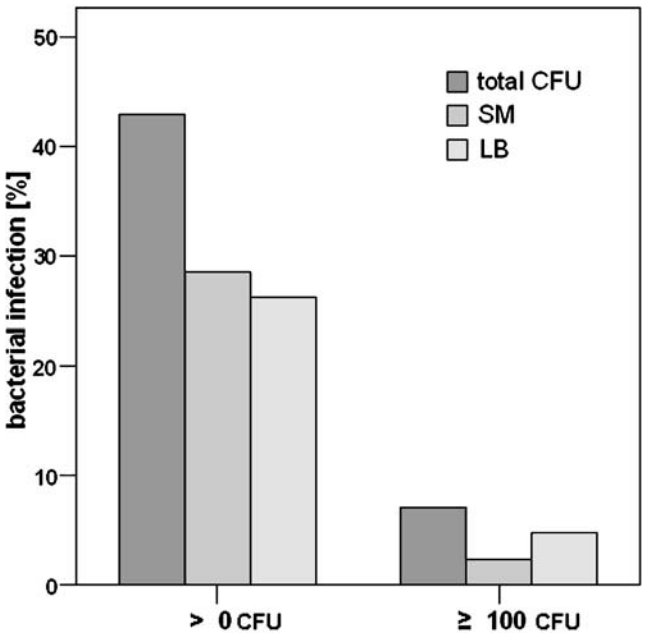

Fig. 2 Total bacterial counts (total CFU), *S. mutans* (*SM*) and *Lactobacilli* (*LB*) obtained in the dentine samples after caries removal. Of all samples, 42.9% harboured bacteria. Regarding a bacterial load higher than 100 CFU per sample, positive dentine samples decreased to a value of 7.1%

cillus in the lesions ($p=0.313$) and in the evaluated dentine samples ($p=0.154$, Table 1).

In 8 out of 79 cases, laser excavated cavities were not judged being caries-free using the conventional tactile criterion for assessing caries tissue. Focussing on these specific teeth, the median log CFU was 0 (min, 0; max, 0.5) for *S. mutans* and 0 (min, 0; max, 1.6) for *Lactobacillus*.

Mean time needed for laser excavation was 2.3±1.2 min. Assessing children's perception of pain and unpleasant sensations of vibration during Er:YAG laser treatment, 93.8% of the children considered laser excavation to be 'comfortable' regarding pain sensations (Table 2). Of all subjects, 6.3% assessed the treatment to be 'very uncomfortable'. Of the patients, 19.4% felt any 'uncomfortable' or 'very uncomfortable' perception of vibration. In one case, no comment was given (Table 2). Ten children were excluded from pain/vibration assessment because the treatment had been performed under local anaesthesia due to low compliance during laser treatment.

Discussion

The results of the present in vivo study in deciduous teeth could demonstrate the capacity of selective caries removal for an Er:YAG laser with a threshold level of 7 units. Although 42.9% of all tested dentine samples showed residual bacteria, only 7.1% of these samples harboured more than 100 CFU/sample after fluorescence feedback laser excavation. Assessing the level of dentine infection after mechanical caries excavation with a carbide bur using an identical sampling procedure for dentine after the completion of cavity preparation, a mean log CFU per sample of 0.78±0.97 was reported [23]. The authors considered a number of less than 1.0×10^2 CFU/ml as clinically insignificant. Therefore, in their study, more than 90% of samples showed no significant level of bacterial infection. Kidd et al. [23] concluded that the conventional optical and tactile criteria were adequate to ensure removal

Table 1 Contingency table for the overall detection of *Streptococcus mutans* and *Lactobacillus* in samples from patients ($n=42$) and individual lesions ($n=79$)

	Lactobacillus		
Streptococcus mutans	No Patients (lesions)	Yes Patients (lesions)	Total Patients (lesions)
No	24 (54)	6 (11)	30 (65)
Yes	7 (10)	5 (4)	12 (14)
Total	31 (64)	11 (15)	42 (79)

[$p=0.154$ (0.313), chi-square test]

Table 2 Patients' perception of pain and unpleasant sensations of vibration during Er:YAG laser treatment (n=32)

Perception	Comfortable	Uncomfortable	Very uncomfortable	No comment
Pain	30	0	2	0
Vibration	25	5	1	1

Ten children were excluded from pain/vibration assessment because treatment was performed under local anaesthesia

of most of the infected dentine leaving, at worst, a small number of microorganisms. Another study revealed a very low level of bacteria after final excavation with a rotary instrument [9], with a median of the total CFU of 1.0×10^1, ranging from 0 to 1.9×10^3. Previous examiners used demineralised histological sections stained for bacteria to assess the persistence of bacteria after excavation. It was found that about 40% of the examined teeth 'had some infected tubules on the pulpal floor' [44], and similar results were found after rigorous excavation of deep carious lesions [13]. Regarding incomplete caries removal, some authors suggest that complete dentine caries removal is not essential to control caries progression when cavity was sufficiently sealed [37, 38].

The application of an Er:YAG laser for the effective ablation of dental hard tissues was demonstrated both in deciduous and in permanent teeth [3, 5, 19, 20, 21]. The search for a more objective caries excavation technique to indicate the endpoint of caries removal has led to the development of a laser fluorescence-controlled Er:YAG laser. However, before feedback-controlled caries excavation with an Er:YAG laser can be recommended clinically, important safety issues need to be addressed. Iatrogenic damage to the vital pulp could occur if circumpulpal dentine showed relatively higher fluorescence [17, 32, 34]. Higher fluorescence values might result from the transmission and scattering properties of the deep dentine [18, 47], the higher organic content of dentine near the pulp [41], the predentine [48] or the pulpal tissue [17, 29, 32]. Recently, an in vivo study assessed fluorescence on the cavity floor after conventional excavation correlating measurements to residual dentine thickness [29]. The fluorescence values obtained by the fluorescence feedback system of the Er:YAG laser increased, while dentine thickness decreased. Thus, approaching the dental pulp, increasing fluorescence values might lead to unwanted pulp exposures. As fluorescence values were never higher than 6 fluorescence units, it could be concluded that using a threshold level of 7 units fluorescence-aided caries removal might be possible without jeopardising vital tooth structures. In an in vitro study evaluating the residual amounts of bacteria on the dentine surface after fluorescence feedback-controlled laser excavation with different threshold levels, no bacteria could be found within the dentine tubules using a threshold level of 7 units [14].

Another fluorescence-aided method for caries excavation is the FACE method that combined caries detection by fluorescence with hard substance removal with a rotary instrument. Visible fluorescence in dental hard tissues is used as a marker for infected dentine. Results of an in vitro study showed that bacteria were present in significantly fewer samples compared to conventionally excavated samples [31]; however, bacteria present in the investigated samples were not quantified because samples were scored positive for residual caries only when bacteria were identified after staining using ethidium bromide and examined by means of confocal laser scanning microscopy.

In the present study, not all cavities were considered as excavated completely after laser treatment with respect to conventional criteria. In these cases, cavity preparation was continued with a conventional bur. As only a low bacterial count could be detected in these samples after Er:YAG laser excavation, it might not have been necessary to perform additional rotary excavation procedures after laser irradiation.

Streptococci mutans and *Lactobacilli* were analysed because they are microorganisms classically associated with dental caries. In the field of microbiological culture methods, *Streptococci mutans* and *Lactobacilli* are identified by means of standard test procedures [15, 39]. Therefore, in the present study, a chair side test system that enables the simultaneous detection of *Streptococci mutans* and *Lactobacilli* by means of selective agars was used for microbiological evaluation [4, 27]. Samples for microbiological analysis were obtained on sterile burs. A 'burful of tissue' represented a standardised sample. According to Kidd et al. [24], the results were therefore expressed by samples rather than by weight of tissue removed.

Isolating the deciduous teeth in the present study using rubber dam was difficult because of their crown anatomy. Therefore, values for *Streptococcus mutans* and *Lactobacillus* could have been be influenced by salivary or plaque contamination. However, the low numbers of bacteria found in this study indicate the successful elimination of bacteria from saliva during sampling procedures. Moreover, the present study was conducted as a first feasibility study, legitimating the lack of a control group of teeth solely treated with a rotary instrument. The beam of the laser device used in this study was emitted in a non-contact mode with a required distance of approximately 12 mm between the exit window and the cavity floor. Thus, application might be sometimes difficult particularly in the posterior region of teeth.

The mean treatment time of the exaction procedure measured in this study was 2.3±1.2 min. This is in contrast to an in vitro study performing different excavation methods in deciduous teeth, reporting a mean time of 5.7±0.5 min for Er:YAG laser excavation [12]. The laser needed almost 2.5 times longer than steel burs. This observation is in accordance with Keller et al. [20]. Mean time for preparation was 7.5±4.6 min. However, the laser devices in these studies were not equipped with a fluorescence feedback system. Thus, because of the lack of an adequate tactile feedback using a non-contact handpiece, dentine hardness had to be checked by means of a dental probe during laser excavation while treatment was discontinued. Regarding other excavation methods like steel bur or hand excavator, mean time needed for the bur ranged between 1.0±0.3 [7], 2.3±0.2 min [12] and 4.3±3.9 min [20]. Time for hand excavation methods were reported between 1.8±0.6 min [7] and 4.1±0.4 min, respectively [12]. These data show that the caries removal procedure is very prone to subjectivity and very dependent on the operator's perception and experience. Evaluating effectiveness for carious dentine excavation, it could also be shown that carbide bur tends to be a fast and rather non-conservative method for dentine caries removal [7, 8, 12].

Considering patient perception and acceptance, it has been reported that the Er:YAG laser produced minimal vibration and noise during cavity preparation, little pain was felt during treatment, and the need for local anaesthesia was none or minimal [19, 21, 22]. These findings could be confirmed in our study: nearly 94% of the children perceived laser treatment as 'comfortable'. Of the patients, 80.6% stated that the excavation procedure caused no unpleasant sensations concerning vibration. The three-option scoring system used in this study was considered more appropriate than the use of an analogue scale for children.

In conclusion, the present clinical study showed that the fluorescence feedback-controlled Er:YAG laser might be an appropriate device for caries removal in deciduous teeth.

An accurate excavation technique should, however, not only successfully remove infected tissue but also conserve sound tissue. The clinically very important aspect of a possible over-excavation employing feedback controlled laser technologies should be addressed in further studies.

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References

1. Alfano RR, Yao SS (1981) Human teeth with and without dental caries studied by visible luminescent spectroscopy. J Dent Res 60:120–122
2. Anusavice KJ, Kincheloe JE (1987) Comparison of pain associated with mechanical and chemomechanical removal of caries. J Dent Res 66:1680–1683
3. Aoki A, Ishikawa I, Yamada T, Otsuki M, Watanabe H, Tagami J, Ando Y, Yamamoto H (1998) Comparison between Er:YAG laser and conventional technique for root caries treatment *in vitro*. J Dent Res 77:1404–1414
4. Aps JKM, Van Maele GOG, Claeys G, Martens LC (2001) *Mutans streptococci*, *Lactobacilli* and caries experience in cystic fibrosis homozygotes, heterozygotes and healthy controls. Caries Res 35:407–411
5. Armengol V, Jean A, Rohanizadeh R, Hamel H (1999) Scanning electron microscopic analysis of diseased and healthy dental hard tissues after Er:YAG laser irradiation: *in vitro* study. J Endod 25:543–546
6. Banerjee A, Gilmour A, Kidd E, Watson T (2004) Relationship between *Streptococcus mutans* and the autofluorescence of carious dentin. Am J Dent 17:233–236
7. Banerjee A, Kidd EA, Watson TF (2000) *In vitro* evaluation of five alternative methods of carious dentine excavation. Caries Res 34:144–150
8. Banerjee A, Watson TF, Kidd EA (2000) Dentine caries excavation: a review of current clinical techniques. Br Dent J 188:476–482
9. Bjørndal L, Larsen T, Thylstrup A (1997) A clinical and microbiological study of deep carious lesions during stepwise excavation using long treatment intervals. Caries Res 31:411–417
10. Braun A, Graefen O, Frentzen M, Nolden R (2000) Comparative study of conventional caries diagnosis versus laser fluorescence measurement. Dtsch Zahnärztl Z 55:248–251
11. Buchalla W, Lennon AM, Attin T (2004) Comparative fluorescence spectroscopy of root caries lesions. Eur J Oral Sci 112: 490–496
12. Celiberti P, Francescut P, Lussi A (2006) Performance of four dentine excavation methods in deciduous teeth. Caries Res 40:117–123
13. Crone FL (1968) Deep dentinal caries from a microbiological point of view. Int Dent J 18:481–488
14. Eberhard J, Eisenbeiss AK, Braun A, Hedderich J, Jepsen S (2005) Evaluation of selective caries removal by a fluorescence feedback-controlled Er:YAG laser *in vitro*. Caries Res 39:496–504
15. Gold OG, Jordan HV, Van Houte J (1973) A selective medium for *Streptococcus mutans*. Arch Oral Biol 18:1357–1364
16. Hibst R, Keller U (1989) Experimental studies of the application of the Er:YAG laser on dental hard substances. I. Measurements on the ablation rate. Lasers Surg Med 9:338–344
17. Iwami Y, Shimizu A, Narimatsu M, Hayashi M, Takeshige F, Ebisu S (2004) Relationship between bacterial infection and evaluation using a laser fluorescence device, DIAGNOdent. Eur J Oral Sci 112:419–423
18. Iwami Y, Shimizu A, Yamamoto H, Hayashi M, Takeshige F, Ebisu S (2003) *In vitro* study of caries detection through sound dentin using laser fluorescence device, DIAGNOdent. Eur J Oral Sci 109:14–19
19. Kato J, Moriya K, Jayawardena JA, Wijeyeweera RL (2003) Clinical application of Er:YAG laser for cavity preparation in children. J Clin Laser Med Surg 21:151–155
20. Keller U, Hibst R, Geurtsen W, Schilke R, Heidemann D, Klaiber B, Raab WH (1998) Erbium:YAG laser application in caries

therapy. Evaluation of patient perception and acceptance. J Dent 26:649–656
21. Keller U, Hibst R (1997) Effects of Er:YAG laser in caries treatment: a clinical pilot study. Lasers Surg Med 20:32–38
22. Keller U, Hibst R (1993) Er:YAG laser in caries therapy. Indications and first clinical results. In: Powell GL (ed) Proceedings of the third International Congress on Lasers in Dentistry. University of Utah Printing Service, Salt Lake City, pp 151–152
23. Kidd EA, Joyston-Bechal S, Beighton D (1993) The use of a caries detector dye during cavity preparation: a microbiological assessment. Br Dent J 174:245–248
24. Kidd EA, Ricketts DN, Beighton D (1996) Criteria for caries removal at the enamel–dentine junction: a clinical and microbiological study. Br Dent J 180:287–291
25. Kidd EAM (2004) How 'clean' must a cavity be before restoration? Caries Res 38:305–313
26. Kirzioglu Z, Gurbuz T, Yilmaz Y (2007) Clinical evaluation of chemomechanical and mechanical caries removal: status of the restorations at 3, 6, 9 and 12 months. Clin Oral Invest 11:69–76
27. Kneist S, Heinrich-Weltzien R, Fischer T, Stösser L (1998) Mikrobiologische Speicheltests—mehr als eine Motivation? Die Quintessenz 49:139–148
28. König K, Schneckenburger H (1994) Laser-induced autofluorescence for medical diagnosis. J Fluoresc 4:17–40
29. Krause F, Braun A, Eberhard J, Jepsen S (2007) Laser fluorescence measurements compared to electrical resistance of residual dentine in excavated cavities *in vivo*. Caries Res 41:135–140
30. Lennon ÁM, Buchalla W, Switalski L, Stookey GK (2002) Residual caries detection using visible fluorescence. Caries Res 36:315–319
31. Lennon ÁM (2003) Fluorescence-aided caries excavation (FACE) compared to conventional method. Oper Dent 28:341–345
32. Lussi A, Francescut P, Achermann F, Reich E, Hotz P, Megert B (2000) The use of DIAGNOdent during cavity preparation. Caries Res 34:327–328
33. Lussi A, Francescut P (2003) Performance of conventional and new methods for the detection of occlusal caries in deciduous teeth. Caries Res 37:2–7
34. Lussi A, Hibst R, Paulus R (2004) DIAGNOdent: an optical method for caries detection. J Dent Res 83(Special Issue C):80–83
35. Lussi A, Megert B, Longbottom C, Reich E, Francescut P (2001) Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. Eur J Oral Sci 109:14–19
36. McComb D (2000) Caries-detector dyes: how accurate and useful are they? J Can Dent Assoc 66:195–198
37. Mertz-Fairhurst EJ, Curtis JW Jr, Ergle JW, Rueggeberg FA, Adair SM (1998) Ultraconservative and cariostatic sealed restorations: results at year 10. J Am Dent Assoc 129:55–66
38. Oliveira EF, Carminatti G, Fontanella V, Maltz M (2006) The monitoring of deep caries lesions after incomplete dentine caries removal: results after 14–18 months. Clin Oral Invest 10:134–139
39. Rogosa M, Mitchell JA, Wiseman RF (1951) A selective medium for the isolation and enumeration of oral lactobacilli. J Dent Res 30:682–689
40. Sato Y, Fusayama T (1976) Removal of dentin by fuchsin staining. J Dent Res 55:678–683
41. Schroeder HE (2000) Oral structure biology. Thieme, Stuttgart New York
42. Shi XQ, Tranæus S, Angmar-Månsson B (2001) Validation of DIAGNOdent for quantification of smooth-surface caries: an *in vitro* study. Acta Odontol Scand 59:74–78
43. Shi XQ, Welander U, Angmar-Mansson B (2000) Occlusal caries detection with KaVo DIAGNOdent and radiography: an *in vitro* comparison. Caries Res 34:151–158
44. Shovelton DS (1968) A study of deep carious dentine. Int Dent J 18:392–405
45. Stuebel H (1911) The fluorescence of animal tissues by irradiation with ultraviolet light. Arch Ges Physiol 142:1–14
46. Thylstrup A, Fejerskov O (1994) Textbook of clinical cariology. Munksgaard, Copenhagen
47. Vaarkamp J, ten Bosch JJ, Verdonschot EH (1995) Propagation of light through human dental enamel and dentine. Caries Res 29: 8–13
48. Yonemoto K, Eguro T, Maeda T, Tanaka H (2006) Application of DIAGNOdent as a guide for removing carious dentin with Er: YAG laser. J Dent 34:269–276