In-Vivo Occlusal Caries Prevention by Pulsed CO_2-Laser and Fluoride Varnish Treatment—A Clinical Pilot Study

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Background and Objectives: High caries prevalence in occlusal pits and fissures warrants novel prevention methods. An 86% reduction in dental enamel smooth surface demineralization *in-vivo* following short-pulsed 9.6 μm-CO_2-laser irradiation was recently reported. The objective of this study was to conduct a blinded 12-month-pilot clinical trial of occlusal pit and fissure caries inhibition using the same CO_2-laser irradiation conditions.

Study Design/Materials and Methods: Twenty subjects, average age 14 years, were recruited. At baseline, second molars were randomized into test and control groups, assessed by International Caries Detection & Assessment System (ICDAS-II), SOPROLIFE light-induced fluorescence evaluator in daylight and blue-fluorescence mode and DIAGNOdent. An independent investigator irradiated test molars with a CO_2-laser, wavelength 9.6 μm, pulse-duration 20 μs, pulse-repetition-rate 20 Hz, beam diameter 800 μm, average fluence 4.5 ± 0.5 J/cm^2, 20 laser pulses per spot. At 3-, 6- and 12-month recall teeth were assessed by ICDAS, SOPROLIFE and DIAGNOdent. All subjects received fluoride varnish applications at baseline and 6-month recall.

Results: All subjects completed the 3-month, 19 the 6-month and 16 the 12-month recall. At all recalls average ICDAS scores had decreased for the test and increased for the control fissures (laser vs. control, 3-month: $-0.10 \pm 0.14, 0.30 \pm 0.18, P > 0.05$; 6-month: $-0.26 \pm 0.13, 0.47 \pm 0.16$, $P = 0.001$; 12-month: -0.31 ± 0.15, 0.75 ± 0.17, $P < 0.0001$; mean ± SE, unpaired *t*-test) being statistically significantly different at 6- and 12-month recalls.

SOPROLIFE daylight evaluation revealed at 6- and 12-months statistically significant differences in changes between baseline and recall for test and control molars, respectively (laser vs. control, 6-month: 0.22 ± 0.13, 0.17 ± 0.09, $P = 0.02$; 12-month: 0.28 ± 0.19, $0.25 \pm 0.17, P = 0.03$). For SOPROLIFE blue-fluorescence evaluation mean changes in comparison to baseline for the control and the laser treated teeth were also statistically significant for the 6- and 12-month recall.

Conclusion: Specific microsecond short-pulsed 9.6 μm CO_2-laser irradiation markedly inhibits caries progression in pits and fissures in comparison to fluoride varnish alone over 12 months. Lasers Surg. Med. 45:302–310, 2013.

Key words: CO_2-laser; microsecond pulsed; *in vivo* occlusal caries prevention; occlusal fissures; fluoride varnish; randomized prospective clinical pilot study

INTRODUCTION

Studies investigating the enhancement of the acid resistance of dental enamel were reported within a few years of the invention of the first laser. Studies from the 1970s to the early 2000s aimed at reducing the acid dissolution of enamel by the use of CO_2-lasers, mostly at 10.6 μm [1–10], all successfully showing various levels of demineralization inhibition of smooth enamel surfaces in the laboratory [11,12]. Other laser wavelengths have been investigated in laboratory studies including Nd:YAG- [13–16], Er:YAG - [17–20], Er,Cr:YSGG- [21–23] as well as argon lasers [24–29] with and without additional topical fluoride application. Small scale *in-vivo* studies using an argon laser around orthodontic brackets [30] or Nd:YAG-laser treatment coupled with initiation dye and acidulated fluoride application in children with the effects assessed by following the development of white spot lesions or fissure caries [31] were reported.

Featherstone et al. [9,10,32] have shown, in several studies, that enhancement of caries resistance of enamel can be achieved in the laboratory by irradiation with short-

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pulsed CO_2-lasers under well-specified irradiation conditions using much lower energy levels than those reported in most of the above studies. The wavelengths most strongly absorbed in dental enamel are the 9.3 and 9.6 μm CO_2-laser wavelengths with up to a 10× higher absorption coefficient compared to a 10.6 μm CO_2-laser [33]. In addition using microsecond instead of millisecond pulses allows a well-defined energy application without harmful side effects. The loss of the carbonate phase from the enamel crystals due to the irradiation heat is reported to be responsible for the reduction in acid dissolution of enamel [34,35]. Using the same laser irradiation conditions in a "pulpal safety study" on teeth in humans evidence was provided that there is no harm to the pulpal tissue of those irradiated teeth [36].

Rechmann et al. [37] used an orthodontic bracket model and showed, for the first time *in-vivo*, in a single blind, prospective clinical trial that enhancing enamel demineralization resistance can be achieved by irradiation with a CO_2 9.6 μm laser, emitting laser pulses in the microsecond range [38]. The quantitative assessment of demineralization by cross sectional microhardness testing of laser treated enamel revealed that using a 9.6 μm CO_2-laser irradiation significantly inhibited the formation of carious lesions around orthodontic brackets. For the first time in vital teeth in humans it was shown that the laser irradiation reduced enamel mineral loss by up to 46% over a time period of 4 weeks. Evaluating the caries resistance enhancing capacity of this CO_2-laser treatment over 12 weeks revealed an 87% reduction in mineral loss in comparison to the control surfaces, which was speculated to be also related to an enhancement of demineralization following the laser irradiation [38].

The study presented here was a single blind, controlled, randomized prospective clinical pilot trial, assessing treatment effects within-person thereby controlling for genetic, nutritional, hygiene, and oral environment factors. The hypothesis to be tested was that the use of a microsecond pulsed 9.6 μm CO_2-laser with additional fluoride varnish applications will significantly inhibit the formation of carious lesions in fissures of molars *in vivo* in comparison to a non-irradiated control tooth in the same arch over a 1 year observation interval.

MATERIALS AND METHODS

Study Inclusion and Exclusion Criteria

The Committee on Human Research at UCSF (IRB# 10-03431) approved the study. Prior to enrolling into the study an independent dental examiner, not otherwise involved in the study, conducted a clinical exam to assess caries status and to determine any treatment needs. An intraoral exam, review of intraoral radiographs, medical history and definitive dental history were performed.

Inclusion criteria for the study were a subject age of 10–17 years, high caries risk status, and having at least two fully erupted second molars in the same arch (contralateral) with untreated, non-carious occlusal surfaces (ICDAS code 0, 1, 2 were allowed; see below). Subjects had to be willing to comply with all study procedures and protocols. They had to be residents of San Francisco or other nearby local communities with water fluoridation (to eliminate water fluoridation as a potential confounding variable). Subjects had to be healthy and subjects/parent had to be willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form. There were no gender restrictions.

Subjects were excluded from the study if they were suffering from systemic diseases, had a significant past or medical history with conditions that may affect oral health (i.e., diabetes, HIV, heart conditions that require antibiotic prophylaxis), were taking medications that may affect the oral flora or salivary flow (e.g., antibiotic use in the past 3 months, drugs associated with dry mouth/xerostomia), had in-office fluoride treatment within the last 3 months prior to being enrolled in the study, were not willing to stop the use of any mouth rinse or other oral hygiene product during the duration of the study, or were planning to leave the area and would not be available for recall visits.

Subjects who met the selection criteria were asked to provide verbal assent/consent and their parent/guardian to provide written informed consent.

Twenty subjects were recruited for the study, comprising six females and 14 males with an average age of 14.2 ± 1.2 years.

The right or left second molar was randomly selected for laser treatment—the tooth on the opposite site in the same jaw served as control. In six subjects the upper and in 14 the lower jaw was available for selection. Randomization resulted in the laser treatment of nine teeth on the right and 11 teeth on the left side. The randomization list was created by a random number generator (QuickCalcs online random numbers by GraphPad Software, Inc.).

Study Procedure

After enrollment, before evaluating the occlusal surfaces the second molars were cleaned with a disposable tapered rotating brush (Denticator, Earth City, MO) 10–20 seconds per tooth and then rinsed with an air–water spray. Prophy paste was not used. Cotton rolls were placed and the occlusal surface was briefly air-dried (3 seconds per tooth) immediately before performing a caries lesion assessment (detailed description below). Then the study tooth was laser treated and the lesion assessment was repeated. The participants were instructed to brush twice daily with a dentifrice containing 1,100 ppm F as NaF for 1 minute each brushing.

All subjects received fluoride varnish applications (Omni Vanish fluoride varnish, Omni Preventive Care, West Palm Beach, FL) at baseline and 6-month recall.

For caries lesion assessment recalls were scheduled at 3-, 6-, and 12 months after laser treatment.

As entrance criteria only subjects with no caries or precavitated lesions (ICDAS code 0, 1, 2) were allowed into the study. If at any recall appointment a higher ICDAS code was registered a sealant or filling was placed and the subject's participation in the study was terminated. At the

end of the study the control and test teeth were sealed with a sealant (Helioseal, Ivoclar Vivadent, Amherst, NY).

Laser Settings

The laser used in the study was a CO_2-laser, Pulse System, Inc (PSI) (Model #LPS-500, Los Alamos, NM), wavelength 9.6 μm, pulse duration 20 μseconds, pulse repetition rate 20 Hz, beam diameter at focus approximately 800 μm delivered through a contra-angle hand piece specifically custom made for this study. The goal was to irradiate each spot of the irradiation area with 20 laser pulses. The laser fluence used in this study-averaged 4.5 ± 0.5 J/cm^2 per pulse (range 4.3–5.9 J/cm^2). The average treatment time per tooth was 95 ± 20 seconds (range 63–134 seconds).

The area of the surface to be irradiated (fissure and adjacent 1 mm surface to each side) was measured and the number of laser pulses and the irradiation time respectively were calculated. High volume evacuation was used and a water coolant was not applied.

Assessment Tools Used

The occlusal surfaces of the study second molars were visually assessed for decalcification using the ICDAS II criteria (International Caries Detection and Assessment System) [39], the SOPROLIFE Light Induced Fluorescence Evaluator system (SOPRO, ACTEON Group, La Ciotat, France) and the DIAGNOdent (KaVo, Biberach, Germany). For each tooth a specific area of interest was noted for the reevaluations, thus at baseline and at all recalls all three assessments occurred exactly at the same point of interest.

Visual Examination and Assessment Using ICDAS Criteria

The ICDAS criteria (International Caries Detection and Assessment System) [39] were applied for assessing the degree of decalcification of the fissure areas of the study teeth. The two examiners (DC, PR) were blinded to each other's evaluation results. On each tooth a specific area of interest was defined and noted for the reevaluations. After independently scoring for ICDAS, the examiners discussed their findings and agreed on one ICDAS score per tooth.

The inter-examiner reliability (DC, PR) for the ICDAS scoring was assessed with a $\kappa = 0.884$, SE of $\kappa = 0.017$, 95% confidence interval from 0.851 to 0.917, at 571 observations in a study occurring before [40]. The strength of agreement is considered to be "very good [41]. The weighted Kappa was calculated at $\kappa = 0.905$ using linear weighting. Assessed this way, the strength of agreement is again considered to be "very good [41].

SOPROLIFE Light Induced Fluorescence Evaluator

The SOPROLIFE Light Induced Fluorescence Evaluator system operates in daylight and in blue fluorescence mode. In the daylight mode the system uses four white LEDs, in the fluorescence mode it uses four blue LEDs emitting a wavelength of 450 nm. In this study the system was used in the LIFE magnification mode with daylight or fluorescence detection mode I—diagnosis aid mode. The handpiece allows for collecting pictures. The images were recorded with the SOPRO IMAGING software. A HP 620 Notebook (HP, Palo Alto, CA; Windows 7, Microsoft Redmond, WA) was used to collect the data for independent evaluation. A lately introduced scoring system was utilized [40,42] to evaluate the images by two independent examiners (BR, PR). The same areas of interested as chosen for the ICDAS scoring were used for the SOPROLIFE scoring. After independently evaluating SOPROLIFE daylight and blue fluorescence scores, the examiners discussed their findings and agreed on one SOPROLIFE daylight and one blue fluorescence score per tooth.

DIAGNOdent Laser Fluorescence

The DIAGNOdent Classic tool (KaVo, Biberach, Germany) emits a red laser light (wavelength 655 nm) and measures the returning fluorescence in the spectral region >680 nm wavelength. Before assessing a new subject the tool was calibrated according to manufacturer's instruction.

The highest score per evaluated fissure area of interest was noted (scores ranged from 3 to 64 in this study).

RESULTS

ICDAS Visual Examination and Assessment—Results

A total of 20 subjects were recruited into the study from which all subjects completed the 3-month recall. At the 6-month recall appointment one subject and at the 12-month recall two subjects were no-shows despite multiple reminders, thus 19 subjects completed the 6-month and 16 the 12-month recall.

One subject presented at the 6-month recall with an ICDAS code 3 lesion in the control tooth, consequently received fissure sealants on the study molars, and was withdrawn from further participating in the study. At the 12-month recall 4 other subjects had developed ICDAS code 3 lesions in their control teeth.

Figure 1 shows the average ICDAS scores for control and laser treated teeth. At baseline the average ICDAS scores were not statistically significant different between the control and the laser group with 1.0 ± 0.18 (mean $\pm$ SE) and 1.15 ± 0.15, respectively (unpaired t-test, $P > 0.5$). At the 3-month recall the average ICDAS score for the control increased to 1.30 ± 0.15 and the score for the laser slightly decreased (1.05 ± 0.14) but they were still not statistically significant different ($P = 0.2$). At the 6- and the 12-month recall the average ICDAS score for the control increased to 1.53 ± 0.19 and 1.69 ± 0.27, respectively. On the contrary, at these recalls the mean ICDAS scores for the laser treated molars had decreased below the starting average to 0.95 ± 0.14 for the 6-month and 0.88 ± 0.16 for the 12-month recall. The differences between control and laser treated scores were statistically significant at both time

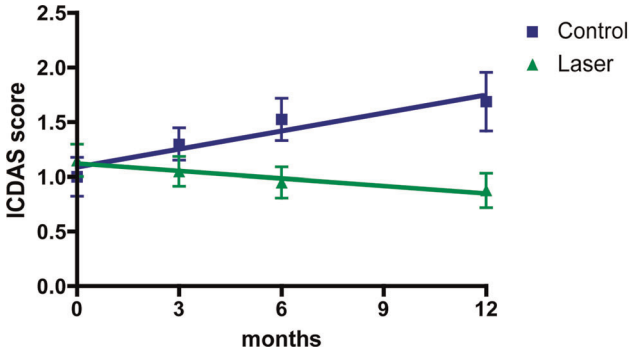

Fig. 1. Average ICDAS scores at baseline, 3-, 6-, and 12-month recall (mean ± SE) for laser treated and control teeth with statistically significant differences at 6- and 12-month recall; linear regression fits being significantly non-zero with a positive slope (increasing—demineralization) for the controls and a negative slope (decreasing—remineralization) for laser treated teeth.

points ($P = 0.021$ at 6-month; $P = 0.016$ at 12-month recall).

Figure 1 also shows the linear regression fit for the average ICDAS scores for control and laser treated teeth. The slopes of the regression lines are significantly non-zero (control $P = 0.048$, laser treated $P = 0.034$) indicating that differences between each average score exist with a goodness of fit of r^2 0.91 and 0.93 for control and laser treated molars, respectively. Furthermore the slope of the regression line for the control teeth is positive (0.06 ± 0.01) and the slope for the laser treated teeth is negative (-0.03 ± 0.004).

Considering average changes of the ICDAS scores between baselines and the 3-, 6- and 12-month recall reveals at the 3-month recall that the laser treated teeth showed a slightly negative ICDAS change while the controls showed slightly positive changes but the differences were not statistically significant (laser treated -0.10 ± 0.14 vs. control 0.30 ± 0.18, mean ± SE; $P = 0.09$). For 6-month and the 12-month recall the tendency continued (6-month: laser treated -0.26 ± 0.13 vs. control 0.47 ± 0.16; 12-month: laser treated -0.31 ± 0.15 vs. control: 0.75 ± 0.17). Those differences in ICDAS changes were statistically significant (6-month $P = 0.001$ and 12-month $P < 0.0001$, unpaired t-test).

SOPROLIFE Light Induced Fluorescence Evaluator

SOPROLIFE daylight mode—results. In addition to the ICDAS assessment system the fissure systems of the study teeth were evaluated with the SOPROLIFE system and scored with a recently introduced scoring system developed for the SOPROLIFE light induced fluorescence evaluator for daylight and for the blue fluorescence mode [40,42]. For the control as well as the laser treated teeth the SOPROLIFE scores ranged between 0 and 3 at baseline.

At baseline the SOPROLIFE daylight scores were not statistically significant different for the two groups (laser 1.45 ± 0.19, control 1.6 ± 0.23 (mean ± SE); unpaired t-test $P > 0.05$). At all recall times using the SOPROLIFE daylight evaluation the control molars showed in average more severe and/or extended lesion while the laser treated teeth exhibited less severe or same lesion scores (3-month: laser 1.70 ± 0.23, control 1.35 ± 0.18; 6-month: laser 1.22 ± 0.22, control 1.83 ± 0.23; 12-month: laser 1.22 ± 0.26, control 1.87 ± 0.27). Nevertheless at each recall time point differences between control and laser teeth were not statistically significant ($P > 0.05$). A linear fit calculation revealed that the deviation from zero for each linear regression line was not significant.

Calculating the changes in SOPROLIFE daylight scores between baseline and each recall (Fig. 2) revealed an increased daylight score for the controls and a decreased score for the laser treated fissures. Those differences were statistically significant for the 6- and 12-month recall (6-month: control 0.17 ± 0.09 vs. laser -0.22 ± 0.13, unpaired t-test $P = 0.02$; 12-month: control 0.25 ± 0.11 vs. laser -0.28 ± 0.19, $P = 0.03$).

SOPROLIFE blue fluorescence mode—results. When evaluating the SOPROLIFE blue fluorescence average scores for baseline and the 3-, 6- and 12-month recall as with the SOPROLIFE daylight scores a similar tendency of increasing control and decreasing laser treated fissure scores was observed (except for the 12-month recall laser group). Nevertheless for each time point the average scores for control and laser treated teeth were statistically not different ($P > 0.05$) (baseline: laser 1.16 ± 0.21, control 1.4 ± 0.3; 3-month: laser 1.05 ± 0.19, control 1.5 ± 0.29; 6-month: laser 1.0 ± 0.24, control

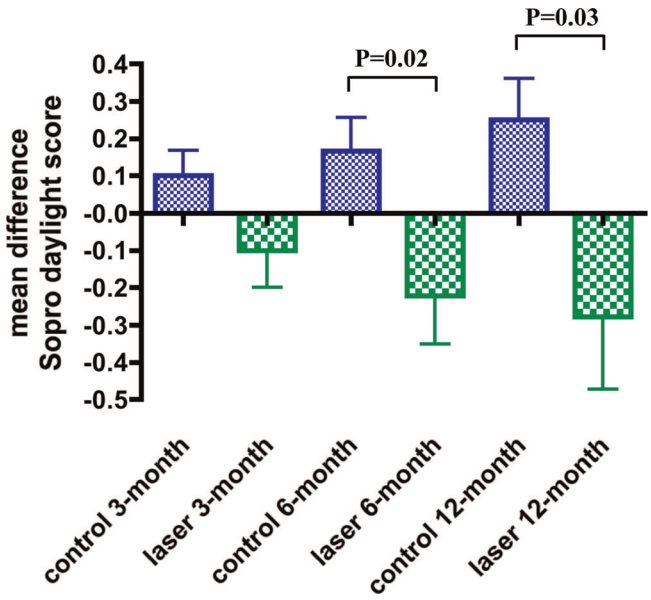

Fig. 2. Average changes of the SOPROLIFE daylight scores for laser treated and control teeth between baseline and the 3-, 6-, and 12-month recall (mean ± SE) with statistically significant differences at the 6- and 12-month recall.

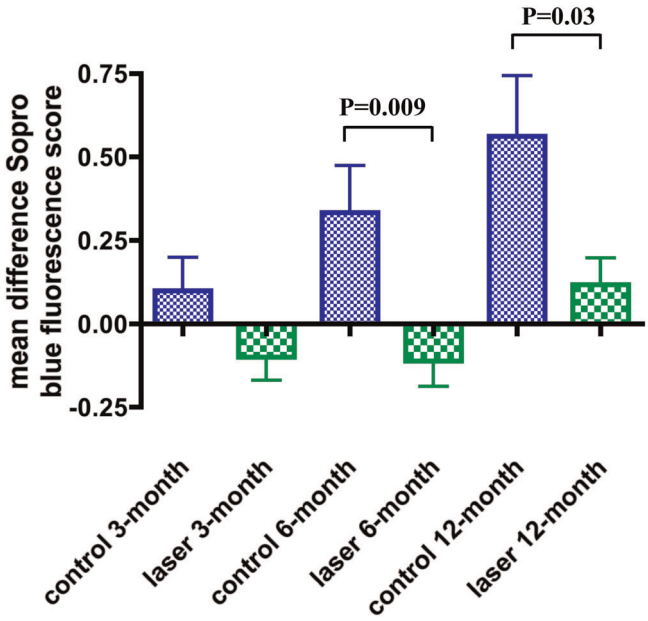

Fig. 3. Average changes of the SOPROLIFE blue fluorescence scores for laser treated and control teeth between baseline and the 3-, 6-, and 12-month recall (mean ± SE) with statistically significant differences at the 6- and 12-month recall.

1.6 ± 0.29; 12-month: laser 1.31 ± 0.24, control 1.8 ± 0.31).

Calculating the changes in SOPROLIFE blue fluorescence scores between baseline and each recall point (Fig. 3) revealed a similar pattern to that for the SOPROLIFE daylight evaluation an increasing average score for the controls, the laser treated teeth showed a score reduction for the 3- and 6-month observation and then slightly increased to the level the control teeth had demonstrated at the 3-month recall. The mean changes in comparison to baseline for the control and the laser treated teeth were statistically significant for the 6- and 12-month recall (Fig. 3) (3-month: control 0.1 ± 0.1 vs. laser −0.1 ± 0.07, unpaired *t*-test $P = 0.1$; 6-month: control 0.33 ± 0.14 vs. laser −0.11 ± 0.08, $P = 0.009$; 12-month: control 0.56 ± 0.18 vs. laser 0.12 ± 0.08, $P = 0.03$).

Figures 4 and 5 are examples of daylight and blue fluorescence pictures of one control and one laser treated tooth of the same subject at baseline and at the 6-month recall. Both molars show obvious changes in the fissure system, which are very distinct in the distal groove. While the area of demineralization in the control tooth appears wider in daylight mode and fluorescence mode after 6 months in the laser treated tooth the demineralization and the red fluorescence, respectively disappeared (left side baseline, right side 6-month recall).

DIAGNOdent—Results

At baseline the DIAGNOdent average values for the control fissures were 23.7 ± 16 and for the laser group 22.4 ± 11.9 (mean ± SD). Over the observation time the

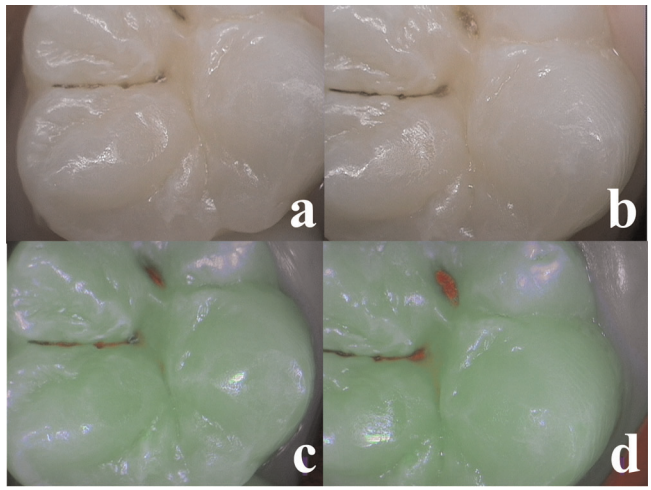

Fig. 4. Example of daylight and blue fluorescence pictures of a control tooth of a subject at baseline and at the 6-month recall. The area of demineralization in the control tooth appears wider in daylight mode and fluorescence mode after 6 month (**a**: daylight, **c**: blue fluorescence mode at baseline; **b** and **d**: at 6-month recall).

average DIAGNOdent values for the controls slightly increased to 25.1 ± 17.8, the value for the laser treated teeth at 12-month recall stayed around 21.0 ± 13.9 (Fig. 6 shows mean ± SE).

Figure 6 also shows a linear regression fit for the average DIAGNOdent scores for control and laser treated teeth over time. The slopes of the regression lines do not deviate significantly from zero indicating that there is no significant change over time for the DIAGNOdent scores. Comparing changes in DIAGNOdent average scores between baseline and each recall point revealed no statistically significant differences for either group.

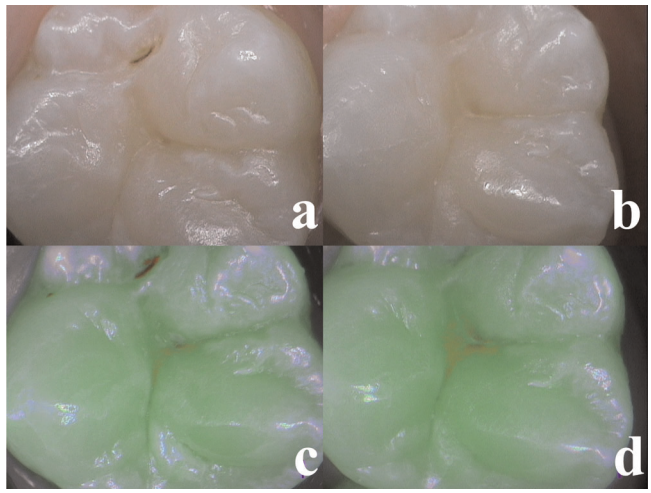

Fig. 5. Daylight and blue fluorescence pictures of the laser treated tooth (same subject as in Fig. 4) at baseline and at the 6-month recall. In the distal fossa of the laser treated tooth in daylight mode and fluorescence mode the demineralization zone and the red fluorescence, respectively is not visible anymore (**a**: daylight and **c**: blue fluorescence mode at baseline; **b** and **d**: at 6-month recall).

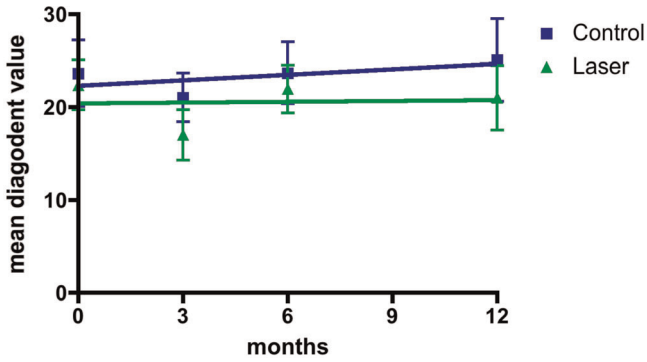

Fig. 6. Average DIAGNOdent scores at baseline, 3-, 6-, and 12-month recall (mean ± SE) for laser treated and control teeth statistically not different at all time points; linear regression fits do not deviate significantly from zero indicating that there is no significant change over time for the DIAGNOdent scores.

DISCUSSION

In the past, several laboratory studies have shown that enhancing enamel demineralization resistance can be achieved by irradiation with microsecond pulsed CO_2-lasers [9,32]. The wavelengths absorbed most strongly in dental enamel are the 9.3 and 9.6 μm CO_2-laser wavelengths [33]. The loss of the carbonate phase from the enamel crystals due to the irradiation heat is responsible for the reduction in acid dissolution of enamel [34,43] transforming carbonated hydroxyapatite into the more acid resistant hydroxyapatite. Adding fluoride at this time leads to the formation of fluorapatite, which is even less acid soluble [44].

Lately we reported that using a 9.6 μm CO_2-laser irradiation (20 μseconds pulse duration) significantly inhibits the formation of carious lesions around orthodontic brackets [38]. Our study showed for the first time in vital teeth in humans that the applied irradiation scheme reduced enamel mineral loss by up to 46% over a time period of 4 weeks and due to enhanced remineralization up to 87% over a 12 weeks period. At the same time demineralization for the controls, as expected, continued to become more severe [38]. This *in-vivo* orthodontic bracket model study confirmed that caries inhibition demonstrated in numerous models and experiments in the laboratory [9,45–47] can also be achieved in humans in vital teeth using short-pulsed 9.6 μm CO_2-laser irradiation.

In this orthodontic bracket model study the test teeth had to be extracted to perform the cross sectional microhardness testing. In the study presented here we irradiated, for the first time, occlusal pits and fissures of second molars in the mouth and assessed a change in mineralization status by visual methods using the International Caries Detection & Assessment System—ICDAS, SOPROLIFE in daylight and in fluorescence mode and the DIAGNOdent tool. The intent of the study was to demonstrate caries inhibition in fissures of molars and this included the additional challenge of irradiating normal fissures as well as deep fissures. In order to reach and irradiate the walls of deep fissures we designed and used a contra-angle laser handpiece specifically custom made for this study.

This single blind, controlled, randomized clinical pilot trial showed that using a microsecond pulsed 9.6 μm CO_2-laser with additional fluoride varnish applications significantly inhibits the formation of carious lesions in fissures of molars *in vivo* in comparison to a non-irradiated control tooth in the same arch over a 1 year observation interval.

However, DIAGNOdent as a spot fluorescence measurement tool, illuminating with a red laser light (655 nm) and creating an infrared fluorescence originated from porphyrins and related compounds from oral bacteria [48–51], was not able to confirm this positive result due to the system's inherent limited capacity of caries detection at the enamel level. The system has shown good performance and reproducibility for detection and quantification of occlusal and smooth surface carious lesions in *in-vitro* studies [48,52,53], but with somewhat more contradictory results *in vivo*, both in the primary and permanent dentition [54–60]. It has also been tried for longitudinal monitoring of the caries process, and for assessing the outcome of preventive interventions [61].

In this laser caries prevention study the average DIAGNOdent score at baseline was 23.0 (±13.9, SD) and thus below the discussed cut-off points for operative interventions (filling) [62–64]. Even if for the controls the value slightly increased over time, those differences were not significant and not expected. All visually observed changes were at a precavitated level or at most a first enamel breakdown (ICDAS code 3) was observed in this study. Thus significant dentin involvement with consequently increased porphyrin levels in dentin did not occur and thus no significant changes in the DIAGNOdent assessments took place. The DIAGNOdent measures the uptake of organic bacterial by-products and does not measure demineralization or remineralization directly.

The International Caries Detection and Assessment System provides a standardized method of lesion detection and assessment, leading to caries diagnosis [39]. ICDAS criteria are based on enamel properties of translucency and microporosity. With numerous demineralization events the microporosity of enamel subsurface increases, which leads to changes in its refractive index. The first sign of carious alteration is a change in translucency and light refraction of the enamel surface. If demineralization continues the enamel microporosity increases, which then leads to further decrease in the refractive index of enamel [65].

Ekstrand et al. [66–68] validated ICDAS by demonstrating an association between the severity of caries lesions (as described by ICDAS codes) and the lesions' histological depth. Other authors have confirmed a close relationship between ICDAS scoring and the histological depth of the caries lesion, especially in precavitated but also in slightly cavitated stages [55,69], endorsing a relationship between the visual topography at surface level and the histological lesion depth.

In this present short-pulsed CO_2-laser caries prevention study only subjects presenting teeth with no caries at all (ICDAS code 0) or with precavitated lesions (code 1, 2) were allowed to participate. Any tooth exhibiting a higher score at any recall received fissure sealants and lead to the withdrawal of the subject from further participating in the study.

One subject presented at the 6-month recall with an ICDAS code 3 lesion—first visible enamel loss—at the control tooth, and at the 12-month recall four other subjects had developed ICDAS code 3 lesions at their control teeth. Thus in total during the observation time of 12 months 5 out of 20 subjects had developed caries lesions at ICDAS 3 code level—all on the non-irradiated control teeth. None of the laser treated teeth changed into ICDAS 3 code.

Regarding the ICDAS score development over time the score for the control teeth constantly increased depicting more severe mineral loss while in the control teeth the ICDAS scores constantly decreased, demonstrating some mineral gain. Comparing average ICDAS scores for each time point and average changes of the ICDAS scores between baselines and the 3-, 6- and 12-month recall showed statistically significant levels at 6- and 12-month and that over the observation period of 1 year the control teeth increased the ICDAS score by almost $^3/_4$ of an ICDAS score from an average ICDAS code 1 to an ICDAS 1.7 code. At the same time the laser treated fissures at the contralateral side of the mouth showed for a similar ICDAS code at study start (average code 1.05) a reduction of almost 1/5 of an ICDAS score, down to an average ICDAS 0.88 code.

These trends were confirmed by the linear regression fit, depicting for both groups regression lines significantly deviating from zero with a positive slope (increase) for the control and a negative (decrease) for the laser treated teeth. In the *in-vivo* orthodontic bracket model laser study using short-pulsed 9.6 μm CO_2-laser irradiation the same trends were observed with a lower mineral loss or even mineral gain at the 12-week in comparison to the 4-week interval for the laser treated teeth [38]. From this present study and the orthodontic bracket study the assumption is supported that driving out the carbonated phase from the enamel crystal due to the irradiation decreases demineralization of the modified hydroxyapatite in an acid environment. The transformed hydroxyapatite also appears to be prone to higher remineralization specifically when fluoride is present. This phenomenon was observed in this study over a period of 12 months.

Fluorescence is a property of some materials that absorb energy at certain wavelengths and emit light at longer wavelengths. Several caries detection methods engage fluorescence. The SOPROLIFE system is thought to combine the advantages of a visual inspection method (high specificity) with a high magnification oral camera and a laser fluorescence device (high reproducibility and discrimination). In the daylight mode the system uses four white, and in the fluorescence mode it uses four blue LEDs. The fluorescence signal and expression is most probably triggered and modified by bacteria and bacteria by-products. The blue light transmits through healthy enamel and evokes a green fluorescence of the dentin core. The green fluorescence light coming back from the dentin core then leads to a red fluorescence from bacteria and bacterial byproducts like porphyrins [40].

A recently published SOPROLIFE daylight and blue fluorescence scoring system with six distinct codes for each detection mode [40,42] was used to evaluate the teeth in this laser study. As with the ICDAS scores SOPROLIFE daylight as well as blue florescence scoring showed over time increasing average scores for the control teeth and decreasing scores for the laser treated fissures. The differences became significant for the 6- and 12-month recall intervals. The trends were obvious for the daylight evaluation, which in the scoring codes strongly correlates with the ICDAS [42] and thus confirmed the ICDAS results for this study. The SOPROLIFE blue fluorescence evaluation despite showing the same significant differences between baseline and follow up for the 6- and 12-month evaluation time points revealed a slightly increased average score at the 12-month recall for the laser treated teeth. Nevertheless the variations in the average code change for the fluorescence codes for the laser treated fissures were extremely low with only around 1/10 of a score while the control teeth showed more prominent average changes of 6/10 of a score. The mechanism as to how porphyrin fluorescence might change over time, specifically in superficial enamel lesions related to remineralization is still not completely understood.

This clinical study has verified that microsecond short pulsed 9.6 μm CO_2-laser irradiation in combination with biannual application of fluoride varnish can, over a 1 year period, efficiently enhance caries resistance of laser treated fissures in comparison to non-treated fissures. The study revealed that using the same laser irradiation conditions, which in a pulpal safety study on teeth in humans had provided evidence that there is no harm to the pulpal tissue of those irradiated teeth [36], even leads to remineralization of the irradiated enamel proven by ICDAS and SOPROLIFE daylight and fluorescence assessments.

Further larger scale clinical studies to ascertain the efficiency of treating fissures and gingival smooth surfaces to reduce demineralization with the short-pulsed CO_2-laser are needed.

REFERENCES

1. Stern RH, Sognnaes RF. Laser inhibition of dental caries suggested by first tests in vivo. J Am Dent Assoc 1972;85 (5):1087–1090.
2. Stern RH, Vahl J, Sognnaes RF. Lased enamel: Ultrastructural observations of pulsed carbon dioxide laser effects. J Dent Res 1972;51(2):455–460.
3. Beeking PO, Herrmann C, Zuhrt R. Examination of laser-treated tooth surfaces after exposure to acid. Dtsch Stomatol 1990;40(12):490–492.
4. Nammour S, Renneboog-Squilbin C, Coomans D, Dourou N. The resistance of the dentine to acid following vaporisation of the caries by CO_2 laser. In: Melcer J, editor. *The Impact of Lasers on Dental Science*. Paris: ITBM Journal; 1990. p 96.

5. Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell GL. Combined effects of laser irradiation and chemical inhibitors on the dissolution of dental enamel. Caries Res 1992;26:333–339.
6. Nammour S, Renneboog-Squilbin C, Nyssen-Behets C. Increased resistance to artificial caries-like lesions in dentin treated with CO_2 laser. Caries Res 1992;26(3):170–175.
7. Hsu J, Fox JL, Wang Z, Powell GL, Otsuka M, Higuchi WI. Combined effects of laser irradiation/solution fluoride ion on enamel demineralization [published erratum appears in J Clin Laser Med Surg 1998 Oct; 16(5) 294–295]. J Clin Laser Med Surg 1998;16(2):93–105.
8. Hossain M, Nakamura Y, Kimura Y, Ito M, Yamada Y, Matsumoto K. Acquired acid resistance of dental hard tissues by CO_2 laser irradiation. J Clin Laser Med Surg 1999;17 (5):223–226.
9. Featherstone JD, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO_2 laser inhibitor of artificial caries-like lesion progression in dental enamel. J Dent Res 1998;77(6):1397–1403.
10. Kantorowitz Z, Featherstone JD, Fried D. Caries prevention by CO_2 laser treatment: Dependency on the number of pulses used. J Am Dent Assoc 1998;129(5):585–591.
11. Hsu CY, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. J Dent Res 2000;79 (9):1725–1730.
12. Hsu CY, Jordan TH, Dederich DN, Wefel JS. Laser-matrix-fluoride effects on enamel demineralization. J Dent Res 2001;80(9):1797–1801.
13. Tagomori S, Morioka T. Combined effects of laser and fluoride on acid resistance of human dental enamel. Caries Res 1989;23:225–231.
14. Huang GF, Lan WH, Guo MK, Chiang CP. Synergistic effect of Nd:YAG laser combined with fluoride varnish on inhibition of caries formation in dental pits and fissures in vitro. J Formos Med Assoc 2001;100(3):181–185.
15. Hashimoto M. Effects of Nd:YAG laser irradiation on acid resistance of defective rat enamel. Shoni Shikagaku Zasshi 1990;28(4):956–967.
16. Hossain M, Nakamura Y, Kimura Y, Yamada Y, Kawanaka T, Matsumoto K. Effect of pulsed Nd:YAG laser irradiation on acid demineralization of enamel and dentin. J Clin Laser Med Surg 2001;19(2):105–108.
17. Kayano T, Ochiai S, Kiyono K, Yamamoto H, Nakajima S, Mochizuki T. Effects of Er:YAG laser irradiation on human extracted teeth. Kokubyo Gakkai Zasshi 1989;56(2):381–392.
18. Hossain M, Nakamura Y, Kimura Y, Yamada Y, Ito M, Matsumoto K. Caries-preventive effect of Er:YAG laser irradiation with or without water mist. J Clin Laser Med Surg 2000;18(2):61–65.
19. Delbem AC, Cury JA, Nakassima CK, Gouveia VG, Theodoro LH. Effect of Er:YAG laser on CaF2 formation and its anti-cariogenic action on human enamel: An in vitro study. J Clin Laser Med Surg 2003;21(4):197–201.
20. Bevilacqua FM, Zezell DM, Magnani R, da Ana PA, Eduardo Cde P. Fluoride uptake and acid resistance of enamel irradiated with Er:YAG laser. Lasers Med Sci 2008;23(2): 141–147.
21. Hossain M, Kimura Y, Nakamura Y, Yamada Y, Kinoshita JI, Matsumoto K. A study on acquired acid resistance of enamel and dentin irradiated by Er, Cr:YSGG laser. J Clin Laser Med Surg 2001;19(3):159–163.
22. Moslemi M, Fekrazad R, Tadayon N, Ghorbani M, Torabzadeh H, Shadkar MM. Effects of ER,Cr:YSGG laser irradiation and fluoride treatment on acid resistance of the enamel. Pediatr Dent 2009;31(5):409–413.
23. de Freitas PM, Rapozo-Hilo M, Eduardo Cde P, Featherstone JD. In vitro evaluation of erbium, chromium:Yttrium–scandium–gallium-garnet laser-treated enamel demineralization. Lasers Med Sci 2010;25(2):165–170.
24. Westerman GH, Hicks MJ, Flaitz CM, Blankenau RJ, Powell GL, Berg JH. Argon laser irradiation in root surface caries: In vitro study examines laser's effects. J Am Dent Assoc 1994;125(4):401–407.
25. Flaitz CM, Hicks MJ, Westerman GH, Berg JH, Blankenau RJ, Powell GL. Argon laser irradiation and acidulated phosphate fluoride treatment in caries-like lesion formation in enamel: An in vitro study. Pediatr Dent 1995;17:31–35.
26. Hicks MJ, Flaitz CM, Westerman GH, Blankenau RJ, Powell GL, Berg JH. Enamel caries initiation and progression following low fluence (energy) argon laser and fluoride treatment. J Clin Pediatr Dent 1995;20(1):9–13.
27. Haider SM, White GE, Rich A. Combined effects of argon laser irradiation and fluoride treatments in prevention of caries-like lesion formation in enamel: An in vitro study. J Clin Pediatr Dent 1999;23(3):247–257.
28. Westerman GH, Flaitz CM, Powell GL, Hicks MJ. Enamel caries initiation and progression after argon laser irradiation: In vitro argon laser systems comparison. J Clin Laser Med Surg 2002;20(5):257–262.
29. Vlacic J, Meyers IA, Kim J, Walsh LJ. Laser-activated fluoride treatment of enamel against an artificial caries challenge: Comparison of five wavelengths. Aust Dent J 2007;52(2): 101–105.
30. Hicks J, Winn D II, Flaitz C, Powell L. In vivo caries formation in enamel following argon laser irradiation and combined fluoride and argon laser treatment: A clinical pilot study. Quint Int 2004;35(1):15–20.
31. Zezell DM, Boari HG, Ana PA, Eduardo Cde P, Powell GL. Nd: YAG laser in caries prevention: A clinical trial. Lasers Surg Med 2009;41(1):31–35.
32. Featherstone JD, Nelson DG. Laser effects on dental hard tissues. Adv Dent Res 1987;1(1):21–26.
33. Fried D, Zuerlein MJ, Le CQ, Featherstone JD. Thermal and chemical modification of dentin by 9–11 microns CO_2 laser pulses of 5–100 microns duration. Lasers Surg Med 2002;31 (4):275–282.
34. Zuerlein MJ, Fried D, Featherstone JD. Modeling the modification depth of carbon dioxide laser-treated dental enamel. Lasers Surg Med 1999;25(4):335–347.
35. Featherstone JDB, Nelson DG. Recent uses of electron microscopy in the study of physico-chemical processes affecting the reactivity of synthetic and biological apatites. Scan Microsc 1989;3:815–827.
36. Goodis HE, Fried D, Gansky S, Rechmann P, Featherstone JD. Pulpal safety of 9.6 μ TEA CO_2 laser used for caries prevention. Lasers Surg Med 2004;35(2):104–110.
37. Gorton J, Featherstone JD. In vivo inhibition of demineralization around orthodontic brackets. Am J Orthod Dentofacial Orthop 2003;123(1):10–14.
38. Rechmann P, Fried D, Le CQ, Nelson G, Rapozo-Hilo M, Rechmann BM, Featherstone JD. Caries inhibition in vital teeth using 9.6-μm CO_2-laser irradiation. J Biomed Opt 2011;16(7):071405.
39. Foundation I. International Caries Detection & Assessment System www.icdas.org.
40. Rechmann P, Charland D, Rechmann BM, Featherstone JD. Performance of laser fluorescence devices and visual examination for the detection of occlusal caries in permanent molars. J Biomed Opt 2012;17(3):036006.
41. GraphPad Software. *Quantify agreement with Kappa Quick-Calcs Online Calculators for Scientists* GraphPad Software Incorporated; www.graphpad.com/quickcalcs/kappa1/, 2013.
42. Rechmann P, Rechmann BM, Featherstone JD. Caries detection using light-based diagnostic tools. Compend Contin Educ Dent 2012;33(8):582–584 586, 588–593; quiz 594, 596.
43. Featherstone JD, Nelson DG. Recent uses of electron microscopy in the study of physico-chemical processes affecting the reactivity of synthetic and biological apatites. Scan Microsc 1989;3(3):815–827 discussion 827-818.
44. Takagi S, Liao H, Chow LC. Effect of tooth-bound fluoride on enamel demineralization/ remineralization in vitro. Caries Res 2000;34(4):281–288.
45. Featherstone JD. Lasers in dentistry 3. The use of lasers for the prevention of dental caries. Ned Tijdschr Tandheelkd 2002;109(5):162–167.
46. Featherstone JDB, Barrett-Vespone NA, Fried D. CO_2 laser inhibition of artificial caries-like lesions progression. J Dent Res 1995;74(470):70.

47. Fried D, Ragadio J, Akrivou M, Featherstone JD, Murray MW, Dickenson KM. Dental hard tissue modification and removal using sealed transverse excited atmospheric-pressure lasers operating at lambda = 9.6 and 10.6 microm. J Biomed Opt 2001;6(2):231–238.
48. Lussi A, Imwinkelried S, Pitts N, Longbottom C, Reich E. Performance and reproducibility of a laser fluorescence system for detection of occlusal caries in vitro. Caries Res 1999;33(4):261–266.
49. Lussi A, Hibst R, Paulus R. DIAGNOdent: An optical method for caries detection. J Dent Res 2004;83 (Spec No C): C80–C83.
50. Verdonschot EH, van der Veen MH. Lasers in dentistry 2. Diagnosis of dental caries with lasers. Ned Tijdschr Tandheelkd 2002;109(4):122–126.
51. Konig K, Flemming G, Hibst R. Laser-induced autofluorescence spectroscopy of dental caries. Cell Mol Biol (Noisy-legrand) 1998;44(8):1293–1300.
52. Shi XQ, Welander U, Angmar MB. Occlusal caries detection with KaVo DIAGNOdent and radiography: An in vitro comparison. Caries Res 2000;34(2):151–158.
53. Shi XQ, Tranaeus S, Angmar-Mansson B. Validation of DIAGNOdent for quantification of smooth-surface caries: An in vitro study. Acta Odontol Scand 2001;59(2):74–78.
54. Rocha RO, Ardenghi TM, Oliveira LB, Rodrigues CR, Ciamponi AL. In vivo effectiveness of laser fluorescence compared to visual inspection and radiography for the detection of occlusal caries in primary teeth. Caries Res 2003;37(6):437–441.
55. Astvaldsdottir A, Holbrook WP, Tranaeus S. Consistency of DIAGNOdent instruments for clinical assessment of fissure caries. Acta Odontol Scand 2004;62(4):193–198.
56. Tranaeus S, Lindgren LE, Karlsson L, Angmar-Mansson B. In vivo validity and reliability of IR fluorescence measurements for caries detection and quantification. Swed Dent J 2004; 28(4):173–182.
57. Bamzahim M, Aljehani A, Shi XQ. Clinical performance of DIAGnodent in the detection of secondary carious lesions. Acta Odontol Scand 2005;63(1):26–30.
58. Angnes V, Angnes G, Batisttella M, Grande RH, Loguercio AD, Reis A. Clinical effectiveness of laser fluorescence, visual inspection and radiography in the detection of occlusal caries. Caries Res 2005;39(6):490–495.
59. Reis A, Mendes FM, Angnes V, Angnes G, Grande RH, Loguercio AD. Performance of methods of occlusal caries detection in permanent teeth under clinical and laboratory conditions. J Dent 2006;34(2):89–96.
60. Akarsu S, Koprulu H. In vivo comparison of the efficacy of DIAGNOdent by visual inspection and radiographic diagnostic techniques in the diagnosis of occlusal caries. J Clin Dent 2006;17(3):53–58.
61. Altenburger MJ, Gmeiner B, Hellwig E, Wrbas KT, Schirrmeister JF. The evaluation of fluorescence changes after application of casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP) on early carious lesions. Am J Dent 2010;23(4):188–192.
62. Jablonski-Momeni A, Ricketts DN, Rolfsen S, Stoll R, Heinzel-Gutenbrunner M, Stachniss V, Pieper K. Performance of laser fluorescence at tooth surface and histological section. Las Med Sci 2011;26(2):171–178.
63. Huth KC, Neuhaus KW, Gygax M, Bucher K, Crispin A, Paschos E, Hickel R, Lussi A. Clinical performance of a new laser fluorescence device for detection of occlusal caries lesions in permanent molars. J Dent 2008;36(12):1033–1040.
64. Eakle S, Gansky S, Zhan L, Featherstone JDB. Clinical evaluation of the diagnodent device. In: Stookey G, editor. *Early detection of dental caries III Indiana conference 2003*. Indiana: Indiana University Press; 2005.
65. Rationale and Evidence for the International Caries Detection and Assessment System (ICDAS II) International Caries Detection and Assessment System (ICDAS) Coordinating Committee September 2005.
66. Ekstrand KR, Kuzmina I, Bjorndal L, Thylstrup A. Relationship between external and histologic features of progressive stages of caries in the occlusal fossa. Caries Res 1995;29(4): 243–250.
67. Ekstrand KR, Ricketts DN, Kidd EA. Reproducibility and accuracy of three methods for assessment of demineralization depth of the occlusal surface: An in vitro examination. Caries Res 1997;31(3):224–231.
68. Tranaeus S, Lindgren LE, Karlsson L, Angmar-Mansson B. In vivo validity and reliability of IR fluorescence measurements for caries detection and quantification. Swedish Dent J 2004;28(4):173–182.
69. Mendes FM, Siqueira WL, Mazzitelli JF, Pinheiro SL, Bengtson AL. Performance of DIAGNOdent for detection and quantification of smooth-surface caries in primary teeth. J Dent 2005;33(1):79–84.