Fissure caries inhibition with a CO_2 9.3-μm short-pulsed laser—a randomized, single-blind, split-mouth controlled, 1-year clinical trial

Peter Rechmann, Maxwell Kubitz, Benjamin W. Chaffee & Beate M. T. Rechmann

Clinical Oral Investigations

ISSN 1432-6981

Clin Oral Invest
DOI 10.1007/s00784-020-03515-x

Springer

Clinical Oral Investigations
https://doi.org/10.1007/s00784-020-03515-x

ORIGINAL ARTICLE

Fissure caries inhibition with a CO_2 9.3-μm short-pulsed laser—a randomized, single-blind, split-mouth controlled, 1-year clinical trial

Peter Rechmann[1] · Maxwell Kubitz[2] · Benjamin W. Chaffee[1] · Beate M. T. Rechmann[1]

Received: 13 May 2020 / Accepted: 7 August 2020

Abstract

Objectives The objective of this randomized, single-blind, split-mouth controlled, clinical trial was to evaluate whether the use of a short-pulsed 9.3-μm CO_2 laser increases the caries resistance of occlusal pit and fissures in addition to fluoride therapy over 12 months.

Materials and methods A total of 60 participants, average age 13.1 years, were enrolled. At baseline, second molars were randomized into test and control, and assessed by ICDAS, SOPROLIFE, and DIAGNOdent. An independent investigator irradiated test molars with a CO_2 laser (wavelength 9.3 μm, pulse duration 4 μs, pulse repetition rate 43 Hz, beam diameter 250 μm, average fluence 3.9 J/cm^2, 20 laser pulses per spot). Test molars received laser and fluoride treatment, control teeth fluoride alone. Fluoride varnish was applied at baseline and at 6 months. After 6 and 12 months, teeth were again assessed.

Results A total of 57 participants completed the 6-month and 51 the 12-month recall. Laser-treated surfaces showed very slight ICDAS improvements over time with ICDAS change − 1 in 11% and 8%, no changes (ICDAS change 0) in 68% and 67%, and slightly worsened (ICDAS change 1) in 19% and 24% at 6- and 12-month recalls, respectively, and worsened by two scores in 2% at both recall time points. Control teeth showed significantly higher ICDAS increases, with 47% and 25% showing ICDAS change 0, ICDAS change 1 in 49% and 55%, and ICDAS change 2 in 4% and 20% at 6- and 12-month recalls, respectively. Differences in ICDAS changes between the groups were statistically significant ($P = 0.0002$ and $P < 0.0001$; Wilcoxon's signed-rank test, exact). A total of 22% of the participants developed ICDAS 3 scores on the control teeth.

Conclusions Microsecond short-pulsed 9.3-μm CO_2 laser irradiation markedly inhibits caries progression in pits and fissures in comparison with fluoride varnish alone.

Clinical relevance The 9.3-μm CO_2 laser irradiation of pits and fissures enhances caries resistance.

Trial registration ClinicalTrials.gov identifier: NCT02357979

Keywords CO_2 laser · Microsecond pulsed · In vivo occlusal caries prevention · Occlusal fissures · Fluoride varnish · Randomized clinical trial

Introduction

Reported in the National Health and Nutrition Examination Survey (NHANES) cycle 2015–2016, the prevalence of total and untreated dental caries in primary or permanent teeth among youth aged 2–19 years was 45.8% and 13.0%, respectively [1]. Despite trends in reduced caries prevalence and severity in the USA [2], obviously, caries is still an issue, and it is strongly related with compliance to oral hygiene measures including brushing frequency [3–6]. A high caries prevalence and progression in children aged 6 to 18 years are reported around the world [7, 8].

Data indicate that approximately 90% of caries in permanent teeth of children occur in tooth surfaces with pits and fissures, and approximately two-thirds are on the chewing surfaces alone [9, 10]. The occlusal surfaces of teeth account for just 12.5% of the at-risk tooth surfaces in the permanent dentition; however, they account for most of the caries [11].

Pits and fissures are more prone to caries development than smooth tooth surfaces, due to the morphological complexity

✉ Peter Rechmann
Peter.Rechmann@ucsf.edu

[1] Department of Preventive and Restorative Dental Sciences, School of Dentistry, University of California at San Francisco, 707 Parnassus, San Francisco, CA 94143, USA

[2] Division of Pediatric Dentistry, Department of Orofacial Sciences, School of Dentistry, University of California at San Francisco, 707 Parnassus, San Francisco, CA 94143, USA

Published online: 15 August 2020

of these surfaces, leading to increased plaque accumulation and decreased levels of caries protection [12]. These areas represent stagnation sites for both biofilm and cariogenic substrates. The pit and fissure surfaces account for a disproportionate amount of the caries experience and result in restorations in permanent tooth surfaces in 12- to 19-year-olds [11]. Consequently, fissure sealants are recommended to prevent the initiation and progression of dental caries [13–15], and these recommendations should be integrated with the practitioner's professional judgment and the patient's needs and preferences [16].

The observed high caries prevalence in occlusal pits and fissures also warrants novel prevention methods. Early on, in laboratory and clinical studies, it was shown that irradiation with microsecond pulsed CO_2 lasers resulted in enhanced caries resistance of enamel [17–20]. Compared with the traditional 10.6-μm CO_2 laser wavelength, the 9.3- and 9.6-μm CO_2 laser wavelengths are absorbed up to ten times stronger in enamel [21]. As a consequence of the irradiation heat, carbonate is driven out from the naturally occurring carbonated hydroxyapatite, resulting in a reduced acid dissolution of the remaining hydroxyapatite [22, 23]. When fluoride is added at this stage, fluorapatite is formed, which is even more acid resistant [24].

Laser settings to achieve enhanced caries resistance had for the first time been tested in vivo in a pulpal safety study. The microsecond short-pulsed CO_2 laser irradiation did not cause any harm to the pulpal tissue of irradiated teeth [25]. In a next step towards clinical application, Rechmann and co-workers tested in vivo the CO_2 9.6-μm laser irradiation in a single-blind, clinical trial using an orthodontic bracket model [26]. Over a 12-week study period, they observed for the smooth surfaces of the bicuspids scheduled for extraction an 86% reduction in demineralization [19].

In the subsequent clinical pilot trial, inhibition of carious lesions was assessed with non-invasive, optical methods. The caries preventive effect of microsecond pulsed 9.6-μm CO_2 laser irradiation with additional fluoride varnish applications on molar fissures was evaluated. The clinical pilot trial showed significantly inhibited formation of carious lesions in fissures of molars in comparison with a non-irradiated contra-lateral control tooth in the same arch over a 1-year observation interval.

Consequently, in the clinical trial presented here, the objective was to evaluate whether the use of a newly developed CO_2 9.3-μm short-pulsed laser increases the caries resistance of occlusal pit and fissure surfaces in patients in addition to fluoride therapy, with test teeth receiving laser and fluoride varnish treatment and control teeth getting only fluoride applications. Clinical status was quantified by visual exams with the International Caries Detection and Assessment System, SOPROLIFE daylight and blue fluorescence, and DIAGNOdent Laser Light–Induced fluorescence in this randomized, single-blind, split-mouth controlled, clinical trial over 12 months.

Materials and methods

Participants

This study was performed between February 2018 and November 2019 at the University of California, San Francisco (UCSF) School of Dentistry. UCSF Institutional Review Board (IRB) approval was obtained (IRB #14-15555), and the study was registered with the US National Institute of Health (ClinicalTrials.gov identifier: NCT02357979). Information about the study was provided to participants in a standardized manner via an informational leaflet and demonstration aids.

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 of the potential participant. Medical history and definitive dental history were evaluated, and an intraoral exam and a review of intraoral radiographs were performed.

Inclusion criteria for the study were (a) a participant age of 6 years and older, (b) moderate or high caries risk status according to CAMBRA (Caries Management by Risk Assessment) [27, 28], and (c) having at least two fully erupted second molars in the same arch (contra-lateral) with untreated, non-cavitated occlusal surfaces (International Caries Detection and Assessment System (ICDAS) scores 0, 1, 2 were allowed; [29] and see below) with deep grooves, (d) participants had to be willing to comply with all study procedures and protocols, (e) they had to reside in San Francisco or other nearby local communities with water fluoridation, (f) participants had to be healthy, (g) participants/parent had 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.

Exclusion criteria included, but were not limited to subjects (a) suffering from systemic diseases, (b) with a significant past or medical history with conditions that may affect oral health (i.e., diabetes, HIV, heart conditions that require antibiotic prophylaxis), (c) using 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), (d) treated with in-office fluoride treatment within the last 3 months prior to being enrolled in the study, (e) were not willing to stop the use of any mouth rinse or other oral hygiene product, besides brushing and flossing, during the duration of the study, or (f) were planning to leave the area and would not be available for recall visits, and (g) showing evidence of extremely poor oral hygiene or (h) had a nut allergy (F-varnish may contain pine

nuts as potential allergens, cross allergies to other nuts are possible).

From the Pediatric Dentistry Clinic at UCSF, 229 potential participants were screened between February 8 and November 21, 2018. From those, 81 were eligible and were invited to participate. Finally, a total of 60 agreed to participate and were enrolled into the study. Participants who met the selection criteria provided verbal assent or written consent and their parent/guardian provided written informed consent.

Randomization

Participants' second molars in the same jaw (upper or lower arch, depending on availability) received a random assignment to the experimental (laser and fluoride) or control (fluoride alone) group. The randomization list was created by a random number generator (QuickCalcs Online Random Numbers by GraphPad Software, Inc.). The randomization list was kept locked and group assignments were kept in separate, closed, opaque, sequentially numbered envelopes. Only after a participant had been enrolled the next-in-line group assignment was revealed. One study investigator was blinded to the group assignment, and the second was partially blinded (the treatment did not result in any visible surface change of the fissure; thus, after 6 and 12 months, the laser treatment allocation side could not be identified clinically). The senior research associate (SRA) (BR) and the doctor providing the laser treatment were not blinded. The SRA provided instructions for homecare and oral hygiene. The randomization list remained secured until the completion of all data collection.

Study procedure, laser and laser settings, clinical visual evaluation, and time points

After enrollment but prior to evaluating, the occlusal surfaces of the second molars were cleaned with a disposable tapered rotating brush (Denticator, Earth City, MO) for 10–20 s per tooth and then rinsed with an air-water spray. Prophy paste was not used in order not to influence fluorescence later on. Cotton rolls were placed, and the occlusal surfaces were air-dried for 3 to 5 s per tooth, immediately before performing a caries lesion assessment (detailed description below). Then, the study tooth was laser treated, and the lesion assessment with the fluorescence tools—SOPROLIFE blue fluorescence and DIAGNOdent—was repeated. Since differences in assessments between before and after laser application did not occur, the before laser treatment records served as baseline. The participants were instructed to brush their teeth for at least 2 min twice daily with a 1100-ppm fluoride-containing dentifrice (as NaF).

All participants received fluoride varnish applications (Vanish, 3M Oral Care, Saint Paul, MN). Fluoride varnish was applied to all teeth in the oral cavity, including the laser-treated as well as the control tooth at baseline and the 6-month recall.

Laser and laser settings—The laser utilized in this study was a microsecond short-pulsed carbon dioxide laser, wavelength 9.3 μm (Solea, Convergent Dental, Inc., Needham, MA). The laser was operated in non-contact mode. The beam diameter was set to 0.25 mm, and the laser focus length was 4 to 10 mm. Irradiation in the distance of the focus length range allowed for a constant spot size of the beam at tissue level. The irradiation beam diameter was verified by using a 1″ FL lens as a relay to magnify the focused spot × 5.5 to an Ophir-Spiricon Pyrocam III pyroelectric camera for detection (Ophir-Spiricon, LLC, North Logan, UT). For the measurement of the beam diameter, BeamGage V5.11 software was used in pulsed mode with 5 ms exposure time, m 90/10 size criteria.

The laser pulse duration was 4 μs, delivering a pulse energy of 1.9 mJ/pulse, resulting in a fluence of 3.9 J/cm^2. The pulse energy was measured with a BeamTrack - Power/Position/Size Thermal Sensor 50(150)A-BB-26-PPS (Ophir-Spiricon) before and after teeth of five participants were irradiated. Energy losses between measurements did not occur. The temporal laser pulse shape was square with an initial sharp energy peak. The pulse repetition rate was set to 43 Hz.

The beam profile had been measured and pictured with an Ophir-Spiricon Pyrocam III, model LBS-100 pyroelectric camera with BeamGage V6.3.0.13 software. The beam profile was basically Gaussian and demonstrated a beam with a centralized sharper, higher energy peak level ("hot spot") resulting in very slight melting of the enamel [30].

To ensure that each spot of a fissure was irradiated with at least 20 laser pulses, a condition that had shown to enhance caries resistance in the past [19], each fissure was irradiated for 2 min with overlapping irradiation. Neither air nor water spray was applied.

Clinical visual evaluations of the study teeth were scheduled at 6 and 12 months after laser treatment. As described above as study entrance criteria, only participants with second molars showing no signs of caries (ICDAS score 0) or only pre-cavitated lesions (ICDAS scores 1, 2) were allowed into the study. If at any recall an ICDAS score 3 or above was registered, a sealant or filling was placed and participation in the study was terminated. At the end of the study, the control and test teeth were sealed with a fissure sealant (Helioseal, Ivoclar Vivadent, Amherst, NY). To evaluate adverse events, side effects or harm at each recall visit participants were asked about any pain or other unusual sensation related to their study teeth or other unusual observations.

Outcome measures

The primary outcome measure was the ICDAS score with (a) differences in change in ICDAS scores between matched case

and control teeth (within patient) from baseline to 6 months and baseline to 12 months, and (b) difference in number of lesion changes into ICDAS score 3 (signifying a cavity) between matched case and control teeth (within patient) from baseline to 6 months and baseline to 12 months.

Secondary outcome measures were differences in change in SOPROLIFE and DIAGNOdent scores between matched case and control teeth (within patient) from baseline to 6 months and baseline to 12 month.

Clinical visual evaluation and assessment tools

The occlusal surfaces of the study second molars were visually assessed for decalcification using the ICDAS criteria [29], 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. Each occlusal fissure area was scored at the mesial, central, and distal location.

Visual examination and assessment using ICDAS criteria To assess the degree of decalcification of the pits and fissure regions of the study molars, ICDAS assessments [29] were performed. The two examiners (MK, PR) were blinded to each other's evaluation results. After independently assessing the ICDAS scores, the findings were discussed, and the examiners agreed on one ICDAS score per area.

The assessed ICDAS scores were reported in two different ways—traditionally as the highest score occurring on the surface evaluated and as an ICDAS surface sum score with all ICDAS score values from all three occlusal evaluation areas of a fissure (mesial, central, distal) added up to one score.

SOPROLIFE Light-Induced Fluorescence Evaluator The daylight mode of the SOPROLIFE Light-Induced Fluorescence Evaluator system uses four white LEDs, and in the fluorescence mode uses four blue LEDs emitting a wavelength of 450 nm (SOPRO, ACTEON Group, La Ciotat, France). For this study, the system was operated in LIFE magnification mode with daylight or fluorescence detection mode I—diagnosis aid mode. The handpiece allows for collecting images, which were recorded with the SOPRO IMAGING software. A MacBook Pro (Apple Inc., Cupertino, CA; OS 10.14) was used to collect the data for independent evaluation. Two independent examiners (MK, PR) utilized an earlier introduced scoring system to evaluate the images [31, 32]. The same three areas of interest on each fissure evaluated by the ICDAS scoring system were used for the SOPROLIFE image 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). Consequently, the intensity of the returning fluorescence in the spectral region of > 680-nm wavelength is measured by the tool. Before assessing the fissure areas, the tool was calibrated according to manufacturer's instruction. Similar to ICDAS, DIAGNOdent scores were again evaluated at three areas of a fissure (scores ranged from 3 to 64 in this study).

Inter- and intra-examiner reliability

While PR and co-workers had trained and calibrated a cohort of 30 dentists in ICDAS scoring for a practice-based research network trial [33], MK was trained for this study on roughly 150 potential study participants during screening. The inter-examiner reliability (MK vs. PR) for the ICDAS scoring was assessed using scoring data available from both examiners over the whole study period for up to 60 study participants at up to 3 different study time points (646 data points). The inter-examiner reliability was determined as a kappa = 0.472, SE of kappa = 0.030, and 95% confidence interval from 0.414 to 0.530. The strength of agreement is considered to be "moderate" [34]. The weighted kappa was calculated at kappa = 0.559 using linear weighting. Assessed this way, the strength of agreement is again considered to be "moderate" [34].

The intra-examiner reliability calculations were based on ICDAS scoring of 54 observations points on extracted molar occlusal fissures. Extracted teeth were chosen since all children in the study had existing sealants on the non-study molars. Consequently, it would have been necessary to recall too many children within a week for re-scoring for the intra-examiner reliability testing. The intra-examiner reliability was determined for MK as a kappa = 0.444, SE of kappa = 0.0097, 95% confidence interval from 0.254 to 0.635, and as weighted kappa = 0.529. For PR, the intra-examiner reliability was determined as a kappa = 0.417, SE of kappa = 0.0095, 95% confidence interval from 0.231 to 0.603, and as weighted kappa = 0.558. The strength of agreement is considered for both examiners to be "moderate" for the kappa as well as the weighted kappa [34].

Power calculation

Power calculations were based on results of a pilot study on caries prevention [20]. To guide calculations, for the primary endpoint change in ICDAS scores, we assumed one pair of molars per participant and compared the proportion of teeth with worsening ICDAS score in laser versus control groups. In the pilot study, we observed 1 participant with worsening ICDAS score in the laser group versus 9 participants in the

control group. The observed difference in percentage for worsening ICDAS score was 56% (6% laser vs. 62% control). Based on a McNemar test to account for the pairing of observations, with a conservative sample size of 50 (after loss to follow-up) and assuming the same proportion of discordant pairs (75%), we would have 80% power to detect a difference in percentages of 35% at the 5% significance level (i.e., allowing for over 35% reduction in the pilot observed difference of 0.56). To account for loss to follow-up, $N = 60$ participants were enrolled.

Statistical methods

The main outcome of interest was change in ICDAS scores between matched treated and control teeth (within participant) from baseline to 6 months and baseline to 12 months. Each occlusal surface was assessed as 3 pit and fissure regions (mesial, central, distal); differences from baseline to follow-up were based on the highest (worst) ICDAS score recorded per surface at that time point. As additional outcomes, highest ICDAS score (regardless of baseline status) and summed scores from all 3 regions were also calculated at each time point. Treated versus control groups were compared by the Wilcoxon signed-rank test. Also assessed was whether any surfaces developed a cavitated lesion (ICDAS score ≥ 3). Percentages of teeth with ICDAS score ≥ 3 were compared between treated and control groups by McNemar's test.

Secondary outcome measures were based on SOPROLIFE scores and DIAGNOdent scores between matched treated and control teeth (within participant) from baseline to 6 months and baseline to 12 months. SOPROLIFE scores (one score obtained per surface) and difference in scores from baseline were compared at each time point (Wilcoxon's signed-rank test). Analogously to ICDAS scores, DIAGNOdent scores were recorded at 3 occlusal regions and calculated as the highest (worst) score per surface per time point, sum of scores per surface per time point, and difference in sum score from baseline (Wilcoxon's signed-rank test), as well as any change in highest score from baseline (McNemar's test). Differences were considered statistically significant at $P < 0.05$, without adjustment for multiple tests. Analyses were completed using Stata 16.1 (StataCorp, College Station, TX).

Results

The mean participant age at baseline was 13.1 ± 1.4 years (mean ± standard deviation [SD]) (range: 10.0–16.7). Table 1 shows the characteristics of the study population with sex, race/ethnicity, the treated quadrant at enrollment, and for the 6- and 12-month recalls. At the 6-month recall, 3 participants did not show for the appointment, and an additional 2 participants were no-shows at the 12-month recall. Additionally, 4 participants who developed cavitated lesions at the 6-month recall were not followed subsequently (Fig. 1).

No adverse events, side effects, or harm was reported.

ICDAS

ICDAS score

Table 2a shows that the highest ICDAS scores for the treated and the control teeth did not significantly differ at baseline. At the 6-month recall for the laser-treated teeth, the percentage of ICDAS score 0 stayed constant with 7%, and the percentage of ICDAS score 1 decreased slightly from 72 to 60%, while for score 2, it increased slightly from 21 to 33%. In contrast, the control teeth showed much higher ICDAS changes. In the control teeth, the percentage of ICDAS score 0 counts decreased from 14 to 5%, and the percentage of ICDAS score 1 decreased from 65 to 33%, while the percentage ICDAS 2 scores increased from 21 to 54%. Moreover, 4 control teeth (7%) showed an ICDAS score 3, but none of the laser-treated teeth did. These observed differences between laser-treated and control teeth are statistically significant ($P = 0.0001$, Wilcoxon's signed-rank test, exact). An ICDAS score 3 terminated the participant's study participation.

At the 12-month recall, differences between laser-treated and control teeth became even more distinct. The percentages of ICDAS scores 0, 1, and 2 stayed nearly the same for the laser-treated teeth, but for the control teeth, the percentage of ICDAS score 0 dropped to 0, the percentage of ICDAS score 1 further dropped from 33 to 24%, the percentage of ICDAS score 2 slightly increased from 54 to 59%, but the number of teeth with ICDAS score 3 grew by additional 9 teeth (18%). The increases in ICDAS scores for the control teeth compared with the laser-treated teeth were again statistically significant with $P < 0.0001$ (Wilcoxon's signed-rank test, exact).

Table 2b shows change in ICDAS scores between baseline and 6- and 12-month recalls expressed in a delta (Δ) ICDAS score with Δ ICDAS score − 1, meaning an improvement of the ICDAS score by one score; Δ ICDAS score 0, the score stayed the same; Δ ICDAS score 1, the score worsened by 1 level; and Δ ICDAS score 2, the score worsened by two levels (for instance, ICDAS 0 changed to ICDAS 2 at follow-up). The laser-treated molar surfaces observed very slight improvements over time (Δ ICDAS − 1, 11% and 8%), no changes (Δ ICDAS 0) in 68% and 67%, and slight changes (Δ ICDAS 1) in 19% and 24%, and changes by two scores (Δ ICDAS score 2) in 2% occurred at the 6- and 12-month recalls, respectively. In contrast, the control teeth showed significantly more Δ ICDAS score increases, with only less than half showing a Δ ICDAS 0 after 6-month and only 25% at the 12-month recall. At 12-month recall, Δ ICDAS score 1 had increased to 55% and worsening of the ICDAS by 2 scores had increased to 20%. The differences in change in the highest

Table 1 Characteristics of the study population

	Enrolled ($N = 60$)	6 months ($N = 57$)	12 months ($N = 51$)
Baseline age, mean (SD)	13.1 (1.4)	13.2 (1.4)	13.2 (1.4)
Sex, *n* (%)			
Female	32 (53)	31 (54)	28 (55)
Male	28 (47)	26 (46)	23 (45)
Race/ethnicity, *n* (%)			
African American	9 (15)	8 (14)	7 (14)
Asian	16 (27)	15 (26)	13 (25)
Hispanic/Latinx	24 (40)	23 (40)	20 (39)
Non-Hispanic White	7 (12)	7 (12)	7 (14)
Other	4 (7)	4 (7)	4 (8)
Treated quadrant, *n* (%)			
Upper right	17 (28)	16 (28)	15 (29)
Upper left	17 (28)	16 (28)	12 (24)
Lower left	14 (23)	14 (25)	13 (25)
Lower right	12 (20)	11 (19)	11 (22)
Days of follow-up, mean (SD)	-	189.0 (16.5)	365.9 (19.9)

ICDAS scores from baseline for both recall intervals were significantly higher for the control than the laser-treated molars with $P = 0.0002$ and $P < 0.0001$ (6-month and 12-month recalls; Wilcoxon's signed-rank test, exact).

ICDAS surface sum score

Table 2c and d show the occlusal ICDAS surface sum score (Table 2c) and the change in ICDAS surface sum score over time (Table 2d). While at baseline for both groups, laser-treated and control, the mean ICDAS sum score was not statistically significantly different (mean 2.0 and 2.2, SD 1.3 for both, treated and control, respectively). At 6 months for the laser-treated teeth, the mean slightly increased to 2.4 ± 1.4 but increased to 3.4 ± 1.7 for the controls ($P < 0.0001$, Wilcoxon's signed-rank test, exact). At 12 months, for the control, the mean ICDAS surface sum score increased even further to 4.2 ± 1.5, but for the laser-treated teeth, it stayed at 2.3 ± 1.3 ($P < 0.0001$) (Table 4a).

With regard to changes of the ICDAS surface sum score over time (Table 4b), the mean increase was only 0.2 ± 1.2 and 0.3 ± 1.4 for the laser-treated teeth at 6 and 12 months, respectively. In contrast, for the controls, the mean heavily increased by 1.4 ± 1.2 and 2.4 ± 1.2 for the same time periods ($P < 0.0001$ for differences between laser-treated and control, at both time points).

Sopro daylight and Sopro blue fluorescence scores

Sopro daylight scores

Table 3a shows the highest Sopro daylight scores per surface for laser-treated and control teeth at baseline and 6- and 12-month recalls. Table 3b shows the changes in scores (Δ) over time. As with ICDAS scores, the Sopro daylight scores showed worsening over time for the control teeth while the laser-treated teeth stayed at a relative stable score count (note that at baseline, the laser-treated teeth started out with worse scores than the control teeth). Control teeth showed higher number of changes in Sopro daylight scores at 6-month as well as even higher Δ numbers at the 12-month recall, resulting in significant differences between the two groups.

Sopro blue fluorescence scores

Table 3c reports Sopro blue light fluorescence scores. Sopro blue light scores were worse in the treatment group at baseline ($P = 0.02$) but by 6 and 12 months, there were no statistically significant differences between groups. However, change in Sopro blue light scores revealed greater worsening over time from baseline to 6 months and from baseline to 12 months for the control teeth. The differences in worsening scores for the controls compared with the laser-treated surfaces were statistically significant (Table 3d).

Correlation between ICDAS score, Sopro daylight, and Sopro blue fluorescence

Taking all measurement time points into account for testing whether any applied score correlates to another, it occurs that ICDAS scores correlated highly with Sopro daylight, and Sopro daylight scores correlated reasonably with Sopro blue fluorescence scores (Spearman's correlation rho = 0.438 and 0.240, respectively, $P < 0.0001$). ICDAS scores also correlated with Sopro blue fluorescence scores with a weaker correlation (rho = 0.14, $P = 0.011$) (Table 4). When considering

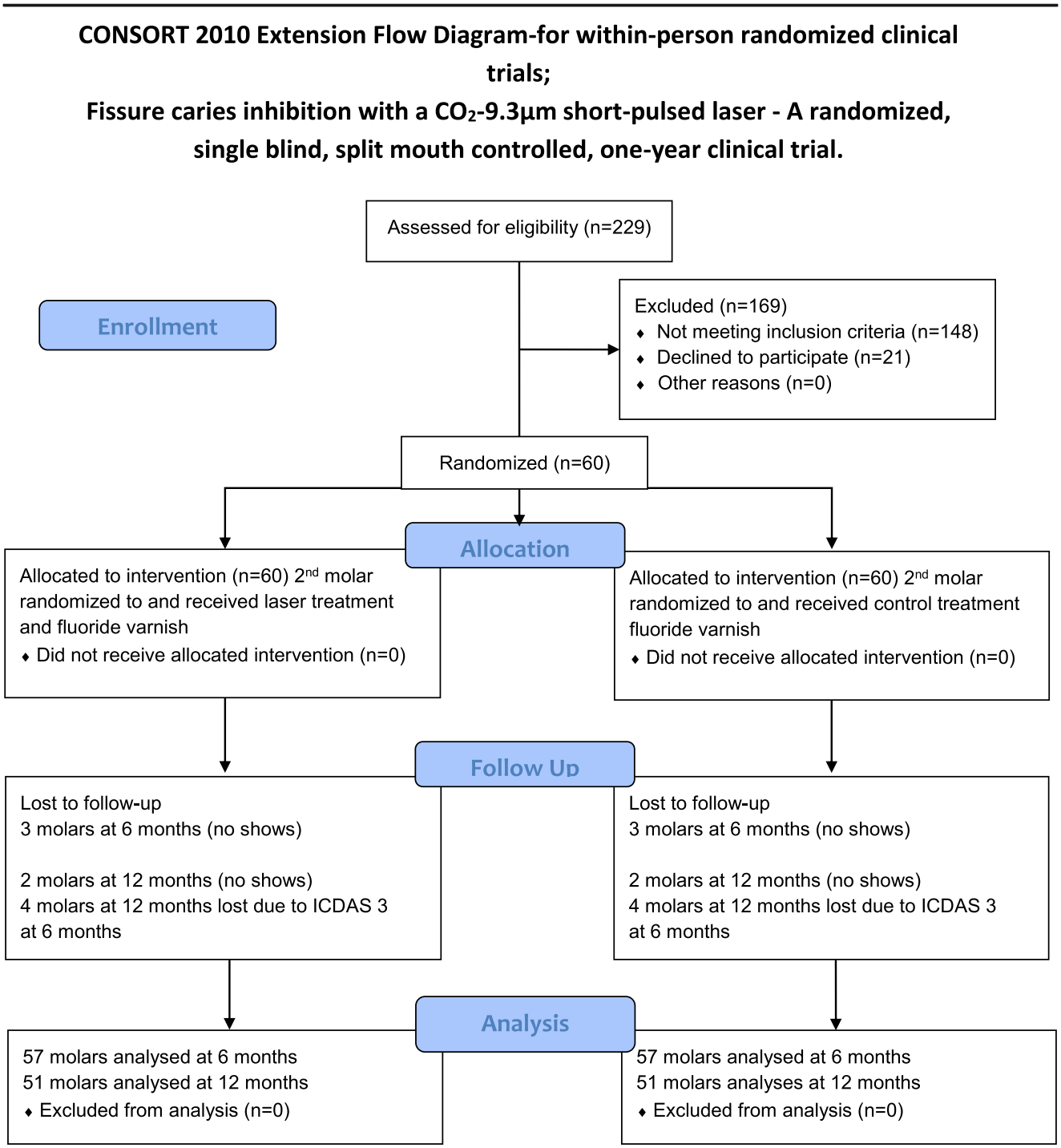

Fig. 1 CONSORT 2010 extension flow diagram for within-person randomized clinical trials, with patients followed from baseline and at each follow-up visit, by intervention status

only baseline scores, ICDAS scores correlated statistically significantly only with Sopro daylight scores (rho = 0.375, $P < 0.001$) (Table 4).

DIAGNOdent scores

Table 5a presents the highest ICDAS scores per surface. While for all time points slight increases in mean DIAGNOdent values occurred, the differences between laser-treated and control teeth were not significant at any time point. Changes in DIAGNOdent scores expressed as the same or better versus worse from baseline show worsening scores for the control teeth only for the interval baseline to 12 months ($P = 0.03$; McNemar's test, exact) (Table 5b).

Table 2 Occlusal surface ICDAS scores by intervention status—highest score per surface, with (a) highest score per surface and (b) change in highest score from baseline, with (c and b) occlusal surface ICDAS scores by intervention status—ICDAS surface sum score, with (c) surface sum score and (d) change in surface sum score from baseline

a. ICDAS: Highest score per surface, *n* (%)

	Treated	**Control**	
Score	Baseline	Baseline	*p* value[a]
0	4 (7)	8 (14)	0.33
1	41 (72)	37 (65)	
2	12 (21)	12 (21)	
Score	6 months	6 months	*p* value[a]
0	4 (7)	3 (5)	0.0001
1	34 (60)	19 (33)	
2	19 (33)	31 (54)	
3	0	4 (7)	
Score	12 months	12 months	*p* value[a]
0	3 (6)	0	< 0.0001
1	31 (61)	12 (24)	
2	17 (33)	30 (59)	
3	0	9 (18)	

b. ICDAS: Change in highest score from baseline, *n* (%)

	Treated	**Control**	
	Baseline	Baseline	
	-	-	
	-	-	
	-	-	
Δ score	6 months	6 months	*p* value[a]
− 1	6 (11)	0	0.0002
0	39 (68)	27 (47)	
1	11 (19)	28 (49)	
2	1 (2)	2 (4)	
Δ score	12 months	12 months	*p* value[a]
− 1	4 (8)	0	< 0.0001
0	34 (67)	13 (25)	
1	12 (24)	28 (55)	
2	1 (2)	10 (20)	

c. ICDAS: Surface sum score

	Treated	**Control**	
	Baseline	Baseline	*p* value[a]
Mean	2.2	2.0	0.25
SD	1.3	1.3	
Median	2	2	
IQR	1, 3	1, 3	
	6 months	6 months	*p* value[a]
Mean	2.4	3.4	< 0.0001
SD	1.4	1.7	
Median	2	3	
IQR	1, 3	2, 5	
	12 months	12 months	*p* value[a]
Mean	2.3	4.2	< 0.0001
SD	1.3	1.5	
Median	2	4	
IQR	1, 3	3, 5	

d. ICDAS: Change in surface sum score from baseline

	Treated	**Control**	
	Baseline	Baseline	
	-	-	
	-	-	
	-	-	
	6 months	6 months	*p* value[a]
Mean	0.2	1.4	< 0.0001
SD	1.2	1.2	
Median	0	1	
IQR	0, 1	1, 2	
	12 months	12 months	*p* value[a]
Mean	0.3	2.4	< 0.0001
SD	1.4	1.2	
Median	0	2	
IQR	− 1, 1	2, 3	

[a] Wilcoxon's signed-rank test (exact)

Discussion

In the past, laboratory and in vivo clinical studies have shown that in the microsecond range, short-pulsed 9.3- and 9.6-μm CO_2 lasers can successfully enhance enamel caries resistance [17–20, 22, 30, 35]. Due to the irradiation heat, the loss of the carbonate phase from the naturally occurring enamel crystals being a carbonated hydroxyapatite leads to a less acid-soluble hydroxyapatite [22, 23], which then in the presence of fluoride can turn in the least acid-soluble fluorapatite [24]. During the first clinical trial using a 20-μs short-pulsed 9.6-μm CO_2 laser, up to 87% reduction in mineral loss around orthodontic brackets was registered with significant reductions in mineral loss over observation periods of up to 12 weeks [19]. In a consequent clinical pilot study, enhanced caries resistance due to irradiation with the same short-pulsed 9.6-μm CO_2 laser of enamel in molar fissures was tested over a 12-month period. Twenty adolescents participated in this 1-year clinical pilot trial. This study revealed that microsecond short-pulsed 9.6-μm CO_2 laser irradiation in combination with biannual application of fluoride varnish efficiently enhanced caries resistance of laser-treated fissures in comparison with non-treated fissures [20]. As evaluation tools, ICDAS, SOPROLIFE, and DIAGNOdent were used [20].

The study presented here was designed with the intention to prove that the use of a new CO_2 9.3-μm short-pulsed laser, commercially available for the use in dental offices, increases the caries resistance of occlusal pit and fissure surfaces in addition to fluoride therapy. The outcome was quantified by visual exams with ICDAS, SOPROLIFE daylight and blue fluorescence, and DIAGNOdent in a randomized, single-blind, split-mouth controlled, clinical trial over 12 months similar to the pilot study mentioned above, but on a large number of participants.

Table 3 Occlusal surface Sopro scores by intervention status, with (a) Sopro daylight: score per surface and (b) Sopro daylight: change in score from baseline; (c) Sopro blue: score per surface and (d) Sopro blue: change in score from baseline

a. Sopro daylight: Score per surface, *n* (%)

	Treated	Control	
Score	Baseline	Baseline	*p* value[a]
0	1 (2)	3 (5)	0.01
1	11 (20)	15 (27)	
2	34 (61)	35 (63)	
3	10 (18)	3 (5)	
Score	6 months	6 months	*p* value[a]
0	1 (2)	0	0.68
1	10 (18)	12 (21)	
2	33 (59)	30 (54)	
3	12 (21)	14 (25)	
Score	12 months	12 months	*p* value[a]
0	1 (2)	0	0.01
1	10 (19)	8 (16)	
2	30 (58)	18 (35)	
3	10 (19)	25 (49)	
4	1 (2)	0	

b. Sopro daylight: Change in score from baseline, *n* (%)

	Treated	Control	
	Baseline	Baseline	
	-	-	
	-	-	
	-	-	
	-	-	
Δ score	6 months	6 months	*p* value[a]
− 1	0	0	0.0001
0	53 (95)	36 (64)	
1	3 (5)	20 (36)	
2	0	0	
Δ score	12 months	12 months	*p* value[a]
− 1	2 (4)	0	< 0.0001
0	47 (90)	19 (37)	
1	1 (2)	30 (59)	
2	2 (4)	2 (4)	

c. Sopro blue: Score per surface, *n* (%)

	Treated	Control	
Score	Baseline	Baseline	*p* value[a]
0	4 (7)	8 (14)	0.02
1	15 (27)	14 (25)	
2	16 (29)	21 (38)	
3	21 (38)	13 (23)	
Score	6 months	6 months	*p* value[a]
0	6 (11)	3 (5)	1.00
1	5 (9)	11 (20)	
2	17 (30)	16 (29)	
3	28 (50)	24 (43)	
4	0	2 (4)	
Score	12 months	12 months	*p* value[a]
0	5 (10)	2 (4)	0.77
1	3 (6)	6 (12)	
2	12 (23)	13 (25)	
3	30 (58)	23 (45)	
4	2 (4)	7 (14)	

d. Sopro blue: Change in score from baseline, *n* (%)

	Treated	Control	
	Baseline	Baseline	
	-	-	
	-	-	
	-	-	
	-	-	
Δ score	6 months	6 months	*p* value[a]
− 2	1 (2)	0	0.02
− 1	2 (4)	1 (2)	
0	40 (71)	30 (54)	
1	9 (16)	21 (38)	
2	4 (7)	4 (7)	
Δ score	12 months	12 months	*p* value[a]
− 2	1 (2)	0	0.03
− 1	1 (2)	2 (4)	
0	30 (58)	19 (37)	
1	14 (27)	19 (37)	
2	5 (10)	9 (18)	
3	1 (2)	2 (4)	

[a] Wilcoxon's signed-rank test (exact)

ICDAS—highest score per surface—Δ change in highest ICDAS score per surface—ICDAS surface sum score

The ICDAS is a widely accepted assessment method for diagnosing caries lesions [29]. ICDAS criteria are based on translucency and microporosity of the enamel. The enamel refractive index changes due to demineralization events as a first sign of carious alteration. The enamel surface appears whitish. If demineralization continues, the enamel microporosity increases, with a further decreased refractive index [36]. ICDAS has been validated by demonstrating an association between the severity of the caries lesions and the lesions' histological depth [37–39]. Especially in pre-cavitated but also in slightly cavitated stages, a relationship between the visual topography at the surface level and the histological lesion depth has been demonstrated [40, 41].

In this present CO_2 9.3-μm short-pulsed laser fissure caries prevention study, participants were only accepted if they presented potential study teeth with no signs of caries at all (ICDAS score 0) or with pre-cavitated lesions only (scores 1, 2). Teeth revealing an ICDAS score 3 or higher at a recall received fissure sealants, and further participating in the study was ended.

Table 4 Correlation between ICDAS score, Sopro daylight, and Sopro blue fluorescence

Technique A	Technique B	Rho	*p* value
Spearman's correlation, all teeth, all measurement time points			
ICDAS	Sopro daylight	0.438	< 0.0001
ICDAS	Sopro blue	0.140	0.011
Sopro daylight	Sopro blue	0.240	< 0.0001
Spearman's correlation, all teeth, baseline only			
ICDAS	Sopro daylight	0.375	< 0.0001
ICDAS	Sopro blue	− 0.024	0.803
Sopro daylight	Sopro blue	0.135	0.155

Similar as reported in the previous pilot study where 25% of the participants had developed an ICDAS 3 lesion on the occlusal surface with all of them occurring in the control teeth [20], in this study, again almost the same percentage of participants (22%) developed an ICDAS 3 lesion, again in the control teeth. Since ICDAS score 3 by definition refers to a physical loss of enamel, not just a loss of mineral as in demineralization, it describes the first visible cavity. Consequently, these teeth received fissure sealants, and the participants were withdrawn from further participation in the study. None of the laser-treated molar fissures showed an ICDAS 3 score. Obviously, the CO_2 9.3-μm short-pulsed laser with additional fluoride use prevented development of first visible cavities over a 1-year period compared with non-laser-irradiated control teeth in the same mouth. Preventing a first cavity avoids a first "drilling action." Indeed, "drilling and filling" starts the "repeat restoration cycle" process that ends with each restoration being less prophylactic and more iatrogenic than the previous one [42].

While at the start of the study the candidates' right and left molars showed very similar ICDAS score counts, after 6 and even further pronounced after 12 months, the control teeth showed worsened ICDAS scores compared with the laser-treated teeth. The differences were statistically significant, supporting that the CO_2 9.3-μm short-pulsed laser treatment prevents demineralization of tooth surfaces better than fluoride treatment alone. Similar high significances for differences between laser-treated and control teeth were also confirmed when regarding the change Δ in highest ICDAS scores per fissure over time. Again, the control teeth showed increased score changes compared with the laser-treated teeth. Differences between treated and control groups were not merely attributable to the ICDAS 3 scores (cavitated lesions) occurring in the controls, as the proportion of non-cavitated lesions (ICDAS scores 1 and 2) were greater in the control group, as well.

Driving out the carbonated phase from the enamel crystal not only enhances demineralization resistance of the modified hydroxyapatite. In addition, the present study showed, although at a much smaller extent than in the aforementioned laser fissure caries prevention pilot study and the orthodontic bracket study [19, 20], a tendency for the transformed hydroxyapatite to be prone to higher remineralization, when

Table 5 Occlusal surface DIAGNOdent scores by intervention status, with (a) DIAGNOdent: highest score per surface and (b) DIAGNOdent: change in highest score per surface from baseline

a. DIAGNOdent: Highest score per surface

	Treated	Control	
	Baseline	Baseline	*p* value[a]
Mean	12.4	12.8	0.57
SD	7.2	8.3	
Median	11	12	
IQR	8, 14	8, 15	
	6 months	6 months	*p* value[a]
Mean	15.1	15.5	0.61
SD	7.3	7.6	
Median	14	15	
IQR	10, 17	10, 20	
	12 months	12 months	*p* value[a]
Mean	17.4	18.6	0.24
SD	9.9	10.7	
Median	16	16	
IQR	10, 22	11, 23	

b. DIAGNOdent: change in highest score per surface from baseline, *n* (%)

	Treated	Control	
	Baseline	Baseline	
	-	-	
	-	-	
	6 months	6 months	*p* value[b]
Same/ Better	18 (32)	18 (32)	1.000
Worse	39 (68)	39 (68)	
	12 months	12 months	*p* value[b]
Same/ Better	19 (38)	9 (18)	0.03
Worse	31 (62)	41 (82)	

[a] Wilcoxon's signed-rank test (exact)

[b] McNemar's test (exact)

fluoride is present. This was supported by the improvements in scoring grades over time, expressed by delta (Δ) ICDAS score changes, showing one or even two score changes into the direction of lower ICDAS scores for laser-treated molars.

By adding up all ICDAS score values from mesial, central, and distal occlusal pit and fissure areas into an ICDAS surface sum score allows receiving an additional aspect of the change in caries burden of the control teeth versus a preventive effect on the laser-treated surfaces. This way of "weighting" the demineralization by accounting for the whole tooth surface and not for only one spot with the worst score showed a very constant low-level ICDAS surface sum score for the laser-treated fissures over the observation time (between 2.2 ± 1.3 and 2.4 ± 1.4) (mean ± SD). While the control fissures had even a nominally slightly lower start of ICDAS surface sum scores than the laser-treated (2.0 ± 1.3), the score for the non-irradiated surfaces more than doubled in 1 year, from ICDAS surface sum score 2.0 ± 1.3 to 4.2 ± 1.5. Obviously, the CO_2 9.3-μm laser irradiation has led to an enhanced caries resistance in every pit and fissure area and not only in the most prone to demineralization fissure areas reported traditional by the ICDAS score.

SOPROLIFE daylight scores and SOPROLIFE blue fluorescence scores

Some caries detection methods engage light fluorescence, which is accomplished in substrates that absorb certain wavelengths and then emit the absorbed energy at a longer wavelength. Fluorescence devices have been intended for early-stage caries diagnosis [32, 43]. The SOPROLIFE system is a combination of a visual inspection method with high specificity using an intraoral camera and a laser fluorescence device offering high reproducibility and discrimination [32, 43, 44].

The SOPROLIFE daylight and blue fluorescence scoring system, as recently published using six distinct codes for each detection mode [31, 32], was used to evaluate the teeth in this laser caries prevention study. Comparable with the ICDAS scores, SOPROLIFE daylight scoring showed increasing scores over time for the control teeth and relatively unchanged scores for the laser-treated fissures. This was expected since SOPROLIFE daylight assessments basically represent a high magnification view of the fissure. Similarly, but less pronounced, the SOPROLIFE blue fluorescence scores, specifically the Δ change in score from baseline to the recall time points, showed significant score worsening for the control teeth. The SOPROLIFE findings support the ICDAS results, stating the caries protective effect of the CO_2 9.3-μm laser irradiation. For both SOPROLIFE daylight and blue fluorescence scores, the earlier pilot study had shown comparable tendencies allowing for similar conclusions [20].

A study comparing the ICDAS system based on histology as the gold standard [45] with SOPROLIFE daylight and blue fluorescence, DIAGNOdent, and Spectra Visix revealed, using linear regression fits, that SOPROLIFE in both detection modes was highly correlated with ICDAS and weakly correlated with DIAGNOdent [19, 31]. In the present study, here testing the correlation between ICDAS and SOPROLIFE daylight scores also revealed a strong correlation for all data points as well as for the smaller number of data points at baseline examinations between the two detection methods. The correlation to SOPROLIFE blue fluorescence scores was existent but much weaker. The blue fluorescence is mainly based on the existence of bacteria by-products like porphyrins accumulating in the enamel pores created by demineralization in the fissures over time [46–48]. In comparison with the pilot study, the observed smaller differences in control and laser-treated surfaces seen with the SOPROLIFE blue fluorescence detection mode in the present study might be based on the fact that in the pilot study the participants had been older. In the pilot study, the average age of participants was 14.2 ± 1.2 years, while in the present study, the average age was 13.1 ± 1.4 years. Since the breakthrough age of second molars is 12 to 13 years, porphyrins could accumulate for roughly 2 years in the pilot study. In the study presented here, the potential accumulation time was roughly 50% shorter, possibly explaining the low level of observed porphyrin fluorescence and as such the low differences between control and laser-treated teeth in SOPROLIFE blue fluorescence scores.

DIAGNOdent

DIAGNOdent has shown a good reproducibility in detecting and quantifying occlusal lesions in in vitro studies [49, 50], but for in vivo studies, the results were to a certain extent contradictory in primary as well in permanent dentition [39, 51–53].

In this CO_2 9.3-μm laser irradiation caries prevention study as well as in the pilot study, DIAGNOdent was not able to confirm the caries preventive results due to the system's innate limited capacity of caries detection at the enamel level. In this laser study, the average DIAGNOdent score at baseline was only 12.4 ± 7.2 for the laser-treated and 12.8 ± 8.3 for the control teeth, and thus below the discussed cut-off points of 20 or 30 for operative interventions like fillings [54–56]. Despite that, over time, for the control teeth, the DIAGNOdent values increased slightly more than for the laser-treated surfaces, but these differences were mainly not significant and also not expected. All observed changes were at a pre-cavitated level or at the most at an ICDAS score 3 level, a first physical enamel loss. Hence, increased porphyrin levels in dentin did not occur, and no significant changes in the DIAGNOdent scores occurred. The DIAGNOdent measures the uptake of organic bacterial by-products and does not measure demineralization or remineralization directly [46].

Applying CO_2 9.3-μm laser irradiation to the highly caries prone pits and fissures can reduce demineralization [30, 35],

and consequently cavities can be prevented. The irradiation measure enhances caries resistance and does not require a patient's compliance. As a requirement to be enrolled into the study, a moderate or high caries risk level according to Caries Management by Risk Assessment (CAMBRA) [27, 28] was prerequisite. Inevitably to be assessed as having a moderate or high caries risk level, most individuals show high levels of microbial plaque, a history of cavities in the last year, and frequent snacking. Obviously, our study participants did not present the highest level of oral hygiene, and their assessed caries risk level suggests that they were not in strong compliance with oral hygiene measures, brushing frequency, and efficiency. Over the study period, by observation, the participants' plaque level and thus caries risk levels did not improve.

As a result of this study on a larger population, CO_2 9.3-μm laser irradiation should be added to the CAMBRA armamentarium as part of the protective factors for patients with moderate or high caries risk. Recommendations like brushing teeth twice a day, reduce or avoid any frequent snacking, and using any additional fluoride rinses or disinfection solutions to reduce bacteria load require the patient's compliance but irradiating enamel with the CO_2 9.3-μm laser can be done quickly in the dental office. The laser treatment does not require further compliance measures. The preventive effect seems to last for at least 12 months.

It is widely supported that sealing pit and fissures of primary and permanent teeth is an effective method for preventing and arresting caries specifically in high caries risk children [15]. The effectiveness of sealant treatment in preventing dental restorations is dependent on the caries risk of individuals and caries prevalence of the country [57]. It is stated that pit and fissure sealants are to be applied to high caries risk children for optimum cost-effectiveness [58]. However, regular checkups must be conducted to avoid advanced tooth decay attributable to leakages in the sealing [13]. In case of a sealant loss [15, 59, 60], an irradiated fissure would still be better protected against caries until the failed sealant is replaced.

In addition, laboratory studies have shown that the caries preventive irradiation significantly enhanced bond to pit and fissure sealants compared with non-laser-irradiated enamel [61]. The risk of a sealant failure due to CO_2 9.3-μm short-pulsed laser irradiation appeared to be reduced. If additional laser ablation is required before placing a sealant, the CO_2 9.3-μm enamel laser-cut showed equivalent or superior bond strength to a flowable sealant in the laboratory study [61, 62].

Limitations of the study

It would be of interest to learn how long the laser caries preventive effect lasts. Consequently, another study might look into the preventive effects over 24 or 36 months. The study was performed on second molars, speculating these teeth can be representative for teeth presenting occlusal fissure surfaces. Since caries in permanent teeth is highly related to caries in deciduous teeth, it would also be of interest to understand the efficiency of the CO_2 9.3-μm laser irradiation in caries prevention in deciduous teeth.

Conclusions and clinical relevance

This randomized, single-blind, split-mouth controlled, clinical trial over 12 months with 60 participants demonstrated that the use of a new CO_2 9.3-μm short-pulsed laser increases the caries resistance of occlusal pit and fissure surfaces in patients in addition to fluoride therapy. This was shown by visual exams using ICDAS, SOPROLIFE daylight and blue fluorescence, and DIAGNOdent Laser Light–Induced Fluorescence.

Acknowledgments Thanks are given to David Lee, Pediatric Dentistry Resident, UCSF School of Dentistry, for helping with sealant placements on the study teeth.

Funding information This work is a principal investigator–initiated study and was funded by Convergent Dental Inc. (Needham, MA) through the University of California, San Francisco's Industry Contracts & Grants Division.

Data availability At this point in time, the datasets used and/or analyzed during the study are available from the corresponding author on reasonable request.

Compliance with ethical standards

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

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Consent to submit the manuscript and contribution Consent to submit has been received explicitly from all co-authors. All authors have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

References

1. Fleming E, Afful J (2018) Prevalence of total and untreated dental caries among youth: United States, 2015-2016. NCHS Data Brief 307:1–8
2. Rozier RG, White BA, Slade GD (2017) Trends in oral diseases in the U.S. population. J Dent Educ 81(8):eS97–eS109. https://doi.org/10.21815/JDE.017.016

3. Erbe C, Klees V, Ferrari-Peron P, Ccahuana-Vasquez RA, Timm H, Grender J, Cunningham P, Adam R, Farrell S, Wehrbein H (2018) A comparative assessment of plaque removal and toothbrushing compliance between a manual and an interactive power toothbrush among adolescents: a single-center, single-blind randomized controlled trial. BMC Oral Health 18(1):130. https://doi.org/10.1186/s12903-018-0588-1
4. Kasmaei P, Amin Shokravi F, Hidarnia A, Hajizadeh E, Atrkar-Roushan Z, Karimzadeh Shirazi K, Montazeri A (2014) Brushing behavior among young adolescents: does perceived severity matter. BMC Public Health 14:8. https://doi.org/10.1186/1471-2458-14-8
5. Kuusela S, Honkala E, Rimpela A, Karvonen S, Rimpela M (1997) Trends in toothbrushing frequency among Finnish adolescents between 1977 and 1995. Community Dent Health 14(2):84–88
6. Llena C, Calabuig E, Sanz JL, Melo M (2020) Risk factors associated with carious lesions in permanent first molars in children: a seven-year retrospective cohort study. Int J Environ Res Public Health 17(4). https://doi.org/10.3390/ijerph17041421
7. Hall-Scullin E, Whitehead H, Milsom K, Tickle M, Su TL, Walsh T (2017) Longitudinal study of caries development from childhood to adolescence. J Dent Res 96(7):762–767. https://doi.org/10.1177/0022034517696457
8. Kassebaum NJ, Smith AGC, Bernabe E, Fleming TD, Reynolds AE, Vos T, Murray CJL, Marcenes W, Collaborators GBDOH (2017) Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990-2015: a systematic analysis for the global burden of diseases, injuries, and risk factors. J Dent Res 96(4):380–387. https://doi.org/10.1177/0022034517693566
9. Brown LJ, Kaste LM, Selwitz RH, Furman LJ (1996) Dental caries and sealant usage in U.S. children, 1988−1991: selected findings from the Third National Health and Nutrition Examination Survey. J Am Dent Assoc 127(3):335–343. https://doi.org/10.14219/jada.archive.1996.0203
10. Centers for Disease C, Prevention (2001) Promoting oral health: interventions for preventing dental caries, oral and pharyngeal cancers, and sports-related craniofacial injuries. A report on recommendations of the task force on community preventive services. MMWR Recomm Rep 50(RR−21):1–13
11. Dye BA, Mitnik GL, Iafolla TJ, Vargas CM (2017) Trends in dental caries in children and adolescents according to poverty status in the United States from 1999 through 2004 and from 2011 through 2014. J Am Dent Assoc 148(8):550–565 e557. https://doi.org/10.1016/j.adaj.2017.04.013
12. Sreedevi A, Mohamed S (2020) Sealants, pit and fissure. StatPearls Publishing, Treasure Island
13. Cvikl B, Moritz A, Bekes K (2018) Pit and fissure sealants-a comprehensive review. Dent J (Basel) 6(2). https://doi.org/10.3390/dj6020018
14. Use of pit-and-fissure sealants (2018) Pediatr Dent 40 (6):162–178
15. Ahovuo-Saloranta A, Forss H, Walsh T, Nordblad A, Makela M, Worthington HV (2017) Pit and fissure sealants for preventing dental decay in permanent teeth. Cochrane Database Syst Rev 7:CD001830. https://doi.org/10.1002/14651858.CD001830.pub5
16. Beauchamp J, Caufield PW, Crall JJ, Donly K, Feigal R, Gooch B, Ismail A, Kohn W, Siegal M, Simonsen R (2008) Evidence-based clinical recommendations for the use of pit-and-fissure sealants: a report of the American Dental Association Council on Scientific Affairs. JADA 139(3):257–268
17. Featherstone JD, Nelson DG (1987) Laser effects on dental hard tissues. Adv Dent Res 1(1):21–26
18. Featherstone JD, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W (1998) CO2 laser inhibitor of artificial caries-like lesion progression in dental enamel. J Dent Res 77(6):1397–1403
19. Rechmann P, Fried D, Le CQ, Nelson G, Rapozo-Hilo M, Rechmann BM, Featherstone JD (2011) Caries inhibition in vital teeth using 9.6-μm CO2-laser irradiation. J Biomed Opt 16(7):071405. https://doi.org/10.1117/1.3564908
20. Rechmann P, Charland DA, Rechmann BM, Le CQ, Featherstone JD (2013) In-vivo occlusal caries prevention by pulsed CO2-laser and fluoride varnish treatment - a clinical pilot study. Lasers Surg Med 45(5):302–310. https://doi.org/10.1002/lsm.22141
21. Fried D, Zuerlein MJ, Le CQ, Featherstone JD (2002) Thermal and chemical modification of dentin by 9-11-microm CO2 laser pulses of 5-100-micros duration. Lasers Surg Med 31(4):275–282
22. Zuerlein MJ, Fried D, Featherstone JD (1999) Modeling the modification depth of carbon dioxide laser-treated dental enamel. Lasers Surg Med 25(4):335–347
23. Featherstone JD, Nelson DG (1989) Recent uses of electron microscopy in the study of physico-chemical processes affecting the reactivity of synthetic and biological apatites. Scanning Microsc 3(3):815–827 discussion 827-818
24. Takagi S, Liao H, Chow LC (2000) Effect of tooth-bound fluoride on enamel demineralization/ remineralization in vitro. Caries Res 34(4):281–288
25. Goodis HE, Fried D, Gansky S, Rechmann P, Featherstone JD (2004) Pulpal safety of 9.6 microm TEA CO2 laser used for caries prevention. Lasers Surg Med 35(2):104–110. https://doi.org/10.1002/lsm.20043
26. Gorton J, Featherstone JD (2003) In vivo inhibition of demineralization around orthodontic brackets. Am J Orthod Dentofac Orthop 123(1):10–14
27. Rechmann P, Chaffee BW, Rechmann BMT, Featherstone JDB (2019) Caries Management by Risk Assessment: results from a practice-based research network study. J Calif Dent Assoc 47(1):15–24
28. Featherstone JDB, Alston P, Chaffee BW, Rechmann P (2019) Caries Management by Risk Assessment (CAMBRA): an update for use in clinical practice for patients aged 6 through adult. J Calif Dent Assoc 47(1):25–36
29. ICDAS-Foundation International Caries Detection & Assessment System www.icdas.org
30. Rechmann P, Le CQ, Kinsel R, Kerbage C, Rechmann BMT (2020) In vitro CO2 9.3-μm short-pulsed laser caries prevention-effects of a newly developed laser irradiation pattern. Lasers Med Sci. https://doi.org/10.1007/s10103-019-02940-z
31. Rechmann P, Rechmann BM, Featherstone JD (2012) Caries detection using light-based diagnostic tools. Compend Contin Educ Dent 33(8):582–584 586, 588-593; quiz 594, 596
32. Rechmann P, Charland D, Rechmann BM, Featherstone JD (2012) Performance of laser fluorescence devices and visual examination for the detection of occlusal caries in permanent molars. J Biomed Opt 17(3):036006. https://doi.org/10.1117/1.JBO.17.3.036006
33. Rechmann P, Jue B, Santo W, Rechmann BMT, Featherstone JDB (2018) Calibration of dentists for Caries Management by Risk Assessment research in a practice based research network - CAMBRA PBRN. BMC Oral Health 18(1):2. https://doi.org/10.1186/s12903-017-0457-3
34. GraphPad (2011) Quantify agreement with kappa - assess how well two observers classify subjects into groups (statistical methods for rates and proportions). GraphPad Software Incorporated
35. Rechmann P, Rechmann BM, Groves WH Jr, Le CQ, Rapozo-Hilo ML, Kinsel R, Featherstone JD (2016) Caries inhibition with a CO2 9.3 μm laser: an in vitro study. Lasers Surg Med 48(5):546–554. https://doi.org/10.1002/lsm.22497
36. Rationale and evidence for the International Caries Detection and Assessment System (ICDAS II) (September 2005). International Caries Detection and Assessment System (ICDAS) Coordinating Committee
37. Ekstrand KR, Kuzmina I, Bjorndal L, Thylstrup A (1995) Relationship between external and histologic features of

progressive stages of caries in the occlusal fossa. Caries Res 29(4): 243–250
38. Ekstrand KR, Ricketts DN, Kidd EA (1997) Reproducibility and accuracy of three methods for assessment of demineralization depth of the occlusal surface: an in vitro examination. Caries Res 31(3): 224–231
39. Tranaeus S, Lindgren LE, Karlsson L, Angmar-Mansson B (2004) In vivo validity and reliability of IR fluorescence measurements for caries detection and quantification. Swed Dent J 28(4):173–182
40. Mendes FM, Siqueira WL, Mazzitelli JF, Pinheiro SL, Bengtson AL (2005) Performance of DIAGNOdent for detection and quantification of smooth-surface caries in primary teeth. J Dent 33(1):79–84. https://doi.org/10.1016/j.jdent.2004.10.010
41. Astvaldsdottir A, Holbrook WP, Tranaeus S (2004) Consistency of DIAGNOdent instruments for clinical assessment of fissure caries. Acta Odontol Scand 62(4):193–198. https://doi.org/10.1080/00016350410001612
42. Brantley CF, Bader JD, Shugars DA, Nesbit SP (1995) Does the cycle of rerestoration lead to larger restorations? J Am Dent Assoc 126(10):1407–1413
43. Terrer E, Slimani A, Giraudeau N, Levallois B, Tramini P, Bonte E, Hua C, Lucchini M, Seux D, Thivichon B, Le Goff A, Cuisinier F, Tassery H (2019) Performance of fluorescence-based systems in early caries detection: a public health issue. J Contemp Dent Pract 20(10):1126–1131
44. Zeitouny M, Feghali M, Nasr A, Abou-Samra P, Saleh N, Bourgeois D, Farge P (2014) SOPROLIFE system: an accurate diagnostic enhancer. Sci World J 2014:924741–924748. https://doi.org/10.1155/2014/924741
45. Jablonski-Momeni A, Rosen SM, Schipper HM, Stoll R, Roggendorf MJ, Heinzel-Gutenbrunner M, Stachniss V, Pieper K (2011) Impact of measuring multiple or single occlusal lesions on estimates of diagnostic accuracy using fluorescence methods. Lasers Med Sci 27:343–352. https://doi.org/10.1007/s10103-011-0881-6
46. Astvaldsdottir A, Tranaeus S, Karlsson L, Peter Holbrook W (2010) DIAGNOdent measurements of cultures of selected oral bacteria and demineralized enamel. Acta Odontol Scand 68(3):148–153. https://doi.org/10.3109/00016350903567176
47. Buchalla W (2005) Comparative fluorescence spectroscopy shows differences in noncavitated enamel lesions. Caries Res 39(2):150–156. https://doi.org/10.1159/000083162
48. Verdonschot EH, van der Veen MH (2002) Lasers in dentistry 2. Diagnosis of dental caries with lasers. Ned Tijdschr Tandheelkd 109(4):122–126
49. Lussi A, Imwinkelried S, Pitts N, Longbottom C, Reich E (1999) Performance and reproducibility of a laser fluorescence system for detection of occlusal caries in vitro. Caries Res 33(4):261–266
50. Shi XQ, Tranaeus S, Angmar-Mansson B (2001) Validation of DIAGNOdent for quantification of smooth-surface caries: an in vitro study. Acta Odontol Scand 59(2):74–78
51. Angnes V, Angnes G, Batisttella M, Grande RH, Loguercio AD, Reis A (2005) Clinical effectiveness of laser fluorescence, visual inspection and radiography in the detection of occlusal caries. Caries Res 39(6):490–495. https://doi.org/10.1159/000088185
52. Bamzahim M, Aljehani A, Shi XQ (2005) Clinical performance of DIAGnodent in the detection of secondary carious lesions. Acta Odontol Scand 63(1):26–30
53. Akarsu S, Koprulu H (2006) In vivo comparison of the efficacy of DIAGNOdent by visual inspection and radiographic diagnostic techniques in the diagnosis of occlusal caries. J Clin Dent 17(3): 53–58
54. Jablonski-Momeni A, Ricketts DN, Rolfsen S, Stoll R, Heinzel-Gutenbrunner M, Stachniss V, Pieper K (2011) Performance of laser fluorescence at tooth surface and histological section. Lasers Med Sci 26(2):171–178. https://doi.org/10.1007/s10103-010-0768-y
55. Huth KC, Neuhaus KW, Gygax M, Bucher K, Crispin A, Paschos E, Hickel R, Lussi A (2008) Clinical performance of a new laser fluorescence device for detection of occlusal caries lesions in permanent molars. J Dent 36(12):1033–1040. https://doi.org/10.1016/j.jdent.2008.08.013
56. Eakle S, Gansky S, Zhan L, Featherstone JDB (2005) Clinical evaluation of the Diagnodent device. In: Stookey G (ed) Early detection of dental caries III. Indiana Conference 2003. Indiana University Press, Indiana,
57. Leskinen K, Ekman A, Oulis C, Forsberg H, Vadiakas G, Larmas M (2008) Comparison of the effectiveness of fissure sealants in Finland, Sweden, and Greece. Acta Odontol Scand 66(2):65–72. https://doi.org/10.1080/00016350801926933
58. Naaman R, El-Housseiny AA, Alamoudi N (2017) The use of pit and fissure sealants-a literature review. Dent J (Basel) 5(4). https://doi.org/10.3390/dj5040034
59. Taneja S, Singh A (2020) Retention of flowable composite resins in comparison to pit and fissure sealants: a systematic review and meta-analysis. Gen Dent 68(4):50–55
60. Simonsen RJ (1991) Retention and effectiveness of dental sealant after 15 years. J Am Dent Assoc 122(10):34–42
61. Rechmann P, Sherathiya K, Kinsel R, Vaderhobli R, Rechmann BM (2017) Influence of irradiation by a novel CO2 9.3-mum short-pulsed laser on sealant bond strength. Lasers Med Sci 32(3): 609–620. https://doi.org/10.1007/s10103-017-2155-4
62. Rechmann P, Bartolome N, Kinsel R, Vaderhobli R, Rechmann BMT (2017) Bond strength of etch-and-rinse and self-etch adhesive systems to enamel and dentin irradiated with a novel CO2 9.3 mum short-pulsed laser for dental restorative procedures. Lasers Med Sci 32(9):1981–1993. https://doi.org/10.1007/s10103-017-2302-y