Fissure Caries Inhibition Study with Solea CO2-9.3µm Short-pulsed Laser

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Study Protocol and Statistical Analysis Plan

1. TITLE PAGE

Study Title:	Fissure Caries Inhibition Study with Solea CO_2-9.3µm short-pulsed laser - A randomized, single blind, prospective, split mouth controlled, clinical trial.
Trial registration	to be registered on ClinicalTrials.gov
Protocol Number:	ClinFCaries05-2014 - Final as of April 2015
Investigational Product:	Convergent CO_2 9.3µm short pulsed laser
Indication:	Prevention of fissure caries
Funding:	Convergent Dental Grant through Office of Innovation, Technology and Alliances at UCSF
Source of Funding:	
Secondary Sponsor:	
Development Phase:	Phase II study
Sponsor's Responsible Medical Officer:	
Sponsor Signatory:	
Effective Date:	

2. SYNOPSIS

Study Title: Fissure Caries Inhibition Study with Solea CO_2-9.3µm short-pulsed laser – A randomized, single blind, prospective, split mouth controlled, clinical trial	
Protocol number: CFCaries05-2014 - Final as of April, 2015	
Investigators: Peter Rechmann, DMD, PhD, Krunal Sherathiya, DDS John D.B. Featherstone MSc, PhD	
Study center: University of California at San Francisco	
Multicenter study [ ] Multicenter Coordinating center or the prime grant holder: **Other Centers:**	
Study Sites:	
Study period: 08-2014 to 04-2017	**Phase of development:** phase II

Investigational Product:	Convergent CO_2 9.3µm short pulsed laser
Indication:	Prevention of dental caries in fissures
IND/IDE numbers:	
Who holds the IND/IDE?	[ ]Sponsor [ ]Investigator

The study design and protocol closely follows the SPIRIT 2013 – "Standard Protocol Items: Recommendations for Interventional Trials" - Recommended items to address in a clinical trial protocol and related documents.[1]
The study results will be reported according to the CONSORT 2010 Statement - guidelines for reporting parallel group randomized trials.[2]

STUDY DESIGN – *Draft*

Objective: The objective of this clinical study is to evaluate whether the use of the new CO_2 - 9.3µm short-pulsed laser increases the caries resistance of occlusal pit and fissure surfaces in patients in addition to fluoride therapy quantified by visual exams with the International Caries Detection and Assessment System (ICDAS II), SOPROLIFE daylight and blue fluorescence, and DIAGNOdent Laser Light-induced Fluorescence in a randomized, single blind, prospective, split mouth controlled, clinical trial over 12 months.

Hypothesis:

The hypothesis to be tested is that treatment with the new CO_2 - 9.3µm short-pulsed laser in addition to fluoride therapy results in changes in crystal composition and structure which increase the resistance of dental mineral to dissolution by acid and will work to better prevent dental caries in the occlusal surface of vital teeth when compared to fluoride therapy alone over 12 months (detailed outcomes see below)**.**

Background and Rational

Over the last two decades caries prevalence in the permanent dentition has declined nearly ten percent in American children aged 9-16.[3] While this remarkable decrease in caries is significant, 42% of the population in this age range is affected by dental caries.[3] Nearly 80% of all of carious lesions in adolescences involve the occlusal pits and fissures.[4] Currently, there are two common professionally delivered techniques towards caries prevention: pit and fissure sealants, and topical fluoride. Other interventions such as diet counseling or oral hygiene instruction are highly dependent on daily patient compliance. The high proportion of occlusal decay suggests that current preventive practices are inadequate whether it is due to the method, technique or patient access to preventive care.

Methods of early caries detection

Current commonly used caries detection methods in the United States include visual inspection, tactile use of the explorer, and radiographs. Studies in Europe have shown that the explorer is only correct less than 50% of the time.[5] Radiographs are good for interproximal caries, but ineffective in detecting occlusal caries before it is well into the dentin due to the amount of sound tissue attenuating the beam[6]. By the time an occlusal caries lesion is detectable radiographically, it is too large to be remineralized[6]. If carious lesions are detected early enough, intervention methods, such as fluoride application, sealants, preventive resin restorations, laser treatment, and antibacterial therapy, can be applied to reverse the caries process.[6]

Visual inspection can be very subjective based on clinician experience and training. Standardized visual inspection systems should be adopted to avoid inconsistencies amongst diagnoses from different dentists. The International Caries Detection and Assessment System (ICDAS) provides a standardized method of lesion detection and assessment, leading to caries diagnosis.[7-10] ICDAS II assigns scores to lesions based on apparent caries status and lesion severity of plaque-free teeth when visualized wet and when air dried.[7] Of particular interest to this study are the coronal primary caries detection criteria. The ICDAS II detection codes for coronal pits and fissures caries range from 0 to 6 as follows[7] (pictures and details see below).

Fluorescence is a property of some manmade and natural materials that absorb energy at certain wavelengths and emit light at longer wavelengths. Laser fluorescence is a new method for early caries detection. By analyzing the fluorescence emission spectrum of carious regions versus sound tissues, studies have shown that laser fluorescence is useful as a quantitative measure distinguishing carious from non-carious surfaces.[6]

Three main methods of laser fluorescence have been studied: quantitative light-induced fluorescence (QLF) (blue light),[11-16] KaVo DIAGNOdent (red light),[17-23] and SOPROLIFE in

blue fluorescence mode.[24, 25]
The QLF method has been tested in several in vitro,[26-28] in situ,[29] and in vivo[11-16] studies for smooth surface caries lesions. The possibility of adapting the QLF method for occlusal caries diagnosis is under investigation.[30] On occlusal lesions the discrimination between the severities of carious lesions has been shown to be insufficient.[24]
The DIAGNOdent system has shown good performance and reproducibility for detection and quantification of occlusal and smooth surface carious lesions in in vitro studies,[31-33] but with somewhat more contradictory results in vivo, both in the primary and permanent dentition.[17-23] It has also been tried for longitudinal monitoring of the caries process, and for assessing the outcome of preventive interventions.[34]
SOPROLIFE in daylight and blue fluorescence mode has demonstrated its capability to efficiently visualize caries lesions in early, pre-cavitated stages and thus allows scoring of pre-cavitated lesion stages.[24, 25]

Evidence regarding rendering enamel caries resistant by use of a short-pulse CO_2-Laser

Featherstone and co-workers have shown, in several studies, that enhancement of caries resistance of enamel can be achieved in the laboratory by irradiation with short-pulsed CO_2-lasers under well-specified irradiation conditions [35-37] using much lower energy levels than those reported in other studies. 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.[38]

Rechmann and co-workers used an orthodontic bracket model [39] 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.[40] 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. It was shown that the laser irradiation reduced enamel mineral loss by up to 46% over a time period of 4 weeks, and up to an 87% over 12 weeks in comparison to the control surfaces, which was speculated to be also related to an enhancement of remineralization following the laser irradiation.[40]

Lately, the group has shown in a single blind, controlled, randomized, clinical split mouth pilot trial on 20 subjects 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 one year observation interval.[41] At all recalls, average ICDAS scores had decreased for the test and increased for the control fissures (laser vs. control, 3-month: -0.10±0.14, 0.30±0.18, $P>0.05$; 6-month: -0.26±0.13, 0.47±0.16, $P=0.001$; 12-month: -0.31±0.15, 0.75±0.17, $P<0.0001$; mean ± SE, t-test). SOPROLIFE daylight evaluation revealed at 6- and 12-month 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±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.

Explanation of Rationale with justification for undertaking the trial

In summary there is sufficient evidence that during CO_2 short-pulsed laser irradiation the outer few micrometers of enamel are thermally transformed from a carbonated hydroxyapatite into a more acid resistant hydroxyapatite.[42-44] This is advantageous since it increases resistance to acid dissolution and thus inhibits tooth decay.

The clinical study presented here will show that using a CO_2 9.3µm short pulsed laser irradiation markedly inhibits caries progression in pits and fissures in comparison to fluoride varnish alone over 12 months.

Study Design

[x]Randomized [ ]Investigational intervention without random assignment
[x]Blinded, Single, [ ]Blinded, Double
[x]Prospective [x]Control [ ]Behavioral

Study's formal designation:
[ ]Phase I [x]Phase II [ ]Phase III [ ]Phase IV

Framework:
[x] superiority [ ] equivalence [ ] non inferiority [ ] exploratory

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Study type

The CHR application will be a minimal risk, full committee review application.

Methodology

Specific Aims

A) The aim of the study is to assess if the use of the new CO_2 - 9.3µm short-pulsed laser in addition to fluoride therapy allows rendering occlusal pit and fissures caries resistant in comparison to fluoride therapy alone in participants in a randomized, single blind, prospective, split mouth controlled, clinical trial. The fissure mineral loss will be quantified by visual exams with the International Caries Detection and Assessment System (ICDAS II), SOPROLIFE daylight and blue fluorescence, and by DIAGNOdent Laser Light-induced Fluorescence.

Primary study outcome measures

Differences in change in ICDAS scores between matched case and control teeth (within patient) from baseline to 6 months and baseline to 12 months

Differences in occurrence of ICDAS 3 scores between matched case and control teeth (within patient) from baseline to 6 months and baseline to 12 months

Secondary study outcome measures

Differences in change in SOPROlife scores between matched case and control teeth (within patient) from baseline to 6 months and baseline to 12 month

Material & Methods

Clinical Study:
This project will be a single blind, controlled clinical split mouth study. Patients meeting the inclusion criteria from the UCSF Predoctoral, Postgraduate Pediatric Dental and Postgraduate orthodontic clinics will have the study explained to them and be invited to participate. The Caries Management By Risk Assessment (CAMBRA) questionnaire will be used to access the caries risk status at baseline. Patients with moderate/high caries risk will be invited to enroll into the study. The molar on their dominant side (right or left) will be randomly assigned to either the test or the control group with the contralateral non-dominant side receiving the other treatment. A baseline visual inspection using ICDAS II, white and blue light digital photographs (SOPROLIFE), and DIAGNOdent assessments will be made by a dentist blinded to randomized group prior to treatment. The test tooth/~~teeth~~ will be treated with CO_2 laser irradiation and fluoride varnish; the control tooth~~/teeth~~ will receive fluoride varnish treatment alone. The patient will be asked to return for a 6-month and a 12-month follow up exam, at which time visual inspection with all testing methods will be conducted by the dentist who originally completed the baseline exam. The endpoint of the study for each participant will be when either the control or test tooth is found to have significant demineralization by ICDAS assessment (ICDAS code 3 – "localized enamel breakdown without clinical visual signs of dentinal involvement") or at the 12 month exam, whichever comes first. The control and test teeth might be sealed with a dental sealant at the end of the study. All data obtained will be analyzed for statistical significance.

Subjects
Participants in the study will be patients, who have at least one pair of contralateral unsealed molars (first) showing ICDAS score 0, 1, or 2.
Subjects will be of moderate or high caries risk according to Caries Risk Assessment. Information on the child's dental visit history, tooth brushing habits, and snacking habits will be collected through a questionnaire. The appropriate UCSF questionnaire[45] and/or the MyCAMBRA^R App (Apple AppStore, Cupertino, CA) will be used to assess the Caries Risk. Age limitation for the study is ≥ 6 years.

Settings and locations where data will be collected
The subjects will be recruited from the UCSF School of Dentistry. They will be recruited from the UCSF Predoctoral, Postgraduate Pediatric Dental and Postgraduate orthodontic clinics. The Pediatric Dentistry Clinic sees 200 to 300 patients in need of multiple fissure sealants per year. Approximately 2,500 sealants are placed every year. (There are reasons why sealants are not placed; these patients will be eligible for this study described here – see below.)

Subjects will be visiting the dental clinics for a new patient visit or a periodic oral exam. Subjects will have at least one pair of contralateral molars (first) in need of fissure sealants. Subjects will be treated in the Pediatric Dentistry and in the PRDS facilities.

Experimental Design

Enrollment, Consenting & Randomization: Flyers posted in the School of Dentistry will be

used to recruit patients and study investigators (and/or staff) will recruit their own patients directly in person (outside the direct treatment area).
When patients are seen by their dentist and the treating dentist believes that the patient might fit into the study, he/she will ask the potential participant (and parent/guardian for minors) whether she/he might be interested in being in a dental study. If the subject is interested the treating dentist will ask the potential participant (and parent/guardian for minors) whether she/he gives permission to disclose her/his name to the researchers. If the patient agrees (and parent/guardian for minors), a Clinical Investigator (Peter Rechmann, Krunal Sherathiya) and/or the Clinical Study Coordinator (Beate Rechmann) will ask the potential subject whether she/he is interested in participating in this study. If so the Clinical Investigator/Clinical Study Coordinator will explain the whole study and will further check for eligibility.

Consenting all study subjects and their parents/guardians will take place prior to entering the study. Potential participants will be consented and enrolled by the PI/I, and a senior research associate, respectively.

To achieve adequate participant enrolment to reach targeted sample size, advertisements will be placed in the Pediatric Dentistry Clinic area as well as in other patient areas of the School of Dentistry.
In the Division of Pediatric Dentistry the recommendations are to treat first molars with fissure sealants. Nevertheless, sometimes patients cannot afford or do not want the sealant treatment. In addition, anatomical conditions with tissue overgrowth on the distal side of the molar sometimes do not allow placing a sealant. These are potential situations for using the CO_2-laser to render the fissure enamel caries resistant. Thus the intended study does not interfere with the typical treatment.

<u>The interventions - treatment groups</u>
Each tooth of a molar pair in the same jaw will be randomly assigned to one of the 2 different treatments.

The study treatments are:

1) CO_2 9.3 µm short pulsed laser rendering fissure areas more caries resistant (using proven laser settings) plus fluoride treatment (experimental group)
2) Fluoride treatment alone (control group)

- All study teeth will receive F-varnish application at baseline and every 6 months

<u>Randomization of study teeth – allocation concealment</u>

After enrollment subject's molars will be randomly assigned to the experimental or control group. The molar on the dominant side (right or left) will be randomly assigned to either the test or the control group with the non-dominant (contralateral) side receiving the other treatment. This is because dominant side determined by handedness may relate to oral hygiene.

A study independent person will create a randomization list, which will be created by a random number generator (QuickCalcs Online Random Numbers by GraphPad Software, Inc.).[46] The randomization list will be kept locked by a dental assistant (DA), group assignments will be in separate, closed opaque, sequentially numbered envelopes; only after

a subject has been enrolled the next-in-line group assignment will be revealed.

Blinding

The doctor performing the laser treatment and the dental assistant (DA) will not be blinded to subjects' group assignment and will explain the assignment to the subjects. The participant will also not be blind to the treatment. The DA will inform the laser treating doctor, which tooth will be the intervention and which the control tooth.

An independent study investigator will be blinded to subjects' group assignments. The DA will serve as the go-to person for subjects.

The statistician will be blinded to treatment assignment by (randomly) labeling the 2 groups as 0 and 1, without giving the statistician the key.

Study duration

The total study duration for each subject will be 12 months.

Testing methods

The following tools for assessments will be used:

a. Filling out the UCSF CAMBRA form (standard form used in the UCSF predoctoral clinic – evaluating habits and risks related to caries).
b. Visual clinical examination and assessment using 2x magnification loops, dental mirror and dental unit light for caries assessment using ICDAS II criteria* (at baseline ICDAS 0 – 2);
c. SOPROlife**daylight intraoral camera; SOPROlife day light mode, picture recorded with Sopro-imaging software (Acteon, Sopro, La Ciotat, France) - allowing independent observers to judge SOPROlife daylight score[25] later on;
d. SOPROlife fluorescence mode, picture recorded with Sopro-imaging software fluorescence measurements using blue light fluorescence (SOPROlife) – SOPROlife blue fluorescence score[25];
e. DIAGNOdent (KaVo, Biberach, Germany) light fluorescence device (red fluorescence);
f. digital photography.

* Table 1– ICDAS classification and definition

ICDAS code	**0**	**1**	**2**	**3**
				
Definitions	Sound tooth surface; no caries change after air drying (5 sec); or hypoplasia, wear, erosion and other non-caries	First visual change in enamel; seen only after air drying, or colored change "thin" limited to the confines of the pit	Distinct visual change in enamel; seen when wet, white or colored, "wider" than the fissure/fossa.	Localized enamel breakdown, with no visible dentin or underlying shadow; discontinuity of surface enamel,

	phenomena.	and fissure area.		widening of fissure.

** SOPROLIFE - The SOPROLIFE system (SOPRO, ACTEON Group, La Ciotat, France) combines the advantages of a visual inspection method with a high magnification oral camera and a laser fluorescence device. This technique is based on the LIFE DT concept (Light-Induced Fluorescence Evaluator - Diagnostic and Treatment).[47, 48] In the "daylight mode" white LEDs illuminate the tooth while in the "fluorescence mode" the excitation is managed by four blue LEDs at 450nm wavelength. The intense blue light shines through the enamel and induces a green fluorescence from the dentin core, which then consequently initiates on its way back through dentin/enamel a red fluorescence due to porphyrins and related compounds from oral bacteria remaining at a caries lesion site.[25]

Testing times

The evaluation of the fissures will occur

- at baseline ((before and after laser treatment)
- at the end of 6 and 12 months

Study duration

The total study duration for each subject will be 12 months

Data collection methods

Data will be collected on individualized Case Report Forms (CRF). Case report forms will be detailed for baseline and all recall intervals. Data will be transferred into electronic files for data analyses. After data transfer, a second person will check for correctness of data transfer.

Inter and intra-examiner reliability

The independent evaluator(s) and a study doctor/evaluator will be trained and calibrated on the ICDAS criteria. The training and calibration will occur on pictures as well as on patients in the Pediatric Dentistry Clinic or in PRDS. Evaluators will examine the same patients to establish an intra-examiner reliability score. They also will examine the same participant at two different time intervals, ideally two to four weeks separate. This will allow for intra examiner reliability score evaluation. The training and calibration will occur before this study will be started as well as during the study.

Data management

To ensure confidentiality of the data, the data will be labeled with study numbers in place of subject identifiers, the data will be stored in a locked cabinet within a locked office, and any electronic data will be stored on a password protected computer. Data will be kept in locked files, which are in locked offices accessible only for the PI and authorized study personal.

Data monitoring

There is no need for monitoring data or safety. The study is neither invasive nor will any invasive treatments be performed. There is no testing of any drugs. The CO_2 short-pulsed laser has been tested and proven for clinical safety[49], has FDA approval for hard tissue removal and has been shown efficient for enhancing caries resistance in clinical studies.[40, 50]

As regular Pediatric Dentistry patients, the subjects will be monitored closely every 6 months. Thus any unusual issues will be evaluated in a timely fashion. In addition, simple dental indices will be evaluated concerning the visible occlusal surfaces of permanent molars. "Light" diagnostic with blue light camera tools and digital photography will be performed.
As always required, the PI will review all adverse events as they occur and will report any serious and/or unexpected ones to the CHR within 10 working days, as required by the CHR.

Auditing
The Sponsor will audit the study.

Eligibility criteria for participants

1. Inclusion Criteria

- age 6 or older, in good general health
- subject is of moderate or high caries risk according to CAMBRA
- has at least one pair of unsealed molars in at least one jaw with need for a sealant
- teeth with an ICDAS code 0, 1 and 2 with deep grooves and fissures providing an anatomical stick for an explorer
- willing to comply with all study procedures and protocols
- must be able to read and understand English
- have an understanding of the study
- residing in San Francisco or other nearby locales with community water fluoridation (to eliminate water fluoridation as a potential confounding variable)
- patient and parent/guardian able to provide written informed consent in English
- willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form; data will only be used for research.

2. Exclusion Criteria

- show evidence of extremely poor oral hygiene
- subjects suffering from systemic diseases, significant past or medical history with conditions that may affect oral health or oral flora (i.e. diabetes, HIV, heart conditions that require antibiotic prophylaxis),
- subjects with Nut Allergy
- taking medications that may affect the oral flora or salivary flow (e.g. antibiotic use in the past three months, drugs associated with dry mouth / xerostomia [extreme high caries risk])
- other conditions that may decrease the likelihood of adhering to study protocol
- subjects who will leave the area and are unable to complete the study

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Sample size calculation & statistics

Sample size calculations are performed by UCSF Department of Epidemiology and Biostatistics using PASS 12 Power Analysis & Sample Size Software (http://www.ncss.com/software/pass/).

We assume that we will enroll into the suggested in-vivo occlusal caries prevention by short-pulsed CO_2 9.3 µm laser prospective, randomized, single blinded clinical study

roughly 70 to 80 subjects.

Power calculations below are based on results obtained from our pilot study on caries prevention [51] (Rechmann, P., et al, "In-vivo occlusal caries prevention by pulsed CO_2 -laser and fluoride varnish treatment - a clinical pilot study," Lasers in Surgery and Medicine 45(5), 302-310 (2013)). For the purpose of sample size calculation, we assume a simple measure based on our primary endpoint of change in ICDAS scores with only one pair of molars per subject, by comparing the proportion of teeth with worsening ICDAS score in laser vs. control groups. (We actually expect some subjects to provide more than one pair of molars, which will add to the power.) Note that we will actually use a generalized linear mixed model (proportional odds) for the primary analysis based on differences in scores, but there are no general power analysis software procedures for that test. Therefore, we use this simpler and less powerful metric to conservatively guide power considerations. In the pilot study we observed 1 participant with worsening ICDAS score in the laser group vs. 9 participants in the control group. We determine power based on a McNemar test to account for the pairing of observations. The observed difference in ratio for worsening ICDAS score was 0.56 (0.06 laser vs. 0.62 control). 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 proportions of 0.35 at the 5% significance level (i.e. allowing for over 35% reduction in the pilot observed difference of 0.56). Furthermore, we expect to improve power further by working with the actual ICDAS scores and using the Wilcoxon signed-rank test (the extra power comes from being able to capture the extent of worsening in each case).

Data Analysis - by UCSF Department of Epidemiology and Biostatistics.

The primary analysis of this study will compare ICDAS scores for the laser group vs. controls. To this end, we will perform mixed effects proportional odds regression modeling with outcomes of ICDAS and SOPROLIFE scores. These models will explicitly account for within patient clustering and will include group (laser vs. control) as the main predictor along with tooth site and additional potential moderating variables such as age, sex, snack habit measures, tooth brushing habit, and dental history predictors. Importantly, we will include interactions of these additional predictors with the group predictor as well as time, which may indicate whether there is possible differential protectiveness of laser (over time) in specific subgroups. The models will be explored in a bottom up approach with each predictor considered in models one at a time along with group, group by predictor interaction and tooth site. If we see important effects, we will then combine a small number of predictors based on scientific plausibility in a single model, though we note that these will be far from definitive models given the sample size and scope of the proposed study. $P \leq 0.05$ will be considered statistically significant though we will emphasize that any model results are highly speculative and will require independent verification.

IRB approval

Investigational Review Board approval (UCSF Committee on Human Research CHR) will be achieved before the study will be started.

If requested the IRB can additionally scrutinize the study and will state that the study was conducted under the rules of GCP - Good Clinical Practice.

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