Title

Fissure Caries Inhibition with Solea CO 2 -9.3µm short-pulsed laser - A randomized, single blind, prospective, split mouth controlled, clinical trial.

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Fissure Caries Inhibition with Solea CO 2 -9.3µm short-pulsed laser - A randomized, single blind, prospective, split mouth controlled, clinical trial.

by
Maxwell Kubitz

THESIS
Submitted in partial satisfaction of the requirements for degree of
MASTER OF SCIENCE

in

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in the

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of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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DEDICATION AND ACKNOLWEDGEMENTS

I'd like to dedicate this work to my family who have supported me, the mentors who have pushed me to achieve my goals, and the friends who have helped me enjoy my journey.

SPECIAL ACKNOWLEDGEMENTS

I would not have been able to take on this project without the help and support of my professional community. I appreciate, immensely, the time, guidance and encouragement that has been afforded to me by those close to this project.

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CONFLICT OF INTEREST DISCLOSURES

This study is a Principle Investigator Initiated Study and was funded by Convergent Dental, Inc. through the UCSF Contracts & Grants Division.

ABSTRACT

Fissure Caries Inhibition with Solea CO_2-9.3µm short-pulsed laser - A randomized, single blind, prospective, split mouth controlled, clinical trial.

By

Maxwell Kubitz

BACKGROUND:

LASERs continue to gain popularity in dentistry. There continues to be new data on the efficacy of short-pulsed CO_2 LASERs in treating caries, increasing bond strengths and preventing demineralization due to caries. Recently, a pilot study has been conducted that showed increased resistance to caries demineralization after treatment with a short-pulsed CO_2 LASER.

OBJECTIVES:

The aim of this 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 over 12 months. The fissure mineral loss will be evaluated by visual exams using the International Caries Detection and Assessment System (ICDAS), SOPROLIFE daylight and blue fluorescence, and by DIAGNOdent Laser Light-induced Fluorescence.

METHODS:

This study was carried out in three appointments. The first involved screening, consenting and assenting, recording of initial values, treatment with carbonate removal settings of the Solea CO_2 LASER, and fluoride varnish applications. The second appointment at the 6-month recall

consisted of a routine periodic examination and prophylaxis, cleaning of the study surfaces, collection of data values and application of fluoride varnish. If, at the second appointment, any of the study surfaces of interest had progressed to an ICDAS 3 score or greater, the subject's participation in the study was terminated and the necessary treatment was rendered. At the third appointment at 12 months, again a routine examination and prophylaxis, cleaning of the study surfaces, collection of data values, was performed and additional placement of sealants or minimally invasive resin restorations on the treatment surfaces occurred, plus application of fluoride varnish.

RESULTS:

A total of 229 patients were screened for this study. Sixty patients were recruited and enrolled to participate in the study: 32 females and 28 males. The average age of the patients at enrollment was 13.2 years old. The attrition rate at time of writing this thesis has been 8.3%; five subjects were lost and unable to be evaluated at follow-up, three male and two female patients. This included two subjects who returned for the 6-month follow up but moved out of the country prior to the 12-month follow-up. Two subjects were unable to be contacted after the baseline appointment. One subject had sealants placed over the treatment and control teeth in the pre-doctoral clinic prior to the 6-month follow up appointment.

Thus far, 10 participants have had at least one location on one tooth progress to ICDAS 3 or greater, consequently finishing their participation in the study. Four subjects had at least one ICDAS 3 or greater score at the 6-month follow-up appointment. Six subjects presented with at least one ICDAS 3 or greater at the 12-month follow-up appointment.

Maxillary molars were picked as study teeth slightly more often than mandibular molars – 32 participants provided maxillary molars and 28 participants provided mandibular molars for the study.

At the time of submission of this thesis the study evaluators were still blinded as there were still participants requiring 12-month follow up visits. When data collection is completed in December of 2019 and unblinding occurs this thesis and specifically the results and conclusion sections will be amended to contain the obtained data.

CONCLUSION:

Although the enrollment is closed for this study, at this time follow-up is still underway and as such the hypothesis cannot be confirmed nor rejected. Although no conclusion can yet be drawn it can be noted, interestingly, that to this point in time 10 subjects have had lesion progression to ICDAS 3 scores in one tooth only. The distribution of male and female participants was nearly equal, indicating that there seems to be generalized equal acceptance of laser treatment from both male and female patients. The distribution between the upper and lower arches as location for treatment were also nearly equal.

KEYWORDS:

Solea LASER, CO_2 9.3 μm short-pulsed laser, carbonate removal, fluoride varnish, caries inhibition

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1. INTRODUCTION

1.1 Background

The use of LASER technology in dentistry has become more commonplace. Some of the major advantages of LASER technology include reduced need for anesthetic injections, reduced sensations related to noise and sound, expedited soft tissue procedures, and more conservative restorative dentistry (1). As a result, patient and provider use and acceptance of these technologies has grown (2). LASER technologies have been introduced that have the ability to aid in caries detection and identification, and remove and modify caries and dental hard tissues.

1.2 LASER Wavelength Type

LASERs are capable of producing beams of varying wavelengths depending on their construction and the stimulation medium used (e.g. CO_2, erbium, argon, etc.). The wavelength of a LASER beam plays a crucial role in how it interacts with a human tissue. In order for a LASER beam to have an effect on a tissue, it must be effectively absorbed by a molecular group in the tissue. Different human tissues, for example hydroxyapatite, will more effectively absorb certain wavelengths compared to others due to the types of molecular groups present in the tissue. It has been shown that dental enamel absorbs LASER beams at several different wavelengths although it most effectively absorbs wavelengths of 9.3 and 9.6μm while conventional 10.6μm CO_2 LASERs are not absorbed nearly as effectively (3,4). The absorption percentages for molecular groups in hydroxyapatite and water at the various LASER wavelengths produced by differing base LASER mediums can be appreciated in **Figure 1.1**. The

highest absorption percentages for hydroxyapatite crystals are seen in the 9.0 to 10.6μm range with the most absorption occurring between 9.3 and 9.6μm (5,6).

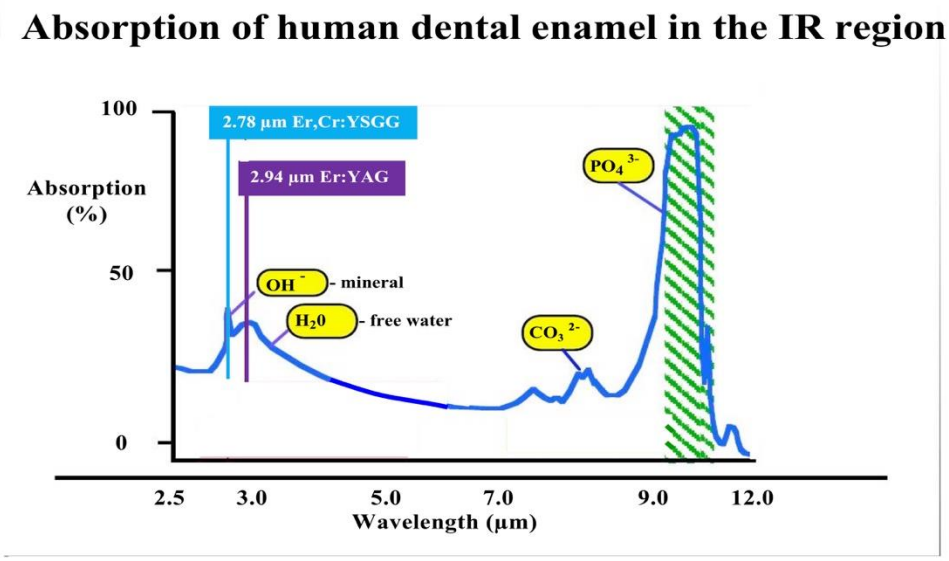

Figure 1.1: Absorption Curves of Various Tissue Components (Rechmann et. al Lasers in Dentistry – Current Concepts; Editors: Coluzzi, et. Al. 2017; Pages 361-375

1.3 Selection of Wavelength and Pulse Duration

In decades of LASER research, many different LASERs with many different wavelengths have been evaluated. When a LASER beam is directed at a tissue such as dental enamel, there are several things that can occur. The beam can be absorbed and have a thermal effect, it can be reflected off of the surface, it can be scattered throughout the tissue or it can be transmitted through the tissue. While absorption of a LASER beam is very important in order for an effect on the tissue, the reflection and back scattering of the beam must be taken into account when

assessing its ability to effectively apply energy. Reflection occurs when a beam is redirected off of the surface of the tissue while backscattering occurs when a beam is redirected through a tissue until it is expelled, potentially without absorption. It has been shown that the CO_2 beam wavelengths of 9.6μm has the highest absorption of any wavelength for dental enamel; however dental enamel also has a very high reflection coefficient at this wavelength, which reduces the total amount of energy arriving at the target tissue. While absorption at wavelength 9.3μm is slightly less for dental enamel, the reflection is also reduced relative to 9.6μm, resulting in a greater amount of total energy arriving in the target tissue for the 9.3μm wavelength.

LASER beam energy transferred to enamel is absorbed by some molecular group depending on its wavelength and a that group is heated (7). This thermal increase can be beneficial or detrimental, depending on the depth of the heat penetration and diffusion and the intention to treat. If too much energy is transferred, detrimental effects could occur, like heating the pulpal tissue above a critical temperature. (9). This potential problem has been approached by pulsing the LASER, which allows for thermal relaxation time and limits the thermal effects to the intended tissue only (8,10-12). Pulse durations necessary for safe and selective enamel ablation and modification have been studied and established and are found in the range of microsecond pulses as compared to millisecond pulses (10-14).

1.4 Carbonated Hydroxyapatite Ablation

In recent studies, Featherstone et. al. has shown that enamel crystals include imperfections. The actual enamel is best described as carbonated hydroxyapatite, and the imperfections result in increased susceptibility to acid demineralization which then results in caries formation. In contrast, fluorapatite is a very acid resistant crystal found in dental enamel that occurs both naturally and forms over time with exposure to fluoride ions. When carbonate is

removed from the hydroxyapatite crystals and fluoride is present the resulting fluorapatite is more resistant to acid attack (15-19). Using cross sectional microhardness measurements, Rechmann and others have shown in vivo that removal of the carbonated phase of enamel by treatment with a specific LASER irradiation and subsequent fluoride varnish application reduced the susceptibility of enamel to acid demineralization when orthodontic brackets were present (18,19).

1.5 LASER Treatment Rendering Enamel Caries Resistant with Additional Fluoride Application

Fluoride treatment alone is known to increase enamel resistance to caries (37). Recently, it has been proposed and investigated that laser irradiation in combination with fluoride treatments can further increase enamel resistance to caries (38-40). Featherstone et. al. has shown in several studies that enamel caries resistance can be enhanced in the laboratory by laser irradiation with CO_2 9.6μm wavelength laser with several different pulse durations (41-43). Rechmann et. al. has shown in vivo using an orthodontic bracket model in a single blind, prospective clinical trial using a CO_2 9.6μm LASER and quantitative assessment of demineralization by cross sectional microhardness testing that LASER irradiation can significantly inhibit carious lesion formation around brackets. The reduction in enamel mineral loss was up to 46% over a 4-week period and up to 87% over a 12-week observation period in comparison to control surfaces (18,44). More recently, in a single blind, controlled, randomized, clinical split mouth pilot trial in 20 subjects using a CO_2 LASER with a wavelength of 9.6 μm and fluoride varnish application, it was shown that carious lesion inhibition can be achieved in fissures of molars in vivo compared to a non-irradiated control tooth over one year (39). ICDAS scores decreased at all recalls for the irradiated treatment tooth and increased for the controls

(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 scores for 6 and 12-month evaluations showed statistically significant differences between baseline and recall for both the test and the control teeth (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).

1.6 CAMBRA

The Caries Risk Assessment (CRA) form the Caries Management by Risk Assessment system that has been developed for use in assessing an individual's personal caries risk (22). It has demonstrated predictive validity in identifying the caries risk of any age. On the CRA tool the individual's disease indicators, risk factors and protective factors are collected (**Figure 1.2).** After collection and analysis of this information a provider can assess the individual's caries risk as being either extreme, high, moderate or low.

Updated CAMBRA* Caries Risk Assessment Form for Patients Aged 6 to Adult (January 2019)
(Refer to the second page of this form for details and instructions for use.)

Patient name: Reference number:
Provider name: Date:

Caries risk component			
Disease indicators	Check if Yes		
1. New cavities or lesion(s) into dentin (radiographically)			
2. New white spot lesions on smooth surfaces			
3. New noncavitated lesion(s) in enamel (radiographically)			
4. Existing restorations in last three years (new patient) or the last year (patient of record)			
Biological or environmental risk factors		Check if Yes	
1. Cariogenic bacteria quantity — **not currently available**			
2. Heavy plaque on the teeth			
3. Frequent snacking (> 3 times daily)			
4. Hyposalivatory medications			
5. Reduced salivary function (measured low flow rate)**			
6. Deep pits and fissures			
7. Recreational drug use			
8. Exposed tooth roots			
9. Orthodontic appliances			
Protective factors			Check if Yes
1. Fluoridated water			
2. F toothpaste once a day			
3. F toothpaste 2X daily or more			
4. 5000 ppm F toothpaste			
5. F varnish last 6 months			
6. 0.05% sodium fluoride mouthrinse daily			
7. 0.12% chlorhexidine gluconate mouthrinse daily seven days monthly			
8. Normal salivary function			
	Column 1	Column 2	Column 3
Final Score: **Yes in column 1:Iindicates high or extreme risk** **Yes in columns 2 and 3: Consider the caries balance** **** **Hyposalivation plus high risk factors equals extreme risk**			
Final Overall Caries Risk Assessment Category (check) determined as per guidelines below **EXTREME** ☐ **HIGH** ☐ **MODERATE** ☐ **LOW** ☐			

*CAMBRA is a registered trademark of the University of California, San Francisco, School of Dentistry

Figure 1.2: Caries Risk Assessment Form Used by UCSF School of Dentistry

1.7 Caries Detection Methods (ICDAS, SOPRO Life, DIAGNOdent)

The International Caries and Detection System (ICDAS) is a visual scoring system developed for use in clinical assessment and clinical research of caries development and progression (19, 20). It uses a numbered scoring system, 0-6, to grade enamel demineralization and cavitation, with a score of "0" representing unaffected/sound enamel, "1" representing initial demineralization confined to the deepest portion of the pit/fissure that is visible only after air drying, "2" representing a distinct visual change in the enamel that is visible when the surface is wet, "3" representing localized loss or breakdown of enamel with widening of the pit or fissure but no visible underlying dentin or shadow, and "4-6" representing larger carious lesions (**Figure 1.3)**. The ICDAS visual scoring system is accompanied by treatment recommendations, with a score of "3" representing the first diagnosis that is indicated for a preventive (resin sealant) or minimally invasive functional treatment (resin restoration) (**Figure 1.4**).

Quantitative light-induced fluorescence has been tested in vitro and in vivo as a method of caries detection and progression in occlusal surfaces (23-29). The SOPROlife daylight intraoral camera (SOPROlife day light mode) is used to record an intraoral picture with Sopro-imaging software (Acteon, Sopro, La Ciotat, France). This allows for a digital record of enamel surfaces which can allowing independent observers to judge SOPROlife daylight scores later. Additionally, the SOPROlife fluorescence mode uses four blue LEDs to illuminate the tooth with 450nm wavelength blue light. Fluorescence measurements using blue light fluorescence (SOPROlife) – SOPROlife blue fluorescence score can also be used to provide a visual comparison of enamel surfaces using known fluorescence properties of enamel and dentin and additionally can identify the presence or absence of bacterial porphyrins which accumulate in the porous enamel surfaces after demineralization and fluoresce red under exposure to blue light

(**Figure 1.5**) (30-32). **Figure 1.5** depicts this fluoresce – blue light with a wavelength of 450nm is transmitted through enamel and green light fluoresces off of healthy dental as green light while red light fluoresces off of porous mineral that harbors porphyrins.

The KaVo DIAGNOdent (KaVo, Biberach, Germany) system uses red LASER light with a wavelength of 655nm to aid in caries detection (30-36). The system is also based on light fluorescence. Some of the LASER red light is re-emitted as fluorescence in the near-infrared. Increased fluorescence has been shown to be associated with the progression of caries in enamel and dentin. The system will measure this amount of re-emitted near-infrared fluorescence light. Higher values are correlated to lesion progression and a clinical recommendation (**Figure 1.6**) are as followed: at values of 0-13 is not advised perform any invasive treatment, a value of 14-20 is advised to be treated with preventive measures, a value of 21-29 is advised to be approached with preventive and possibly operative treatment, and a value greater than 30 is advised to be treated with operative care.

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 phenomena.	First visual change in enamel; seen only after air drying, or colored change "thin" limited to the confines of the pit and fissure area.	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, widening of fissure.

Figure 1.3: ICDAS Scores 0 to 3

ICDAS code	0	1	2	3	4	5	6
							
Definitions	Sound tooth surface; no caries change after air drying (5 sec); or hypoplasia, wear, erosion, and other noncaries phenomena	First visual change in enamel; seen only after air drying, or colored change "thin" limited to the confines of the pit and fissure area	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, widening of fissure	Underlying dark shadow from dentin, with or without localized enamel breakdown	Distinct cavity with visible dentin; frank cavitation involving less than half of a tooth surface	Extensive distinct cavity with dentin; cavity is deep and wide involving more than half of the tooth
Histologic depth		Lesion depth in P/F was 90% in the outer enamel with only 10% into dentin	Lesion depth in P/F was 50% inner enamel and 50% into the outer 1/3 dentin)	Lesion depth in P/F with 77% in dentin	Lesion depth in P/F with 88% into dentin	Lesion depth in P/F with 100% in dentin	Lesion depth in P/F 100% reaching inner 1/3 dentin
Sealant/restoration Recommendation for low risk	Sealant optional DIAGNOdent may be helpful	Sealant optional DIAGNOdent may be helpful	Sealant optional or caries biopsy if DIAGNOdent is 20-30	Sealant or minimally invasive restoration needed	Minimally invasive restoration	Minimally invasive restoration	Minimally invasive restoration
Sealant/restoration Recommendation for moderate risk	Sealant optional DIAGNOdent may be helpful	Sealant recommended DIAGNOdent may be helpful	Sealant recommended or caries biopsy if DIAGNOdent is 20-30	Sealant or minimally invasive restoration needed	Minimally invasive restoration	Minimally invasive restoration	Minimally invasive restoration
Sealant/restoration Recommendation for high risk *	Sealant recommended DIAGNOdent may be helpful	Sealant recommended DIAGNOdent may be helpful	Sealant recommended or caries biopsy if DIAGNOdent is 20-30	Sealant or minimally invasive restoration needed	Minimally invasive restoration	Minimally invasive restoration	Minimally invasive restoration
Sealant/restoration Recommendation for extreme risk **	Sealant recommended DIAGNOdent may be helpful	Sealant recommended DIAGNOdent may be helpful	Sealant recommended or caries biopsy if DIAGNOdent is 20-30	Sealant or minimally invasive restoration needed	Minimally invasive restoration	Minimally invasive restoration	Minimally invasive restoration

* Patients with one (or more) cavitated lesion(s) are high-risk patients. ** Patients with one (or more) cavitated lesion(s) and xerostomia are extreme-risk patients.

*** All sealants and restorations to be done with a minimally invasive philosophy in mind. Sealants are defined as confined to enamel. Restoration is defined as in dentin. A two-surface restoration is defined as a preparation that has one part of the preparation in dentin and the preparation extends to a second surface (note: the second surface does not have to be in dentin). A sealant can be either resin-based or glass ionomer. Resin-based sealants should have the most conservatively prepared fissures for proper bonding. Glass ionomer should be considered where the enamel is immature, or where fissure preparation is not desired, or where rubber dam isolation is not possible. Patients should be given a choice in material selection.

Figure 1.4: ICDAS Scores and Treatment Recommendations

Figure 1.5: SOPROLife with Blue Light Fluorescence Technology

Values of measurement	Clinical treatment
0-13	Non active care is advised
14-20	Preventive care is advised
21-29	Preventive or operative care is advised depending on the patient's caries risk, recalls, etc.
>30	Operative care is advised

Figure 1.6: DIAGNOdent Values and Associated Treatment Recommendations

2. SIGNIFICANCE, AIMS, AND HYPOTHESIS

2.1 Significance

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), SOPRO Life daylight and blue fluorescence, and DIAGNOdent Laser Light-induced Fluorescence in a randomized, single blind, prospective, split mouth controlled, clinical trial over 12 months.

2.2 Hypothesis

Treatment with the new CO_2 -9.3µm, short-pulsed laser irradiation (Solea, Convergent Dental Inc., Natick, MA) on sound human enamel in addition to fluoride varnish therapy results in changes in crystal composition and structure which increase resistance of dental enamel to dissolution by acid and will work to better prevent dental caries formation on the occlusal surface of vital human teeth when compared to fluoride therapy alone over 12 months.

2.3 Null Hypothesis

Treatment with the new CO_2 -9.3µm, short-pulsed laser irradiation (Solea, Convergent Dental Inc., Needham, MA) on sound human enamel in addition to fluoride varnish therapy

results in changes in crystal composition and structure which does not increase resistance of dental enamel to dissolution by acid and will not work to better prevent dental caries formation on the occlusal surface of vital human teeth when compared to fluoride therapy alone over 12 months.

2.4 Specific Aims

Specific Aim: 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 over 12 months. The fissure mineral loss will be quantified by visual exams with the International Caries Detection and Assessment System (ICDAS), SOPROLIFE daylight and blue fluorescence, and by DIAGNOdent Laser Light-induced Fluorescence.

2.5 Outcome Measures

Primary Study Outcome Measure: Differences in change in ICDAS 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

3. MATERIALS & METHODS

Institutional Review Board (IRB) approval was obtained from the UCSF Committee on Human Research (IRB #: 14-15555, and the study was registered with ClinicalTrials.Gov. All phases of the study were conducted at the UCSF School of Dentistry Clinics (707 Parnassus

Avenue, Department of Pediatric Dentistry, San Francisco, CA 94122). Funding was provided by Solea, Convergent Dental Inc. (Needham, MA) through the UCSF Contracts & Grants Division. Each study subject was compensated with up to $100 in cash for his/her time and effort spent in the study. The compensation was staggered: $20 at baseline, $35 at the 6-month visit, and $45 at the 12-month visit.

3.1 Study Population

Subjects were recruited from the UCSF School of Dentistry Pediatric Dentistry Clinic from February 2019 to November 2019.

3.1.1 Inclusion Criteria

To be included, participants had to fulfill the following 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.

3.1.2 Exclusion Criteria

Individuals meeting the following criteria were excluded from participation:

- 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

3.1.3 Patient Screening

Subject enrollment required study investigator MK, a postdoctoral pediatric dentistry resident, to screen charts of patients being treated in the UCSF Pediatric Dentistry Clinics for candidates that would fulfill the inclusion criteria. MK screened patients from both the post-doctoral clinics and the pre-doctoral clinics and noted their upcoming appointments. On the day of their dental appointment, the patients and their guardians were approached by MK or BR, a senior research associated and dental assistant, and were informed about the study. If the patient and their guardian were interested in the study, then they were screened by either MK or PR, the

Principle Investigator of the study, for qualification. If patients fulfilled the necessary criteria and agreed to participation in the study, MK and/or BR obtained assent from the participant and consent from the parent/guardian.

229 pediatric patients were screened for participation in this study. Appointments and follow-ups were monitored by MK and BR to ensure appropriate follow-up in accordance with the study protocol. When possible, patients were schedule for their regular periodic oral examinations at the time of their follow-up appointments. In several instances where this coordination was not possible, the patient returned for an additional appointment for either periodic examination or treatment follow-up.

3.1.4 Sample Size Calculation

Sample size calculations were performed by Dr. John Kornak, UCSF Department of Epidemiology and Biostatistics using PASS 12 Power Analysis & Sample Size Software (http://www.ncss.com/software/pass/). Power calculations below were based on results obtained from a pilot study on caries prevention (20). For the purpose of sample size calculation, it was assumed 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. In the pilot study 1 participant with worsening ICDAS score was observed in the laser group vs. 9 participants in the control group. The power was determine 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%) there would be a 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, it was expected 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).

3.2 Consent

Consent was obtained from the participant's parent or legal guardian. They were then consented and offered several opportunities to ask questions and for any clarification needed by both BR and MK. The consent form was given in paper form in English to the parent or legal guardian with an unlimited amount of time for review and questions, after which the consent form was signed.

3.3 Assent

Assent was obtained from the participant. The participant was verbally consented and offered several opportunities to ask questions and for any clarification need by both BR and MK. The assent form was given in paper form, written in basic and common language English, with an unlimited amount of time for review and questions, after which the assent form was signed by the participant.

3.4 Experimental Design

3.4.1 Randomization

After it was decided that the participant met inclusion criteria, the participant's molars were randomly assigned to the experimental (laser treatment and fluoride) or control group (fluoride only). A randomization list had been created by a random generator (QuickCalcs Online Random Numbers by GraphPad Software, Inc.) by BR and kept in a locked drawer. Assignments were kept in separate, closed opaque, sequentially numbered envelopes.

3.4.2 Blinding

The doctor performing the laser treatment, PR, and the dental assistant, BR, were not be blinded to subjects' group assignment as they were participating in the treatment. The participant was also not blind to the treatment. BR informed the laser treating doctor which tooth would be the treatment tooth and which would be the control tooth. An independent study investigator, MK, was blinded to subjects' group assignments. BR and MK served as the go-to person for subjects. The statistician will be blinded to treatment assignment by labeling the 2 groups as 0 and 1, without giving the statistician the key.

3.4.3 Materials and Methods

The laser used in this study was a Solea hard tissue CO_2 LASER (Solea, Convergent Dental Inc., Needham, MA). The varnish applied every 6 months was 3M™ Vanish™ 5% Sodium Fluoride White Varnish, 12151CL, Cherry, Mint & Melon Flavors, 1000 0.5mL Unit Dose.

3.4.4 ICDAS

At the baseline appointment, the participant had both the experimental and control teeth cleaned with a slow-speed rotational brush head until the occlusal surface was visibly clear of debris by either PR or MK. Dental loupes with 2x magnification were used to assess the occlusal surface of both teeth by PR and MK independently in separate clinics with the other investigator absent. ICDAS values were recorded for both teeth on individual case report forms that were managed solely by BR. After baseline assessment, BR escorted the participant to a separate clinic where they were re-assessed by the other investigator and the ICDAS values were again recorded on a separate case report form managed by BR.

3.4.5 SOPROLife and Diagnodent

After ICDAS values we recorded by PR, SOPROLife images were taken of the occlusal surfaces of both treatment and control teeth by PR. Both SOPROLife Daylight and SOPROLife Blue Fluorescence images were taken and stored on a secure laptop. Diagnodent readings were then made by PR in the pits and fissures of the occlusal surfaces and recorded by BR on a case report form.

3.4.6 Treatment, LASER Energy and Beam Size/Pattern Settings

The experimental tooth received LASER irradiation at baseline followed by fluoride varnish treatment; at baseline, the control tooth received only fluoride varnish treatment. At 6 month and 12-month follow ups both experimental and control teeth received standard prophylaxis cleaning and fluoride varnish treatment. Isolation of the treatment tooth and soft tissue retraction were achieved with the help of the dental assistant. A timer was set and the treatment tooth was irradiated for 120 seconds to insure that all pit/fissure surfaces on the treatment tooth were sufficiently irradiated (see below). Immediately after, the treatment tooth, control tooth and all teeth were treated with fluoride varnish.

During treatment, a pulse duration of 4μs was used that delivered a pulse energy of 1 1.9mJ/pulse that resulted in a fluence of 3.9 J/cm^2. The pulse energy was measured using a BeamTrack – Power/Position/Size Thermal Sensor 50(150) A-BB-26-PPS (Ophir-Spiricon) before and after five patient's teeth were irradiated in order to confirm correct settings and energy delivery. Pulse repetition rate was set to 43 Hz. The beam diameter was 250 μm with a focus distance of 10 to 15mm. To ensure that each spot was irradiated with a minimum of 20 LASER pulses, each fissure was irradiated for 120 seconds with a continuous and overlapping motion of the laser hand piece.

3.4.7 6 Month Follow-up Appointment

At 6-month follow-up appointments, the patient was seen by either PR or MK initially, and then was transferred to the other evaluator. The provider that saw the participant first cleaned the occlusal surfaces of the enrolled teeth as described above, dried the occlusal surfaces, and recorded ICDAS scores. Additionally, PR recorded SOPROLife images and DIAGNOdent values. The evaluator that saw the patient last performed the fluoride treatment. The patient was then reimbursed by BR and was given a payment receipt.

3.4.8 12 Month Follow-up Appointment

At 12-month follow-up appointments the same model was followed as with the 6-month appointments outlined above. Additionally, MK completed any indicated treatment (sealants or minimally invasive resin restorations) on the enrolled teeth. The evaluator that saw the patient last performed the fluoride treatments. The patient was then paid by BR and was given a payment receipt.

3.4.9 ICDAS 3 Values

After evaluation by both PR and MK if any patient was deemed to have a lesion of ICDAS 3 or greater the patient was informed of their lesion progression and that they would be removed from the study. In these cases, MK treated both the treatment and control teeth with either sealants or minimally invasive resin restorations.

3.4.10 Compliance

To ensure compliance with follow-up appointments, BR and MK made periodic phone calls to the parent or legal guardian of the participants. Appointments were set by both BR and MK and patients were instructed to only set appointments with BR and MK to ensure that

additional treatment of the enrolled surfaces by others did not occur and that additional prophylaxis or fluoride treatment was not performed.

3.4.11 Reliability

Intra-examiner and inter-examiner reliability of ICDAS scores were assigned by PR and MK were assessed periodically. Comparison of several sets of dentition with a range of established ICDAS scores were used. PR and MK evaluated and recorded ICDAS scores for the established teeth once before patient enrollment and once after recruitment and treatment of the study subjects. ICDAS scores by PR and MK were compared to each other and to their own recorded ICDAS scores and a Kappa/weighted Kappa score was generated.

3.5 Data Analysis

Data analysis will be performed by Dr. John Kornak, UCSF Department of Epidemiology and Biostatistics. The primary analysis of this study will compare ICDAS scores for the laser group vs. controls. Mixed effects proportional odds regression modeling with outcomes of ICDAS and SOPROLIFE scores will be performed. These models will 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. Interactions of these additional predictors with the group predictor as well as time will be included, 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 important effects are identified, then a small number of predictors will be combined based on scientific plausibility in a single model. It is noted that these will be far from definitive models given the sample size and scope of the

proposed study. P≤0.05 will be considered statistically significant though it will be emphasized that any model results are highly speculative and will require independent verification.

The complete analysis of the data from this study require further data to be collected and analyzed. At the time of publication of this thesis, their remain 24 patients to present for a 12-month follow-up appointment. Subject recruitment was not completed until December 2018; as each study subject had to be followed for one year, data from the final 12-month time point would not be available until the end of the Fall 2019 term. For this reason, the results reported below will deviate slightly from the original goals of the project described above. As the study investigators were required to remain blinded for the collection of data at the final 12-month time point, the thesis committee agreed that the analysis presented here would exclude ICDAS, SOPROLife and DIAGNOdent data and would be limited to an evaluation of the subject pool as a collective, irrespective of their assignment to the experimental or control groups. A second manuscript will be presented at the completion of all data collection and subsequent unblinding of the study investigators for comparison of changes between the experimental and control groups.

4. RESULTS

4.1 Participant Demographics

Enrollment of subjects took place from February 2018 to December 2018, a total recruitment period of nine months. Sixty patients were recruited to participate in the study: 32 females and 28 males. The average age of the patients when they were enrolled and treated was 13.15 years old (Appendix, Table 1).

4.2 Participant Attrition

The attrition rate thus far has been 8.3%; five subjects were lost. Three were male and two were female. Subjects 8 and 20 returned for the 6-month follow up but moved out of the country prior to the 12-month follow-up. Subjects 13 and 32 were unable to be contacted after the baseline appointment. Subject 47 had sealants placed over the treatment and control teeth in the pre-doctoral clinic prior to the 6-month follow up appointment (Appendix, Table 2).

4.3 Progression to ICDAS 3 Resulting in Participation Cessation

Thus far, 10 participants have had at least one location on one tooth progress to ICDAS 3 or greater, forcing them to be terminated from the study pool. Subject 23, 26, 30 and 39 had at least one ICDAS 3 or greater value at the 6-month follow-up appointment. Subjects 2, 4, 5, 10, 14 and 25 presented with at least one ICDAS 3 or greater value at the 12-month follow-up appointment (Appendix, Table 7).

4.4 Enrollment and Arch Selection

Total, 229 patients were screened for this study, and 60 were enrolled, a 26% enrollment (Appendix, Table 3). The maxillary molars were treated slightly more often than mandibular – 32 participants had in maxillary molars enrolled and 28 participants had the mandibular molars enrolled (Appendix, Table 4).

5. DISCUSSION

5.1 Enrollment

Of the 229 patients screened for this study, only 26% (60 patients) met inclusion criteria and were willing to participate in the study. Enrollment for this study proved difficult due to several factors. First, the number of patients presenting with eligible molars was lower than initially

expected. Many of the new patients who presented for exams that were screened had already had their second molars sealed or, more commonly, had carious lesions on one or both contralateral molars that disqualified them from the study. This is likely related to the demographics of the patient population that presents to UCSF School of Dentistry for treatment. Additionally, a small number of patients that were screened for participation were not interested in the additional chair-time required to participate in the study.

5.2 Limitations

In split mouth designs, it is always possible that there are individual discrepancies in how patients treat each side of their mouth. It is possible that patients are inconsistent in their oral hygiene, brushing one side more thoroughly than the other, or eating food on a dominant side, for example. That said, it was not observed in the enrollment of this study that any of the patients exhibited evidence of this type of behavior.

In a split mouth study that requires treatment and follow up by the same provider, it may be possible for bias to manifest in the provider's potential ability to recall which tooth is the treatment tooth and which is the control tooth. It seems unlikely, however, in a study with a total of 60 patients treated, over 200 screened, and with time gaps of 6 months, that a provider would be able to recall such specifics. In addition a second examiner who had always been blinded to the treatment also evaluated the ICDAS scores independently.

6. CONCLUSION

While the majority of clinically relevant conclusions will be made after unblinding and data analysis, several minor conclusions can be draw from evaluation of the participant data. The distribution of male and female participants was nearly equal, indicating that there seems to be generalized equal acceptance of laser treatment from both male and female patients. Additionally, the distribution between the upper and lower arches as location for treatment were also nearly equal.

Enrollment is closed for this study, however final follow-up appointments are still pending for one third of the subjects. As such, the hypothesis cannot currently be confirmed nor rejected. However, it is known that at least 10 subjects have had lesion progression to ICDAS 3 scores on one tooth only.

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APPENDIX

Table 1: Subject Demographics

Females	32
Males	28
Age at Baseline	13.15ys

Table 2: Causes of Attrition

Unable to Contact	2
Moved Away	2
Sealants Placed on Teeth	1

Table 3: Patients Screened and Enrolled

Patients Screened	229
Enrolled	60 (26%)
Not Enrolled	169 (74%)

Table 4: Arch Enrolled

Maxilla	32 (53%)
Mandible	28 (47%)

Table 5: 6-Month Recall Outcomes

Routine	53 (88%)
Lost	3 (5%)
ICDAS 3 Progression	4 (7%)

Table 6: 12-Month Recall Outcomes Thus Far

Routine	28 (32%)
Lost	2 (4%)
ICDAS 3 Progression	6 (11%)
Remaining	28 (53%)

Table 7: Current Enrollment

Total Lost	5 (8%)
ICDAS 3 Progression	10 (17%)
Enrolled	45 (75%)

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