Title
In vivo occlusal caries prevention by pulsed CO2 laser treatment quantified by QLF

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Publication Date
2009

Peer reviewed|Thesis/dissertation

In vivo occlusal caries prevention by pulsed CO_2 laser treatment quantified by QLF

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

[redacted]

DEDICATION

To my Mom, Uncle Frank, and my husband, Alex

ACKNOWLEDGEMENTS

This work was primarily supported by NIH/NIDCR Grant #RO1DE09958 and Featherstone lab.

I would like to thank my mentors, Dr. John D.B. Featherstone and Dr. Peter Rechmann, for their tireless effort in supporting and guiding me through my research journey during the last three years.

My deepest gratitude goes to my thesis committee, Dr. John D.B. Featherstone, Dr. Peter Rechmann, Dr. Jane A. Weintraub, and Dr. Brent P.J. Lin, for their encouragement and knowledge.

I would also like to acknowledge Beate Rechmann for her assistance during patient treatment. Many thanks to Charles Le for his technical laser support. I am also grateful to Dr. Ling Zhan for her help in handling my petty cash account. Thank you to my co-residents who have helped me screen and recruit patients and even volunteered themselves to help calibrate the examiners.

The author would like to acknowledge the UCSF Committee on Human Research for approving my clinical research. (CHR# H9136-30702-01)

I would like to thank my husband, Dr. Alexander Ham, for his countless hours of keeping me company while I worked on my research and helping me with cleaning and organizing many, many pictures of teeth.

In vivo occlusal caries prevention by pulsed CO_2 laser treatment quantified by QLF
Tiffany H. Hsu

Purpose: High caries prevalence in occlusal pits and fissures of permanent molars warrants novel caries prevention methods. Rechmann et. al. reported 86% reduction in smooth surface caries following treatment by specific CO_2 laser irradiation.[1] This study's purpose was to conduct a blinded 12-month pilot scale clinical trial of occlusal pit and fissure caries inhibition in humans using the same CO_2 laser irradiation conditions.

Methods: Twenty subjects, average age 13 years, were recruited. At baseline, second molars were randomized into test and control groups, assessed by International Caries Detection & Assessment System (ICDAS) and Quantitative Light-Induced Fluorescence (QLF). An independent investigator irradiated test molars with Pulse Systems (New Mexico) 9.6μm clinical CO_2 laser, average fluence (energy/surface area) $3.4\pm0.3 J/cm^2$ per pulse, pulse duration of 20μs. At 6-month and 12-month recall, teeth were assessed by ICDAS and QLF.

Results: Of 20 subjects, 17 completed the 6-month recall and 14 completed the 12-month recall. ICDAS: At 6-months, average ICDAS score changes were 0.50 (SE 0.15) and 0.53 (SE 0.17) for test and control molars respectively. At 12-months, average ICDAS score changes were 0.40 (SE 0.19) and 0.57 (SE 0.23) for test and control molars respectively. The 6- and 12-month results showed no statistically significant differences ($P>.05$, Student unpaired t-test) in ICDAS score change between test and control molars. QLF: Qualitatively, the QLF images correlated closely to what was observed from ICDAS visual examination. Quantitatively, at 6-months, average ΔF changes were -0.78% (SE 0.38) and 0.36% (SE 0.75) for test and control molars respectively. The 6-month results showed no statistically significant differences ($P>.05$, Student unpaired t-

test) in ΔF changes between test and control molars. At 12-months, average ΔF changes were -1.13% (SE 0.34) and 0.31% (SE 0.38) for test and control molars respectively. The 12-month results showed a statistically significant difference in ΔF changes of test molars compared with control molars (*P=.01*, Student unpaired t-test).

Conclusions: Although this laser treatment markedly inhibited caries progression in the smooth surface laser study, the same result was not found for pits and fissures, likely due to design limitations of the laser delivery system.

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INTRODUCTION/ BACKGROUND

Caries Prevalence in US children

Dental caries continues to be a problem for our nation's school-aged children. It is the most common chronic childhood disease and is the most prevalent unmet health need of children in the United States.[2] More than 50% of children have detectable caries by mid-childhood [3] and approximately 80% of late adolescents have dental caries.[4] Occlusal pit and fissures are the most susceptible surfaces for caries and account for over 80% of all caries in young permanent teeth.[5] This preventable infectious disease poses significant medical and financial consequences. In its earliest stages, dental caries is a reversible process via remineralization and does not require surgical restoration. 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 inhibit the caries process.[6]

The current practice of dentistry has shifted away from traditional G.V. Black restorative techniques of "extension for prevention" toward a non-surgical medical model focusing on tissue remineralization and repair.[7] An effective approach to caries prevention would be to couple early caries detection with innovative methods of caries intervention. This warrants research focus on developing novel methods of caries intervention and early caries detection.

Methods of early caries detection

Current commonly used caries detection methods in the United States include radiographs, visual inspection, and tactile use of the explorer. 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]

Occlusal caries in its early stages is very difficult to detect. By the time an occlusal caries lesion is detectable radiographically, it is too large to be remineralized.[6] Diagnosis and monitoring of incipient occlusal caries using visual inspection is subjective and staining of the pits and fissures can often lead to misdiagnosis and false positives.[8] With the use of fluoride early occlusal caries can remain hidden under a thin layer of intact surface enamel.[9] Due to the hidden nature of occlusal caries, the sensitivity of tactile and visual inspection has been found to be low at approximately 0.3.[10] This makes it even more difficult to detect pit and fissure caries. Studies in Europe have shown that the explorer is only correct less than 50% of the time.[11]

ICDAS

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.[12] ICDAS assigns scores to lesions in occlusal surfaces based on apparent caries status and lesion severity of plaque-free teeth with the use of a ball-ended probe.[12] Of particular interest to this study are the coronal caries detection criteria.[12] (Table 1)

Fluorescence-based Caries Detection

KaVo Diagnodent

Laser fluorescence is a new method of caries detection. By analyzing the emission spectrum of carious regions versus sound tissue, studies have shown that laser fluorescence is useful as a quantitative measure distinguishing carious from non-carious surfaces.[13] The two main methods of using laser fluorescence for caries detection are Diagnodent and Quantitative Light-induced Fluorescence (QLF).

KaVo Diagnodent uses a red diode (λ=655nm) illuminating laser which detects bacteria porphyrins in a carious lesion. Fluorescence occurs when the illuminating light is absorbed by bacteria porphyrins and emitted at a longer wavelength.[13] This method does not really measure lesion depth or mineral changes in the area of the lesion, but rather depends on bacteria activity in the lesion as an indicator of carious change.[14] Early enamel lesions with no bacteria activity will not have porphyrins. Furthermore, according to Lennon et al., cariogenic Mutans streptococci do not significantly produce porphyrins.[15] Instead, porphyrins are usually more significantly produced by co-populating bacteria.[15] Thus, the Diagnodent has low sensitivity for early enamel lesions [16] and is not so useful for detecting early enamel lesions.

Quantitative Light-induced Fluorescence (QLF)

QLF is a second method using laser fluorescence for caries detection. It emits a violet-blue light (λ=290-450nm), which is scattered by demineralized lesions, returning at a longer wavelength, allowing for the quantification of change in fluorescence. When compared to conventional visual examination or other caries detection instruments, QLF can detect twice as many precavitated demineralized enamel areas.[17] Its ability to detect

and quantify changes in clinically visible white spot lesions allows QLF to determine the impact of preventative measures on inhibition of demineralization.[17] QLF has been proven to be an effective method of detecting smooth surface demineralization[18], and a recent in vitro study demonstrated that it can be used for occlusal surfaces.[19]

Methods of occlusal caries prevention

Fluoride

One of the most effective means of caries prevention is water fluoridation.[20] However, the effect of topical fluoride preferentially targets smooth surfaces and does not protect the occlusal pits and fissures as successfully.[21] This leads to a significant problem as occlusal pit and fissure caries now account for over 80% of all caries in young permanent teeth.[5] Because pits and fissures are the earliest and most prevalent surfaces for decay, new methods of caries prevention focus on these susceptible areas.[5]

Sealants

Historically, many creative approaches have been taken in attempts to reduce the risk of occlusal caries.[5] Currently, the most effective way to prevent occlusal caries is the use of dental sealants.[22] Sealants are polymers that are applied to the occlusal surface of the tooth, adhering to it, and providing a barrier against bacterial acids that dissolve the enamel and dentin during the dental caries process. However, according to Feigal, "even under the best of circumstances sealants fail."[23] The 5-10% per year failure rate of sealants account for the need for meticulous recall and maintenance procedures which are vital for long term success.[23] Since the ideal solution for pit and fissure caries prevention has yet to be discovered, creative efforts against pit and fissure caries continue as new technology and materials are developed each year.

Lasers in Caries Prevention

CO_2 laser

One novel approach to occlusal pit and fissure caries prevention is the treatment with a carbon dioxide (CO_2) dental laser with specifically designed parameters of wavelength, pulse duration, repletion rate and fluence (energy/surface area). To safely heat dental enamel to alter its composition and solubility, laser light must be strongly absorbed and efficiently converted to heat without damaging the underlying and surrounding tissues.[6] Enamel, dentin and cementum are composed of carbonated hydroxyapatite that has high absorption bands in the infrared radiation (IR) region of 9.0-11.0μm.[24] When considering caries prevention, focus is placed on altering the solubility characteristics of enamel, which has an extremely high absorption coefficient at 9.6μm.[6] Thus, low-energy, pulsed radiation CO_2 lasers operating at the wavelength 9.6μm are efficiently absorbed within the outer 1μm surface region and have been shown to be the system of choice in preventing demineralization in laboratory experiments.[6, 25, 26]

Previous laboratory, in situ and in vivo safety studies

When pulsed CO_2 laser irradiation interacts with the phosphate groups in enamel, it is absorbed and transformed into heat that can briefly raise temperatures to greater than 400°C, which are capable of driving off carbonate from the carbonated apatite mineral of the tooth.[6, 25] This leaves a hydroxyapatite-like mineral that is less soluble than carbonated apatite.[6] The pulse duration is so short and the absorption coefficient so high that the heat is essentially confined to the outer surface of the tooth and the transfer to the pulp is minimal. Studies in our laboratories have shown that the CO_2 laser treatment of enamel can inhibit caries-like progression by up to 85% in a laboratory setting.[26, 27]

In a safety study in humans, which enrolled only adult subjects, Goodis et al. showed that "the 9.6μm wavelength laser, with irradiation conditions comparable to those proposed in the present study, causes no permanent/serious pulpal damage at the energy levels used and can be used safely for caries prevention treatments in humans."[28]

A recent study by Rodrigues et al. showed that CO_2 laser treatment with 9.6μm wavelength and 5μs pulse duration can inhibit enamel mineral loss in an *in situ* high caries challenge situation on smooth surfaces of enamel in the mouth.[29] Enamel samples, cut from extracted teeth, were irradiated externally with laser conditions comparable to those proposed in the present study. The irradiated and control (non-irradiated) slabs were worn in the mouths of subjects in a "caries challenge" environment and the amount of demineralization measured after the test period.

In 2008, Rechmann et al. reported 86% reduction in smooth surface caries in vivo following treatment by specific CO_2 laser irradiation with 9.6μm wavelength, pulse duration 20μs. Laser irradiation was completed around orthodontic brackets on buccal surfaces of premolars scheduled for extraction. [1] These very promising results were expected to be found in the present study on the occlusal surfaces of vital teeth.

SIGNIFICANCE

High caries prevalence especially in occlusal pits and fissures warrants novel caries prevention methods. Sealants are effective in caries prevention but have limited lifespan and require multiple repairs. CO_2 lasers with the correct wavelength (9.3 or 9.6 μm) and pulse characteristics (pulse duration 2-100 μs) can offer a novel alternative for early caries prevention in pits and fissures. The use of an appropriate CO_2 laser in

combination with QLF caries detection and caries risk assessment is potentially an ideal method of caries prevention in the future.

HYPOTHESIS

Specific wavelength CO_2 laser treatment results in changes in crystal composition and structure which increase resistance of dental mineral to dissolution by acid and will work to prevent dental caries in the occlusal surfaces of vital teeth *in vivo*.

PURPOSE

The purpose of this study was to conduct a single blind, controlled, prospective 12-month pilot scale clinical trial of occlusal pit and fissure caries inhibition in children ages 12-17 years using the same CO_2 laser irradiation conditions as was used in the Rechmann et al. 2008 study. Secondly, this study compared early detection of occlusal enamel demineralization by QLF versus ICDAS. This split-mouth design controls for within-person genetic, hygiene, nutritional, and oral environmental factors.

SPECIFIC AIMS

Aim 1: To compare caries inhibition (change in ICDAS scores) of the occlusal pits and fissures of untreated molars with that of laser treated molars

Aim 2: To compare early caries detection methods in occlusal surfaces by 1) ICDAS visual inspection and 2) QLF *in vivo*

MATERIALS and METHODS

Target Population

The study was conducted on permanent teeth in high caries risk children aged 12-17 years. The study used fully erupted second molars because these teeth are most in need for caries prevention treatment in high caries risk children of this age group.

Children in this age group were used because their dietary habits and their teeth make them well suited for fast caries initiation and progression of caries during the time period of the study.[30, 31] Further, this age group is most likely to have newly erupted second molars requiring sealants and have a generally high level of maturity and cooperation in the dental setting. Consequently, adults were inappropriate for the study. There was no discrimination as regards to gender or race/ethnicity.

Subjects were drawn from the patient pool at the UCSF School of Dentistry. The demographics of the UCSF population are as follows: Of the 10,166 patients of record in the Comprehensive Care Clinic, 50.7% are male and 49.3% are female. The age distribution in the comprehensive care general dentistry clinic is 1.5% 1-17 years old, 9.1% 18-24 years old, 24.3% 25-34 year old, 19.7% 35-44 year old, 17.3% 45-54 year old, 10.5% 55-64 year old, and 16.2% 65+ year old. Ethnicity is varied with 48.8% White, 11.6% Black, 10.0% Asian, 11.0% Hispanic, and 18.6% other, no disclosed or not specified. Demographics in the pediatric clinic are similar, but the age range is exclusively children, as required in the proposed study. Our target enrollment reflected this distribution.

To be included, participants must fulfill the following criteria:

- 12 – 17 years old
- high caries risk status determined using CAMBRA Caries Risk Assessment Form Age 6 to Adult [32, 33]
- two fully erupted second molars with untreated, non-carious occlusal surfaces in the same arch
- healthy and able to cooperate for treatment in dental chair

- parent/guardian able to provide written informed consent in English
- patient provide verbal assent
- residing in San Francisco or other nearby locales with community water fluoridation
- willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form

Individuals meeting the following criteria were excluded from participation:

- low or moderate caries risk status determined using CAMBRA Caries Risk Assessment Form Age 6 to Adult [32, 33]
- no pair of untreated or non-carious occlusal surfaces on second molars in the same arch
- under active orthodontic treatment involving 2nd molar bands
- has limited range of opening
- will leave the area and will not be available for recall visits
- has underlying systemic disease which could alter enamel composition or formation
- a significant medical history with conditions that may affect oral health or flora (i.e. diabetes, HIV, heart conditions that require antibiotic prophylaxis)

Experiment Location/Approval

All experiments were conducted at the UCSF Predoctoral and Postgraduate Pediatric Dental Clinics. Approval for clinical research was obtained by the UCSF Committee on Human Research (CHR# H9136-30702-01). (Appendix 1) Funding was

provided by the Featherstone Laboratory and NIH/NIDCR Grant #RO1DE09958, Principal Investigator: Dr. John D.B. Featherstone.

Experimental Design

Randomization

Using a random number generator, the molars were randomized into test and control groups based on order of involvement. The test molar received intervention with CO_2 laser irradiation and the control molar initially remained unsealed. Under normal conditions, it is the standard of care at UCSF for molars in patients with high caries risk to receive sealants. According to Vanderas et. al., the progression rate of a carious lesion through enamel of permanent teeth takes an average of four years.[34] Furthermore, a periodic six-month oral evaluation was conducted for all study patients such that close monitoring and follow-up of study molars was ensured. Thus, waiting six months to seal a permanent molar will not cause a significant increase in risk for the patient.

Approximately nine hundred sixty patients were screened, out of which twenty-one subjects were enrolled in the study with one subject disqualifying prior to start due to inability to meet inclusion criteria. A total of twenty subjects received treatment, four were disqualified during the course of the study, and the remaining sixteen subjects completed the study at either six-month or twelve-month recall visits.

Blinding

Because this study involved CO_2 laser treatment to the test molar, it was not possible to blind the laser-operating dentist to the treatment (ie. laser treatment or no laser treatment); therefore an independent observer, who was blinded to the treatment, collected the data from the study subjects. Two independent examiners determined

ICDAS coding for the study molars. Both examiners were blinded for ICDAS coding at baseline exam, and one examiner (TH) was blinded for ICDAS coding at six- and twelve-month exams. Due to the obvious observable changes to the CO_2 laser treated molar upon post-treatment QLF analysis, it was impossible to blind both dental examiners.

Materials

CO_2 laser, Pulse Systems, Inc. (PSI) (Model #LPS-500, Los Alamos, New Mexico), at wavelength 9.6 μm, pulse duration 20μs, pulse repetition rate 20Hz, beam diameter 1,100μm, average fluence (energy/surface area) 3.4±0.3J/cm^2 per pulse, 20 laser pulses per spot was used for all test molars.[1] (Figure 6) International Caries Detection & Assessment System (ICDAS) and Quantitative light-induced fluorescence (QLF) Inspektor Pro v.2.0.0.30 (Figure 7) were used to measure caries status around the occlusal pits and fissures. A Canon EOS 10D digital camera with a Canon 100mm macro lens and macro ring flash was used to capture intraoral photographs. 35% Phosphoric acid, Optibond Solo Plus/Clearfil S3, and Tetricflow/Helioseal were used for sealant placement on all study teeth at six months or at the conclusion of the study. Fluoride prophylaxis paste (NUPRO Prophylaxis paste, 1.23% NaF) and acidulated phosphate fluoride (APF) topical foam (Oral B Minute Foam, 1.23% w/w Fluoride ion) were used during baseline, six month, and twelve month exams.

PROCEDURES

Clinical Protocol (Figure 1)

Caries Management By Risk Assessment (CAMBRA) Questionnaire (Appendix 2):

For all subjects, CAMBRA (Caries Risk Assessment) Questionnaire from UCSF Axium (electronic patient record) clinical form was administered to determine caries risk

as is the current procedure in the predoctoral dental clinics. If caries risk was determined to be high, then the patient was screened to be eligible for the study.

Informed Consent:

Written informed consent was obtained from the parent/guardian and verbal assent was obtained from the patient. (Appendix 3) The patient was then scheduled for a baseline exam.

Study Procedures:

Baseline exam

ICDAS Visual Exam

Prior to randomization of molars, a blinded, trained dental examiner (TH) brushed the study molars using a dry prophy brush and conducted a visual exam to assess caries status based on the International Caries Detection and Assessment System (ICDAS) clinical visual criteria for occlusal pit and fissures. A second independent examiner (PR), who was blinded prior to randomization of test and control molars, also conducted a visual exam to assess caries status based on ICDAS clinical visual criteria. Each molar was assigned ICDAS codes for different regions of the tooth based on location of primary occlusal grooves of the tooth. For maxillary molars, each tooth had three main regions, mesial (1), buccal (2), distal (3). (Figure 2) For mandibular molars, each tooth had four main regions, mesial (1), buccal (2), distal (3), lingual (4). (Figure 3) Codes for coronal caries (0-3) were assigned based on severity of caries.[12] (Table 1) If there was a discrepancy of ICDAS codes between the two examiners, a discussion was conducted until agreement was reached. The ICDAS score for the overall tooth was determined based on the worst score from all the tooth regions for that tooth.

QLF Analysis

This was followed by QLF analysis using Inspektor Pro software v.2.0.0.30. Green fluorescent images of the occlusal surfaces of control and test molars were created using an intraoral camera handpiece which emitted a violet-blue light (λ = 290-450nm, average 380nm) and captured through a yellow 520nm high pass filter.[35] The images were analyzed using the White Spot analysis wizard. The area of interest on the occlusal surface was marked. For maxillary molars, the area of interest was confined to the occlusal area mesial to the transverse ridge because CO_2 laser irradiation never reached beyond the transverse ridge of the tooth. (Figure 4) For mandibular molars, the area of interest was the entire occlusal surface, encompassing all primary occlusal grooves. (Figure 5) Areas of unsound enamel were deselected and a reconstructed area of sound enamel was generated. The selected area of interest was analyzed for differences in fluorescence compared to areas of sound enamel, yielding average changes in demineralization (ΔF), average changes in volume of demineralization (ΔQ), and white spot area (WS) values for the overall tooth. The areas of most mineral change were represented with yellow, areas of little mineral change were represented with blue, and areas of moderate change were represented with red and purple.

For maxillary molars, four representative points, one from each tooth region were chosen (Figure 2) and ΔF recorded and followed at 6-month and 12-month exams. For mandibular molars, five representative points, one from each tooth region (Figure 3) were chosen and ΔF recorded and followed at 6-month and 12-month exams.

Pre-treatment intraoral digital photographs:

The occlusal surfaces of study and test molars were photographed prior to laser irradiation using intraoral mirrors and a Canon EOS 10D digital camera with a Canon 100mm macro lens and macro ring flash.

Molar randomization:

The molars were randomized into test and control groups based on order of involvement and a random number generator list as described above. The test molar received intervention with CO_2 laser irradiation and the control molar initially remained unsealed.

Treatment with CO_2 laser irradiation:

Following baseline exam, an independent dental provider (PR), trained in this technique, applied CO_2 laser irradiation to the test molar.

CO_2 laser irradiation (test tooth):

A Pulse Systems (New Mexico) 9.6 μm wavelength clinical laser, described above, was used at an average fluence (energy/surface area) of 3.4±0.3J/cm^2 per pulse, a pulse duration of 20μs, pulse repetition rate 20Hz, beam diameter 1,100μm, for all test molars. This was the same laser conditions used in the 2008 Rechmann et al. caries inhibition study[1], which were sub-ablative and likely effective for caries inhibition. Laser treatment was applied in overlapping spots, approximately 1 mm in diameter, 20 pulses per spot, along the occlusal pits and fissures. The area of the occlusal grooves to be irradiated was measured with a Williams periodontal probe and the number of laser pulses and the irradiation time respectively was calculated. (Table 2) The average laser irradiation time per molar was 61.6 seconds (SD 23.0). Maxillary molars on average

received 53.5 seconds (SD 11.0) CO_2 irradiation, while mandibular molars on average received 77.7 seconds (SD 32.7). The laser irradiation was delivered with the straight handpiece supplied with this prototype laser. (Figure 6) Because the conditions are sub-ablative, no water spray was necessary and only a high vacuum suction was used. The total energy delivered was far below proven safe conditions, no water cooling was needed at all.

Post-treatment QLF analysis:

The laser irradiation was followed by QLF analysis of the test and control molars by the blinded examiner.

Post-treatment intraoral digital photographs:

The occlusal surfaces of test molars were photographed prior to laser irradiation using intraoral mirrors and a Canon EOS 10D digital camera with a Canon 100mm macro lens and macro ring flash.

Periodic oral exam and preventive care:

After the laser study exam and treatment, the patients received a complete oral examination, four bitewing radiographs, dental prophylaxis with NUPRO fluoride prophylaxis paste (NUPRO Prophylaxis paste, 1.23% NaF), and fluoride treatment with acidulated phosphate fluoride (APF) topical foam for four minutes (Oral B Minute Foam, 1.23% w/w Fluoride ion). This is the customary treatment for recall appointments in the UCSF Pediatric Dental Clinic.

Six-month recall

The original dental examiner (TH) brushed the study molars using a dry prophy brush and conducted a visual exam to assess caries status based on the International

Caries Detection and Assessment System (ICDAS) clinical visual criteria for occlusal pit and fissures. A second independent examiner (PR) also conducted a visual exam to assess caries status based on ICDAS clinical visual criteria. Each molar was assigned ICDAS codes for different regions of the tooth based on location of primary occlusal grooves of the tooth as described in detail above.

QLF Analysis

This was followed by QLF analysis using Inspektor Pro software v.2.0.0.30. Green fluorescent images of the occlusal surfaces of control and test molars were created using an intraoral camera handpiece which emitted a violet-blue light (λ = 290-450nm, average 380nm) and captured through a yellow 520nm high pass filter as described above.[35]

Intraoral digital photographs

The occlusal surfaces of study and test molars were photographed using intraoral mirrors and a Canon EOS 10D digital camera with a Canon 100mm macro lens and macro ring flash.

Restoration for ICDAS ≥3

If cavitated change was found per ICDAS visual exam, ICDAS ≥3, on the occlusal surface of one or more of the two study teeth, then sealants or preventive resin restorations were placed on both study teeth, and the patient was considered to have completed the study. If no cavitated change was found per ICDAS visual exam, ICDAS ≤2, the patient continued to the next recall exam in six months (12 months after baseline).

Restoration (for patients with one or more ICDAS ≥3 teeth at recall):

Sealants or preventive resin restorations were placed according to UCSF Pediatric Dental Clinic standard operating procedures. Cotton roll and dri-angle isolation was used. The occlusal surface of the tooth was cleaned with pumice and rinsed. Enameloplasty was performed for occlusal grooves suspect of incipient enamel caries. The teeth were then etched with 35% phosphoric acid for 40 seconds, rinsed and dried thoroughly. The surface was primed and bonded with either Optibond Solo Plus or Clearfil S3, air dried gently and light cured for 20 seconds. Helioseal or Tetricflow, depending on if enameloplasty was not completed or completed respectively, was applied, and light cured for 20 seconds. The margins of the restoration were checked with explorer and occlusion was verified.

12-month recall

The original dental examiner (TH) brushed the study molars using a dry prophy brush and conducted a visual exam to assess caries status based on the International Caries Detection and Assessment System (ICDAS) clinical visual criteria for occlusal pit and fissures in the same manner as described above for previous time periods. A second independent examiner (PR) also conducted a visual exam to assess caries status based on ICDAS clinical visual criteria as described above for previous time points.

QLF Analysis

This was followed by QLF analysis using Inspektor Pro software as described above.

Intraoral digital photographs

The occlusal surfaces of study and test molars were photographed as described above.

Restoration of all remaining study teeth

After completion of the study, sealants or preventive resin restorations were placed on all remaining study teeth, as described above, and the patient was considered to have completed the study.

RESULTS

Patient demographics

A total of twenty subjects participated in the study. Seventeen out of the twenty (ten male and seven female) subjects completed the six-month recall. The average subject age was thirteen years old with a range from eleven years old to sixteen years old. Special permission was granted by the Committee on Human Research to include one eleven year old subject due to her advanced dental age and maturity exceeding her chronologic age. The ratio of maxillary molars to mandibular molars was 24 to 10. Patient attrition at six-month recall due to one patient being deported and two patients lost due to predoctoral students working on the study teeth. Fourteen subjects (eight male and six female) completed the twelve-month recall with two subjects finishing the study at six months due to ICDAS cavitated change and one subject lost due to disinterest. Due to the split-mouth nature of the study, each subject served as their own control, thus there were no significant differences in demographic characteristics, oral hygiene practice, caries risk, or genetic contributions between test and control molars.

Interexaminer & Intraexaminer reliability

Two independent examiners conducted visual exams to assess caries status based on ICDAS clinical visual criteria. The examiners were calibrated using the ICDAS Foundation e-learning software program as well as undergoing ICDAS training under Dr.

Douglas Young at the Arthur A. Dugoni School of Dentistry. Interexaminer and intraexaminer reliability was tested by conducting ICDAS exam and coding on all posterior teeth for seven volunteer subjects on two separate occasions, one week apart.

Cavitated change (ICDAS ≥3)

At six-month recall, two teeth showed cavitated change with ICDAS=3. One molar was a control maxillary molar. The other molar was a laser treated mandibular molar. All study molars for those two subjects were sealed or restored as indicated in the methods section. These two subjects had completed the study at the six-month recall stage and were not asked to return for twelve-month recall.

At twelve-month recall, one tooth showed cavitated change with ICDAS=3. It was a control maxillary molar. The study molars for this subject were sealed or restored as indicated in the methods section.

At the completion of the study, sixteen test molars received sealants and one test molar received a preventive resin restoration, while fifteen control molars received sealants and two control molars received preventive resin restorations.

ICDAS Overall Tooth

Static ICDAS scores taken from baseline exam, six-month recall, and twelve-month recall were compiled (Graph 1). At six-month recall, the average changes in ICDAS scores were 0.50 (Standard Error of the Mean SE 0.15) and 0.53 (SE 0.17) for test and control molars respectively. (Graph 2) The six-month results showed no statistically significant differences ($P>.05$, Student unpaired t-test) in change in ICDAS scores between test and control molars. At twelve-month recall, the average changes in ICDAS scores were 0.40 (SE 0.19) and 0.57 (SE 0.23) for test and control molars

respectively. (Graph 3) The twelve-month results showed a positive trend of test molars displaying less change in ICDAS score compared with control molars; however, this difference was not statistically significant ($P>0.5$, Student unpaired t-test).

QLF Overall Tooth

Qualitatively, the QLF images correlated closely to what was observed from ICDAS visual examination. The QLF images were often even clearer in depicting areas of slight demineralization than could be seen with the naked eye. (Figure 8)

Quantitatively, static QLF (ΔF) values at baseline, six-months, and twelve-months were compiled. (Graph 4) At six-month recall, the average changes in ΔF were -0.78% (SE 0.38) and 0.36% (SE 0.75) for test and control molars respectively. (Graph 5) The six-month results showed no statistically significant differences ($P>.05$, Student unpaired t-test) in change in ΔF values between test and control molars. At twelve-month recall, the average changes in ΔF values were -1.13% (SE 0.34) and 0.31% (SE 0.38) for test and control molars respectively. (Graph 6) The twelve-month results showed a statistically significant difference in change of ΔF values of test molars compared with control molars ($P=.01$, Student unpaired t-test).

DISCUSSION

Potential Caries inhibition with 9.6μm CO_2 Laser irradiation

This pilot study was first to examine the potential caries preventive effects of short pulsed 9.6μm CO_2 laser irradiation of occlusal pits and fissures in vital teeth *in vivo*. The results of this study were not consistent with the findings of past in vitro occlusal laser studies or in vivo smooth surface laser studies that found significant inhibition of enamel demineralization with CO_2 laser treatment. Featherstone et. al. reported up to

85% reduction in caries-like progression in CO_2 laser treated buccal or lingual smooth surface enamel in vitro using various laser parameters.[26] Zhang et. al. showed similar caries inhibition results on occlusal surfaces in a laboratory model following CO_2 irradiation compared to the Featherstone et. al. study.[36] Rechmann et. al. reported 86% reduction in smooth surface demineralization in vital human teeth in vivo following treatment with 9.6μm short pulsed CO_2 laser irradiation using the same laser used in the present study.[1] Although the same laser parameters markedly inhibited mineral loss in the smooth surface laser study, a similar result was not found in the treatment of pits and fissures in the present study.

One possible explanation for this observed lack of significant inhibition of demineralization may be due to limitations in the design of the laser delivery system. The handpiece provided with this prototype laser delivers the beam straight through the handpiece. The handpiece incorporates lenses that focus the beam and provide the desired spot size and fluence. For the previous smooth surface enamel study this was not a limitation. However, due to the straight nosecone handpiece, it was impossible to achieve perpendicular laser beam delivery to the pits and fissures of second molars in the mouths of 11-17 year old children. (Figure 9)

Furthermore, the irregular topography of occlusal pits and fissures made it difficult to ensure that laser beam irradiation reached the depth of the fissure without being first absorbed by the walls of the fissure, especially with this handpiece. The combination of beam angulation error with irregular surface topography likely decreased the effective fluence (energy/surface area) of the laser at the target sites, thus not achieving carbonate ablation from the enamel surface as anticipated. It is possible

instead of ablating carbonate inclusions, the lowered fluence targeted a different substrate resulting in a more disorganized enamel matrix than the innate carbonated hydroxyapatite.

The smooth surface study did not have the same irradiation geometry limitations as was seen in the present study because the teeth in that study were buccal surfaces of premolars scheduled for orthodontic extraction. Easy access to buccal surfaces of premolars and smooth surface topography allowed for well controlled laser beam delivery perpendicular to the enamel surface. This permitted accurate ablation of carbonate inclusions, leading to a more acid-resistant hydroxyapatite-like enamel.

Study limitations include a small sample size that was calculated based on previously found differences of up to 86% reduction in demineralization. A larger sample size may be able to reflect more accurately the minute differences we were trying to detect without allowing possible outliers to influence our results. The twelve-month study duration may not have been long enough to allow for significant enamel demineralization to occur. A longer study follow-up period may be able to show a larger difference between test and control groups.

QLF vs. ICDAS

Qualitatively, QLF images correlated closely to the ICDAS visual examination in simple visual appearance. QLF was often even more sensitive in detection of subtle areas of enamel demineralization than could be seen by the naked eye. (Figure 8)

Quantitatively, QLF results did not correlate with ICDAS results. QLF results at six-months showed a negative trend with laser irradiated molars displaying more change in fluorescence, supposedly indicating more demineralization. Twelve-month QLF

results showed a statistically significant difference in change in delta F values between laser and control molars with the laser-treated molars exhibiting more negative change in delta F values. This directly contradicts the results seen by ICDAS exam. ICDAS exam at both six- and twelve-months showed a positive trend with laser irradiated molars showing less visual changes in enamel compared to control molars. There is no obvious explanation for this difference, and further examination of the QLF data will be necessary.

Immediate post-laser treatment enamel changes could have accounted for a large part of the loss in fluorescence in laser irradiated molars. The laser treatment may have altered the scattering properties, accounting for the measured loss of fluorescence rather than demineralization. If laser irradiation itself caused a loss of enamel fluorescence, then the apparent longitudinal difference in mineral loss, as was indirectly measured from changes in fluorescence, between test and laser-treated molars may not be the correct interpretation of the numbers. Further, charred byproducts of laser irradiation of plaque trapped in the depths of the fissures would be a confounding factor in the accuracy of immediate post-laser treatment QLF measurements. The charred byproducts would cause scattering, thus resulting in an additional false negative change in mineral loss.

Irregular surface topography of occlusal pits and fissures again pose as a challenge. QLF analysis relies on accurate beam angulation and orientation to capture reflected enamel fluorescence. While slight angulation changes will not significantly alter the fluorescence measurement of smooth surfaces, the same cannot be said of occlusal pits and fissures. A minor angulation change of beam geometry at the occlusal surface can potentially change the area of fluorescence measurement to the walls of the

fissure rather than the depth of the fissure. This is similar to the irradiation geometry limitations seen with CO_2 laser irradiation.

CONCLUSION

Although CO_2 laser irradiation markedly inhibited caries progression in the Rechmann et al. smooth surface laser study, the same result was not found thus far in the treatment of occlusal pits and fissures likely due to irradiation geometry limitations. Restrictions in access and beam angle probably led to the decrease in energy delivered. Further study is needed on occlusal pit and fissure caries inhibition using a contra-angle CO_2 laser handpiece to determine whether or not this laser irradiation can inhibit caries progression in these surfaces.

While QLF has been proven to be an effective means in measuring early mineral loss for smooth surfaces in previous studies, further study is needed to determine QLF effectiveness and validity on occlusal pits and fissures, especially when laser irradiation is involved.

TABLE 1: ICDAS Occlusal Protocol Codes 0-3

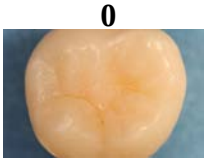
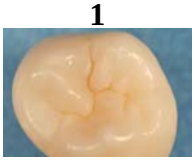
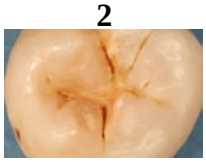
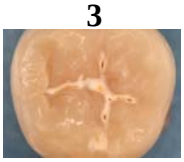
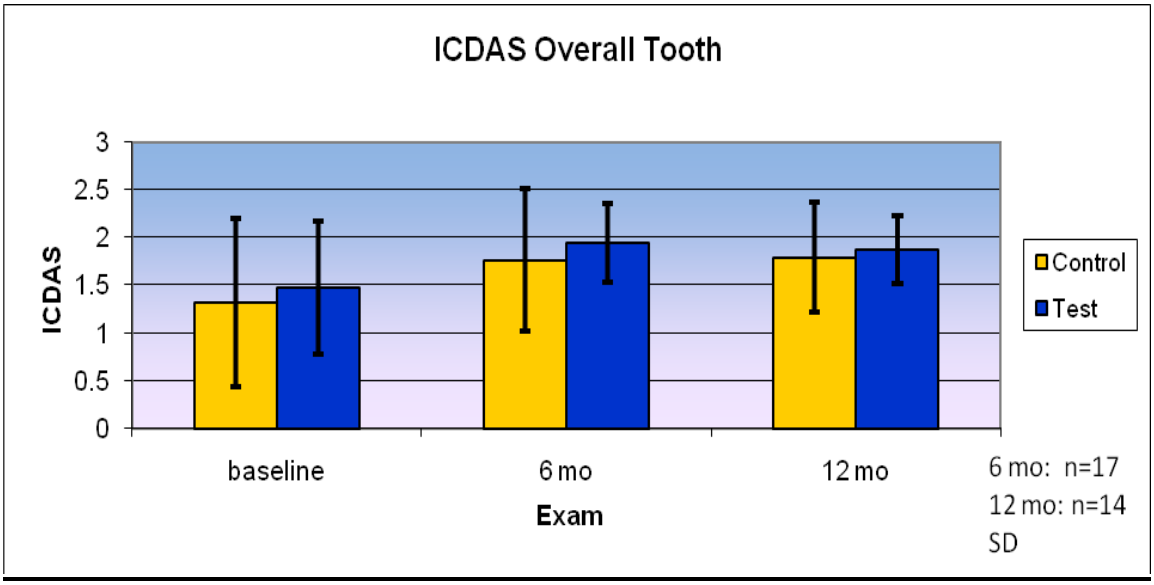
ICDAS code	**0** 	**1** 	**2** 	**3** 
Definitions	Sound tooth surface; no caries change after air drying (5 sec); or hypoplasia, wear, erosion and other non-caries phenomena.	First visual change in enamel; seen only after air drying, or colored change "thin" limited to the confines of the pit and fissure 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.
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.
Sealant/restoration Recommedation 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**
Sealant/restoration Recommedation 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**
Sealant/restoration Recommedation 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**
Sealant/restoration Recommedation 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**

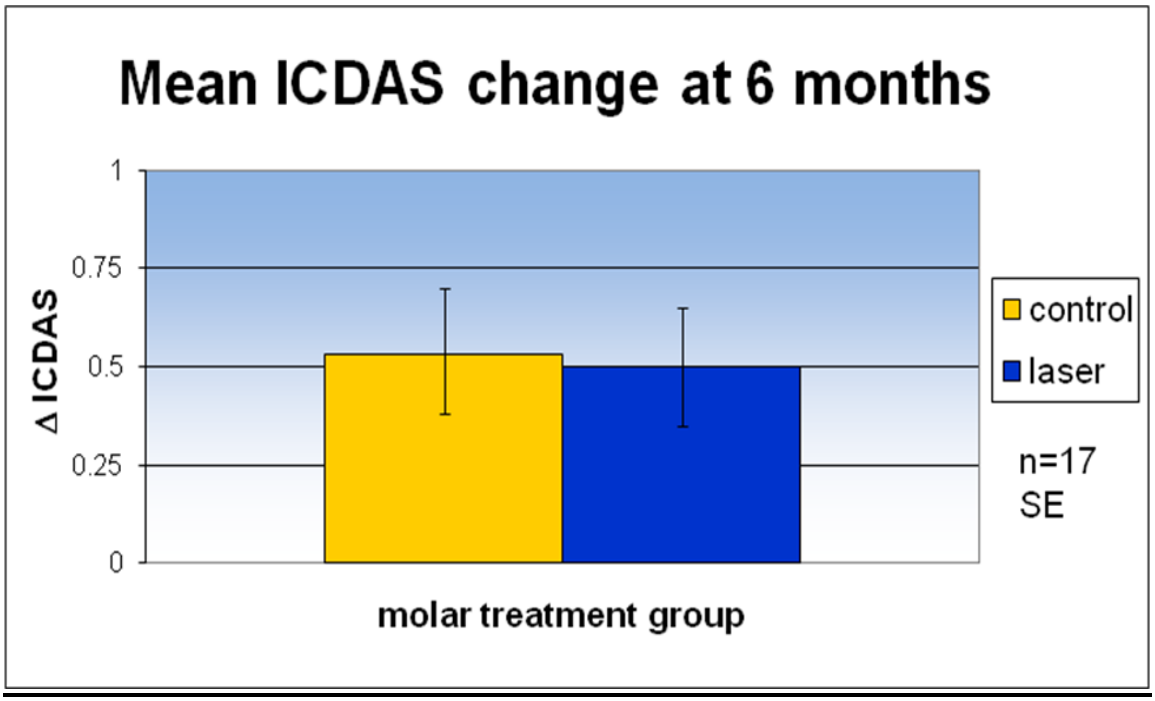
TABLE 2: Laser irradiation time per tooth

ID	Tooth #	Irradiation time (sec)
LS01	31	60
LS02	15	55
LS04	2	30
LS06	18	49
LS07	31	77
LS08	15	63
LS09	2	56
LS10	2	35
LS11	15	56
LS12	15	56
LS13	15	56
LS14	31	60
LS15	15	50
LS17	2	55
LS18	31	140
LS19	2	70
LS20	15	60
LS21	31	80

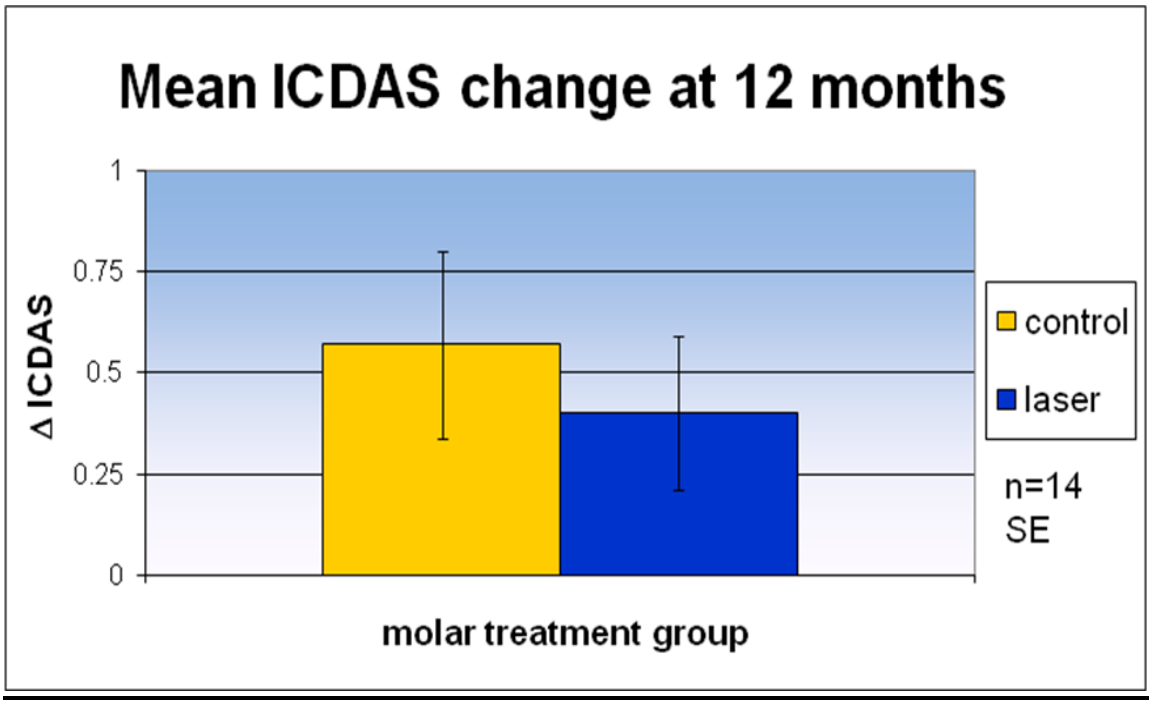
GRAPH 1: Static ICDAS Scores for Overall Tooth at Baseline, 6-mo., 12-mo.



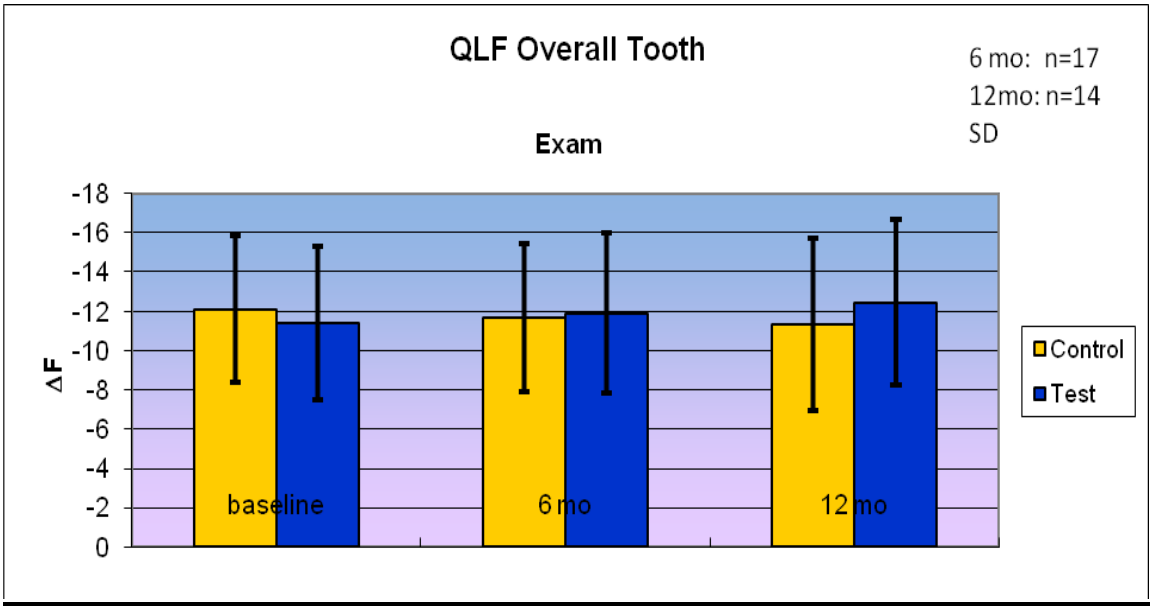
GRAPH 2: Mean change in ICDAS scores for Overall Tooth at 6-month recall



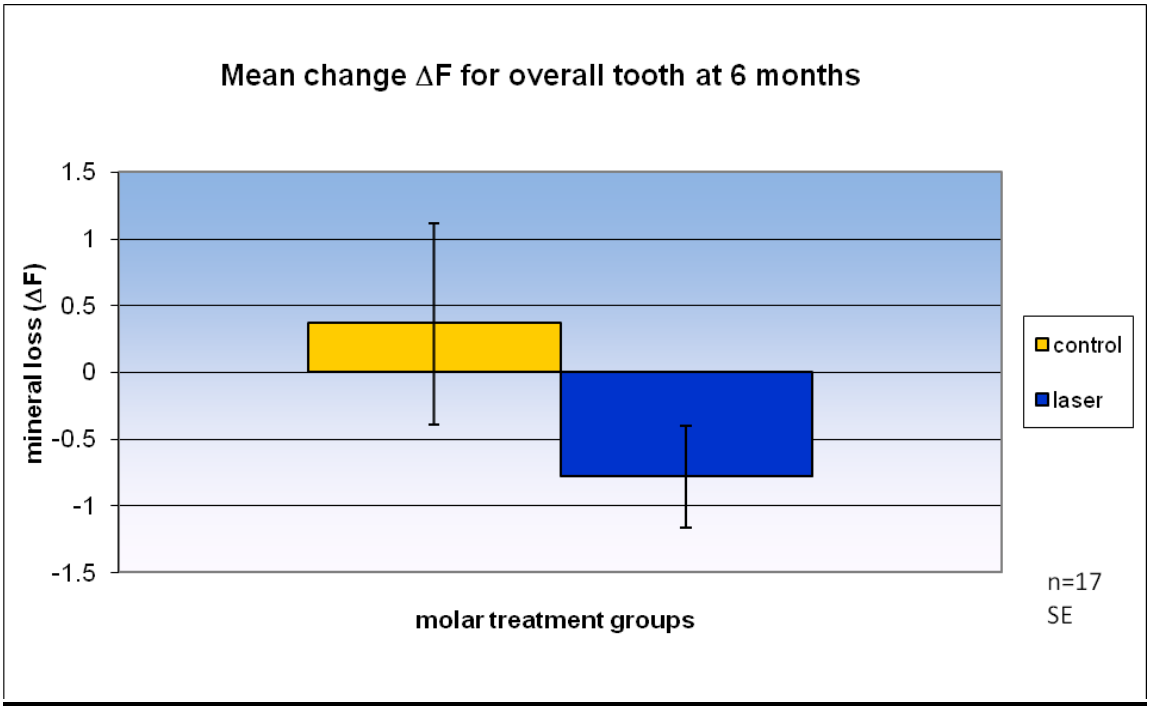
GRAPH 3: Mean change in ICDAS scores for Overall Tooth at 12-month recall



GRAPH 4: Static QLF (ΔF) for Overall Tooth at Baseline, 6-mo., 12-mo.



GRAPH 5: Mean change in QLF (ΔF) for Overall Tooth at 6-mo.



GRAPH 6: Mean change in QLF (ΔF) for Overall Tooth at 12-mo.

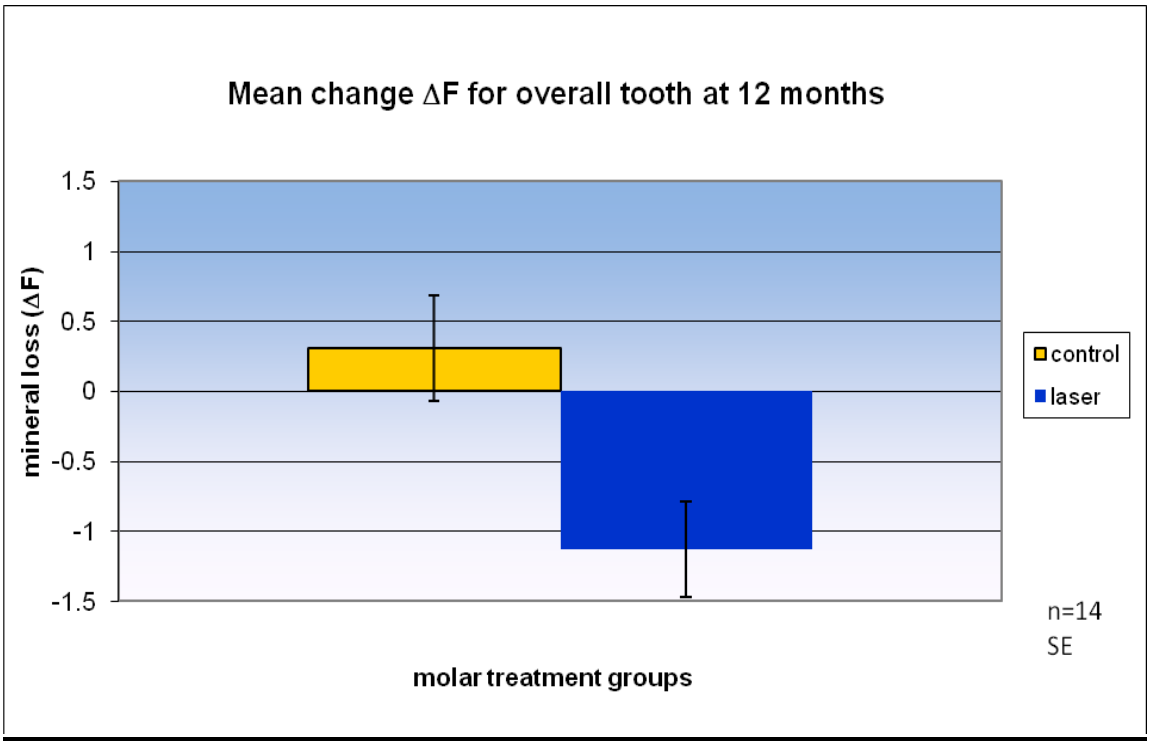


FIGURE 1: Clinical Protocol

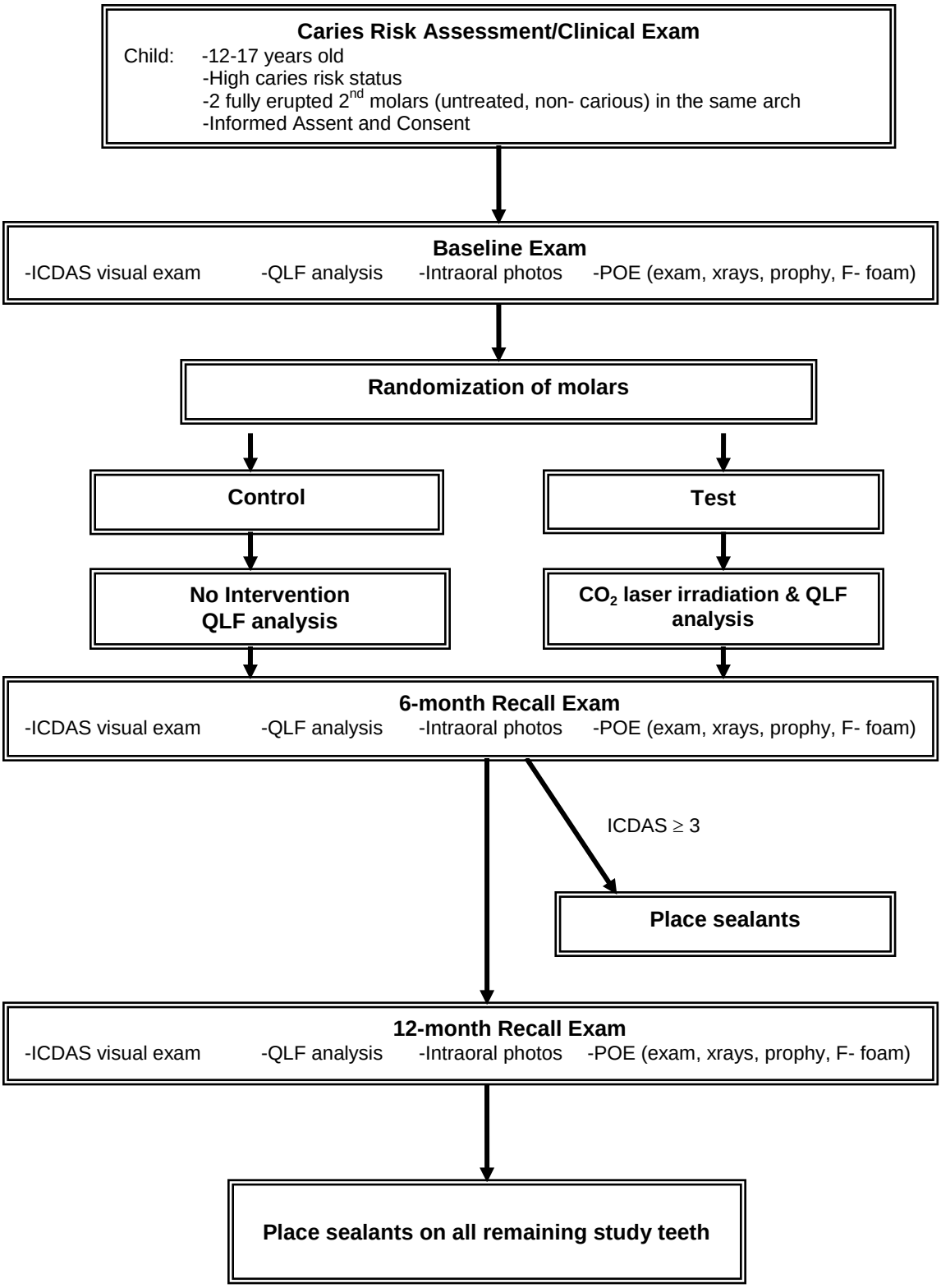
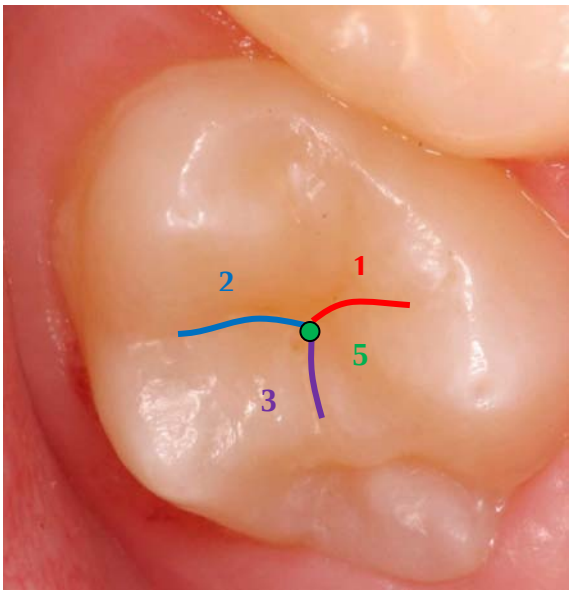
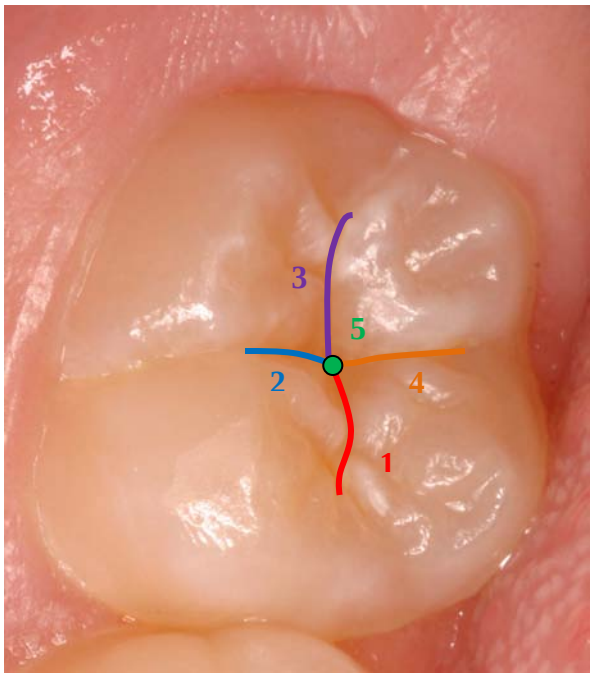


FIGURE 2: ICDAS & QLF Maxillary Tooth Regions



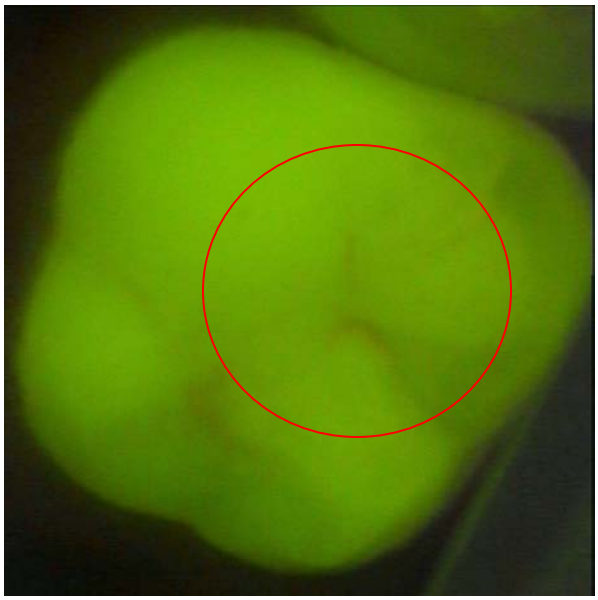
For maxillary molars, regions were assigned based on primary groove locations.
1= Mesial, 2=Buccal, 3=Distal, 5=Central pit

FIGURE 3: ICDAS & QLF Mandibular Tooth Regions



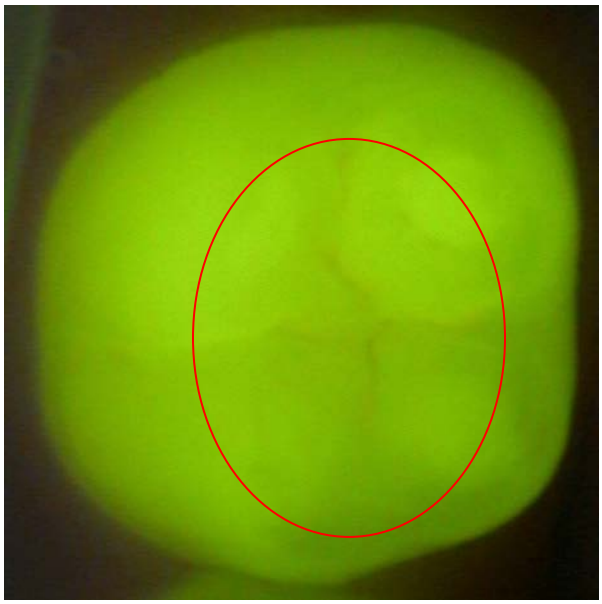
For mandibular molars, regions were assigned based on primary groove locations.
1= Mesial, 2=Buccal, 3=Distal, 4=Lingual, 5=Central pit

FIGURE 4: QLF analysis area of maxillary molars



For maxillary molars, the area of interest was confined to the occlusal area mesial to the transverse ridge.

FIGURE 5: QLF analysis area of mandibular molars



For mandibular molars, the area of interest was the entire occlusal surface, encompassing all primary occlusal grooves.

FIGURE 6: CO_2 Laser with straight handpiece

CO_2 laser, Pulse Systems, Inc. (PSI) (Model #LPS-500, Los Alamos, New Mexico)[37]

FIGURE 7: QLF Inspektor Pro

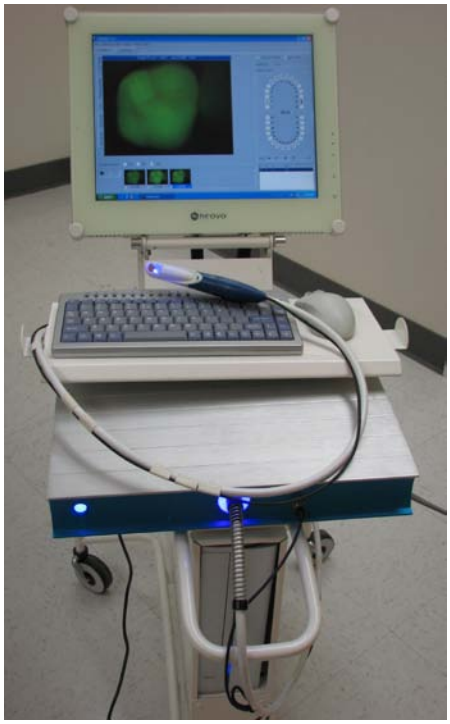
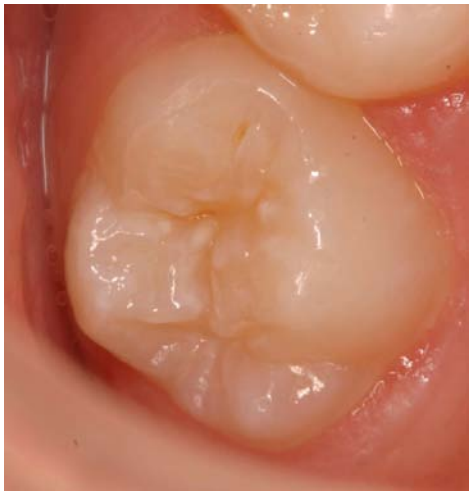
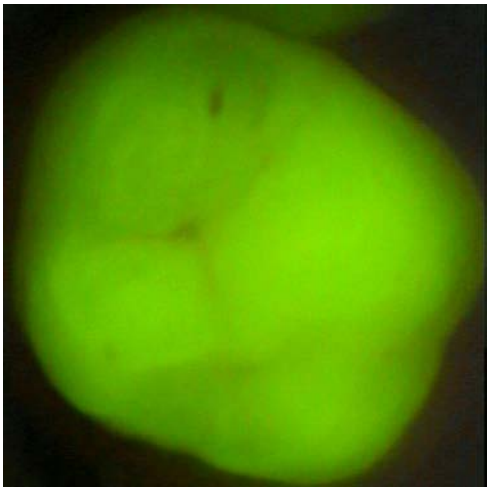


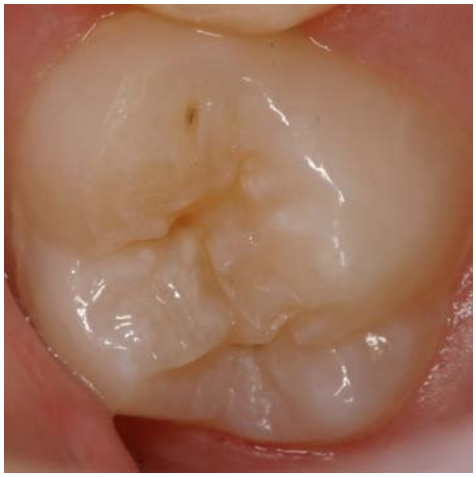
FIGURE 8: Qualitative QLF Results



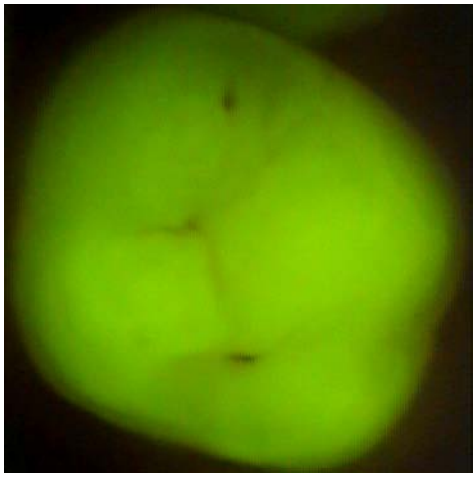
LS11 #2 ICDAS at baseline



LS11 #2 QLF at baseline

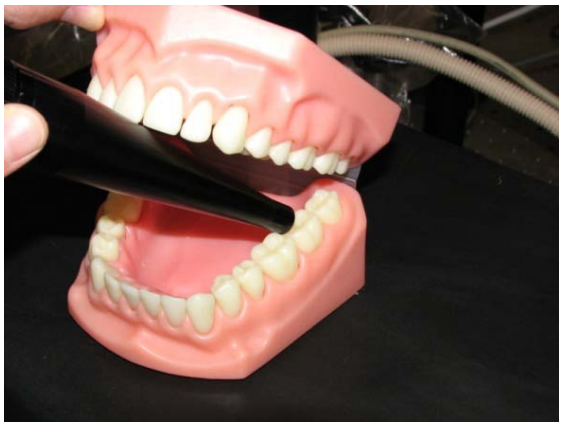


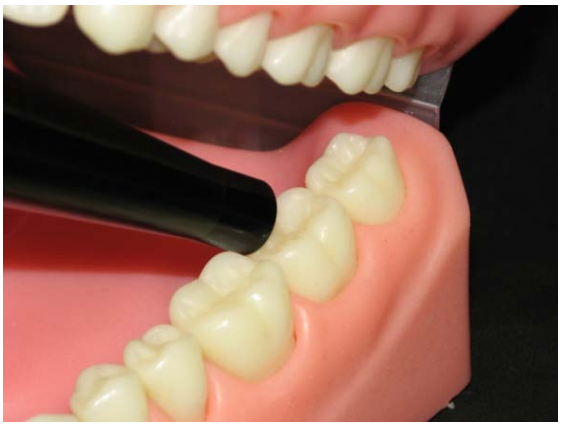
LS11 #2 ICDAS at 6-month



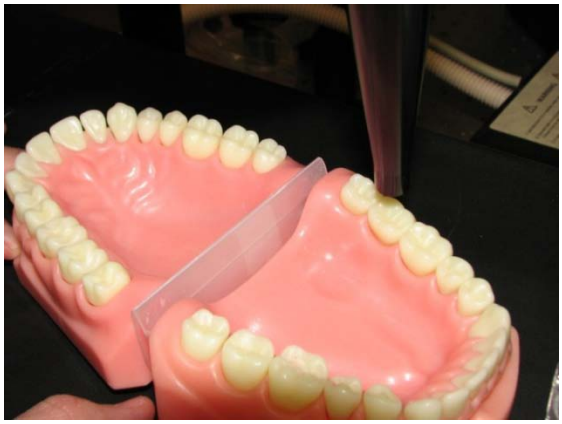
LS11 #2 QLF at 6-month

Figure 9: Irradiation Geometry Limitations





Due to the straight nosecone handpiece, it was impossible to achieve perpendicular laser beam delivery to the pits and fissures of second molars in the mouths of 11-17 year old children.



Patients would need to open 180° to allow perpendicular laser beam delivery to the pits and fissures of second molars.

REFERENCES

1. Rechmann P, Fried, D., Le, C.Q., Nelson, G., Rapozo-Hilo, M., Rechmann, B.M.T., Featherstone, J.D. Inhibition of caries in vital teeth by CO2 laser treatment. San Francisco: University of California San Francisco; 2008. p. 7.
2. Mouradian WE, Wehr E, Crall JJ. Disparities in children's oral health and access to dental care. JAMA 2000;284(20):2625-31.
3. Service UPH. Healthy People 2000 Progress Report on Oral Health. In: Services UDoHaH, editor. Washington D.C.; 1995.
4. Research NIoD. Oral Health of the United States Children: The National Survey of Dental Caries in School Children, 1986-86; National and Regional Findings. In: Research NIoD, editor. Bethesda, MD: DHHS; 1989. p. 89-2247.
5. Feigal RJ, Donly KJ. The use of pit and fissure sealants. Pediatr Dent 2006;28(2):143-50; discussion 92-8.
6. Featherstone JD. Caries detection and prevention with laser energy. Dent Clin North Am 2000;44(4):955-69, ix.
7. Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc 2000;131(7):887-99.
8. Lussi A. Comparison of different methods for the diagnosis of fissure caries without cavitation. Caries Res 1993;27(5):409-16.
9. Pitts NB. Diagnostic tools and measurements--impact on appropriate care. Community Dent Oral Epidemiol 1997;25(1):24-35.
10. ten Cate J, van Amerongen JP Caries Diagnosis: Conventional Methods. Paper presented at: Early Detection of dental caries, 1996; Indianapolis: Indiana University.
11. Lussi A. Validity of diagnostic and treatment decisions of fissure caries. Caries Res 1991;25(4):296-303.
12. Committee ICDaASC. International Caries Detection & Assessment System; 2006.
13. Lussi A, Hibst R, Paulus R. DIAGNOdent: an optical method for caries detection. J Dent Res 2004;83 Spec No C:C80-3.
14. Jones RS. Near Infrared optical imaging of early dental caries [San Francisco: University of California San Francisco; 2006.
15. Lennon AM, Buchalla W, Brune L, Zimmermann O, Gross U, Attin T. The ability of selected oral microorganisms to emit red fluorescence. Caries Res 2006;40(1):2-5.
16. Shi XQ, Welander U, Angmar-Mansson B. Occlusal caries detection with KaVo DIAGNOdent and radiography: an in vitro comparison. Caries Res 2000;34(2):151-8.
17. Stookey GK. Quantitative light fluorescence: a technology for early monitoring of the caries process. Dent Clin North Am 2005;49(4):753-70, vi.
18. Tranaeus S, Shi XQ, Angmar-Mansson B. Caries risk assessment: methods available to clinicians for caries detection. Community Dent Oral Epidemiol 2005;33(4):265-73.

19. Kuhnisch J, Ifland S, Tranaeus S, Angmar-Mansson B, Hickel R, Stosser L, et al. Establishing quantitative light-induced fluorescence cut-offs for the detection of occlusal dentine lesions. Eur J Oral Sci 2006;114(6):483-8.
20. CDC. Recommendations for Using Fluoride to Prevent and Control Dental Caries in the United States. Morbidity and Mortality Weekly Report 2001;50(RR-14):1-42.
21. Pedodontics ASoDfCaAAo. Rationale and guidelines for pit and fissure sealants. Pediatric Dentistry 1983;5(1):89-90.
22. Beauchamp J, Caufield PW, Crall JJ, Donly K, Feigal R, Gooch B, et al. Evidence-based clinical recommendations for the use of pit-and-fissure sealants: a report of the American Dental Association Council on Scientific Affairs. J Am Dent Assoc 2008;139(3):257-68.
23. Feigal RJ. Sealants and preventive restorations: review of effectiveness and clinical changes for improvement. Pediatr Dent 1998;20(2):85-92.
24. Wigdor HA, Walsh JT, Jr., Featherstone JD, Visuri SR, Fried D, Waldvogel JL. Lasers in dentistry. Lasers Surg Med 1995;16(2):103-33.
25. Fried D, Ragadio J, Akrivou M, Featherstone JD, Murray MW, Dickenson KM. Dental hard tissue modification and removal using sealed transverse excited atmospheric-pressure lasers operating at lambda=9.6 and 10.6 microm. J Biomed Opt 2001;6(2):231-8.
26. Featherstone JD, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO2 laser inhibitor of artificial caries-like lesion progression in dental enamel. J Dent Res 1998;77(6):1397-403.
27. Kantorowitz Z, Featherstone JD, Fried D. Caries prevention by CO2 laser treatment: dependency on the number of pulses used. J Am Dent Assoc 1998;129(5):585-91.
28. Goodis HE, Fried D, Gansky S, Rechmann P, Featherstone JD. Pulpal safety of 9.6 microm TEA CO2 laser used for caries prevention. Lasers Surg Med 2004;35(2):104-10.
29. Rodrigues LK, Nobre Dos Santos M, Featherstone JD. In situ mineral loss inhibition by CO2 laser and fluoride. J Dent Res 2006;85(7):617-21.
30. Al-Ansari JM, Al-Jairan LY, Gillespie GM. Dietary habits of the primary to secondary school population and implications for oral health. J Allied Health 2006;35(2):75-80.
31. CDC. Guidelines for School Health Programs to Promote Lifelong Healthy Eating. Morbidity and Mortality Weekly Report 1996;45(RR-9):1-33.
32. Featherstone JD, Domejean-Orliaguet S, Jenson L, Wolff M, Young DA. Caries risk assessment in practice for age 6 through adult. J Calif Dent Assoc 2007;35(10):703-7, 10-3.
33. Young DA, Featherstone JD, Roth JR, Anderson M, Autio-Gold J, Christensen GJ, et al. Caries management by risk assessment: implementation guidelines. J Calif Dent Assoc 2007;35(11):799-805.
34. Vanderas AP, Manetas C, Koulatzidou M, Papagiannoulis L. Progression of proximal caries in the mixed dentition: a 4-year prospective study. Pediatr Dent 2003;25(3):229-34.

35. Pereira AC, Eggertsson H, Martinez-Mier EA, Mialhe FL, Eckert GJ, Zero DT. Validity of caries detection on occlusal surfaces and treatment decisions based on results from multiple caries-detection methods. Eur J Oral Sci 2009;117(1):51-7.
36. Zhang S FJ, Shariati M, McCormack SM. Treatment of tooth enamel fissures by combined CO2 laser/fluoride. Paper presented at: Proceedings of the Third International Congress on Lasers in Dentistry, 1993; Salt Lake City, UT.
37. Pulse Systems I. Pulsed CO2 Lasers. Los Alamos, NM; 2009.

Appendix 1: CHR Application

UCSF COMMITTEE ON HUMAN RESEARCH

Please date form:
_08/03/07_

FULL COMMITTEE REVIEW APPLICATION

General Instructions | Submission Checklist

Street Address:
Committee on Human Research (CHR)
Office of Research
3333 California Street, Suite 315
University of California
San Francisco, CA 94118

Campus Mailbox:
CHR
Box 0962

Office Contact for questions:
Office: (415) 476-1814
Facsimile: (415) 502-1347
e-mail: chr@ucsf.edu

PART 1: ADMINISTRATIVE REQUIREMENTS

- Eligibility requirements for Principal Investigator, Co-Principal Investigator and Contact Person
- Training requirements

A. Principal Investigator:		
Name and degree John D.B. Featherstone, MSc., Ph.D.	University Title Interim Dean	Department School of Dentistry
Campus Mailing Address (Box No.) Box 0758	Phone Number 415-476-0456	E-mail Address jdbf@ucsf.edu
Co-Principal Investigator:		
Name and degree Tiffany H. Hsu, DDS	University Title Resident	Department Orofacial Sciences
Campus Mailing Address (Box No.) Box 0753	Phone Number 925-768-1679	E-mail Address tiffhsu.dds@gmail.com
Additional Contact Person (if any):		
Name Peter Rechmann, DDS, PhD	University Title Professor	Department PRDS
Campus Mailing Address (Box No.) Box 0758	Phone Number 415-514-3225	E-mail Address Rechmannp@dentistry.ucsf.edu
Send correspondence to (check *one*):	[ ]PI only	[X]PI and Co-PI [ ]PI and Additional Contact Person

Study Title:	Application Type:
In vivo occlusal caries prevention by pulsed CO_2 laser treatment quantified by QLF	[ ]New Full Committee Application [ ]Response to "Contingent" or "Return" letter [X]Modification [ ]Renewal Current CHR #: _H9136-30702-01_ Expiration date: _05/03/08_

UCSF Sites (Check all that apply):

[X]UCSF [ ]Cancer Center [ ]PCRC [ ]GCRC (Moffitt/Mt. Zion) [ ]GCRC (SFGH) [ ]SFGH [ ]ITN [ ]Fresno

UCSF Affiliated Sites (Check all that apply):

[ ]VAMC [ ]Gladstone [ ]Gallo [ ]SFDPH [ ]IOA [ ]BSRI [ ]BCP

Non-UCSF Affiliated Sites - Attach IRB Approval Certification Supplement for all items checked below:

[ ] Other UC Campus (please identify): [ ]Foreign Country [ ]Other

B. Funding: If this study is eligible for "Just in Time" NIH review, do not submit your application to the CHR until you have received notification from the federal granting agency that your study appears to be in a fundable range. If this study is federally funded please complete section B.6.

Check all that apply:

1. Type of funding:	2. Source of funding:	3. Funds will be awarded to/through:
[X]Contract/Grant [ ]Subcontract [ ]Drug/device donation [ ]Departmental [ ]Gift [X]Student project [ ]Other: __ Have funds been awarded? [ ]Yes [X]Pending [ ]No Award No.: __ **Proposal Express** number(s): __	[ ]Federal Government [ ]Other Gov. (e.g., State, local) [ ]Industry [ ]Other Private [ ]Campus/UC-Wide program [X]Departmental Funds [ ]Other: **Specify name of source designated above: __**	Dept./ORU: *Institution* — *Federal Wide Assurance (FWA) No.* [X]UCSF ..... 00000068 [ ]Blood Centers of the Pacific ..... 00002111 [ ]Blood Systems Research Institute ..... 00006454 [ ]Gallo Institute ..... 00000304 [ ]Gladstone Institute ..... 00000087 [ ]Institute on Aging ..... 00002525 [ ]NCIRE ..... 00000256 [ ]S.F. Dept. of Public Health ..... 00000162 [ ] SFVAMC Research Office 00000280

4. UCSF (or affiliate) financial contact person for IRB review recharge:	
5. Grant Title and PI (if different from above):	

6. CHR Protocol/Federal Grant or Contract Comparison (New CHR Studies Only)

If this study is federally funded, please submit one copy of one of the following documents (unless there is more than one grant or contract involved; in that case, submit one copy for each associated grant or contract). Please indicate which document you have attached:

[ ] The Research Plan, including the Human Subjects, Section E of your NIH grant

[ ] For other federal proposals (contracts or grants), the section of the proposal describing human subjects work, or

[ ] The section of your progress report if it provides the most current information about your human subjects work.
7. If there are any significant discrepancies between this CHR application and the grant or contract or if this is a training grant please explain here:
8. Secondary sponsors: If there are multiple sources of funding for this study, please describe the additional funding:

C. Scientific Merit Review: This study has received or will receive scientific merit review from (check all that apply):
[ ]NIH [ ]Cancer Center* [ ]GCRC or PCRC [ ]SFVAMC [ ]Dept. Review
*Required prior to final CHR approval for oncology studies.

D. Key Personnel: *All key personnel including the PI and Co-PI must be listed below along with a brief statement of their qualifications and study role(s).* If the SF VAMC is a study site*, please identify the principal VAMC investigator, unless already listed as PI or Co-PI above. For questions regarding the VAMC application process, please contact the VA Clinical Research Office at 221-4810 ext.4655.* **Please note:** *All Key Personnel at UCSF or UCSF affiliated sites must complete the online UCSF Human Subject Protections Training program (https://www.researchonline.ucsf.edu/).*

Investigators and other personnel [and institution(s)]:	*Qualifications:*	*Study role(s):*

Dr. John D. Featherstone	Dr. Featherstone is a Professor in the Department of Preventive and Restorative Dental Sciences at UCSF. He is internationally recognized for studies relating to dental caries prevention and risk assessment. He has been PI or co-investigator on numerous NIH-funded grants	P.I.
Dr. Tiffany H. Hsu	Dr. Hsu is a first year pediatric dental resident. She will be responsible for the overall clinical aspects of this study.	Co-P.I.
Dr. Jane A. Weintraub	Dr. Weintraub is Professor and Chair of the Division of Oral Epidemiology and Dental Public Health. She has been PI or co-investigator on numerous NIH-funded grants.	
Dr. Peter Rechmann	Dr. Rechmann is an experienced clinical scientist and dental laser researcher in the PRDS department. He will supervise the clinical laser aspects of the study.	
Dr. Brent Lin	Dr. Lin is a Clinical Associate Professor in the Division of Pediatric Dentistry and the Director of Pre-doctoral Pediatric Dentistry. He has been involved in several clinical studies and has extensive experience in pediatric dentistry. He will work closely with Dr. Hsu on dental examinations and research subjects.	
Dr. Stuart Gansky	Dr. Gansky is an Associate Professor in the Division of Oral Epidemiology in the Dept. of PRDS. He is a biostatistician with metholodological research in health study design and statistical analysis and with applied research in dentistry, public health, health services, medicine, and pharmaceuticals.	

E. Statement of Financial Interest: Does the PI or any investigator have any financial interests related to this clinical study? **If Yes, Attach Disclosure Of Investigators' Financial Interests Supplement**	[ ]Yes [X]No

F. Drugs, Devices and Biologics:		
List any investigational drugs, biologics and IND Numbers:	Name	IND #
	Refer to non-significant risk determination in attachment	
List any investigational devices and IDE Numbers:	Name	IDE#
	Pulse Systems (NM) 9.6 μm carbon dioxide clinical laser as currently in use in project CHR approval H9136-25290-03 "Laser Effects on Dental Hard Tissue"	
	[X]Non-Significant Risk Determination Requested **Attach - NSR Supplement**	
Who holds the IND/IDE?	[ ]Sponsor [ ]Investigator	
Are investigational drugs or biologics controlled by a pharmacy?	[ ]Yes [X]No If "Yes," identify the pharmacy/ies:	

Are investigational drugs, devices, or biologics (test articles) controlled by the Principal Investigator? (See HRPP IND and IDE Guidance for information regarding test article control)	[ ]Yes [X]No If "Yes," describe your plan for control of the test article:
Are investigational drugs, devices, or biologics prepared or manufactured in UCSF research labs?	[ ]Yes [X]No If "Yes," identify the lab:
List any approved drugs, biologics and/or devices being *studied*:	

G. Other Approvals/Regulated Materials: Does this study require approval or authorization from any of the following regulatory committees, or involve the use of the regulated materials listed below? Follow the hyperlinks for more information. If "Yes," complete the applicable section(s) below.	[ ]Yes [X]No		
[ ]	Biological Safety Committee		BUA #:
	[ ]	Human Gene Transfer/Recombinant DNA Research	**Attach -**

			Human Gene Transfer / Recombinant DNA Research Supplement
[ ]	Institutional Animal Care and Use Committee		IACUC #:
	[ ]	Xenotransplantation	
[ ]	Controlled Substances		
[ ]	Human Stem Cells		**Attach - Human Stem Cell Supplement**
[ ]	Radiation Safety Committee		RUA #:

H. Principal Investigator's Certification:

- I certify that the information provided in this application is complete and correct.
- I accept ultimate responsibility for the conduct of this study, the ethical performance of the project, and the protection of the rights and welfare of the human subjects who are directly or indirectly involved in this project.
- I will comply with all policies and guidelines of UCSF and affiliated institutions where this study will be conducted, as well as with all applicable federal, state and local laws regarding the protection of human subjects in research.
- I will ensure that personnel performing this study are qualified, appropriately trained and will adhere to the provisions of the CHR-approved protocol.
- I will not modify this CHR-certified protocol or any attached materials without first obtaining CHR approval for an amendment to the previously approved protocol.
- I assure that the protected health information requested, if any, is the minimum necessary to meet the research objectives.
- I assure that the protected health information I obtain, if any, as part of this research will not be reused or disclosed to any parties other than those described in the CHR-approved protocol, except as required by law.
- I assure that adequate resources to protect participants (i.e., personnel, funding, time, equipment and space) are in place *before* implementing the research project, and that the research will *stop* if adequate resources become unavailable.

Principal Investigator's Signature _____ Date _____

PART 2: STUDY DESIGN

Complete items A-E using clear, concise, non-technical, lay language (i.e., the type of language used in a newspaper article for the general public) wherever possible. Define all acronyms. Use caution when cutting and pasting from another application or protocol to ensure that information is complete, supplemented where necessary, is pasted in a logical order, and is relevant to the specific section.

Space limits are recommendations and should be adjusted as needed, but the total length for sections A-E should not exceed 5 pages.

For modifications and renewals, please highlight in *italics* all changes from previously approved version.

A. Synopsis (Briefly summarize the study.)

The aim of the study is to demonstrate for the first time in vivo, that caries inhibition in occlusal pits and fissures can be achieved by the use of specific carbon dioxide (CO_2) laser irradiation (wavelength 9.6 µm). This is a pilot study, which, if successful, will lead to a larger clinical trial. To achieve this aim, occlusal pits and fissures of the test molar will be irradiated according to a protocol established in Dr. Featherstone's published laboratory studies, the other molar will serve as control. Subjects will be asked to return for 6-month and 12-month recalls, where the occlusal surface will be examined by ICDAS visual inspection and Quantitative Light-Induced Fluorescence (QLF). This model allows us to measure caries inhibition around the occlusal pits and fissures by determining the ΔZ value (relative loss of mineral = "caries") in vivo. We know that the occlusal pits and fissures of the teeth (biting surface) are at high risk for demineralization and that the use of typical fluoride toothpaste will not totally prevent the loss of mineral in this surface. It is anticipated that the laser irradiation will alter the enamel near the tooth surface and make it chemically resistant to acid thereby inhibiting mineral loss. We will enroll 20 subjects for this pilot study. Subjects who are patients in the UCSF Pre-doctoral and Postgraduate pediatric dental clinics will be invited to participate.

B. Purpose (Specify the hypotheses, aims and/or objectives.)

The purpose of this study is to conduct a one-year pilot scale clinical study of caries inhibition in teeth in humans on occlusal pit and fissure surfaces following CO_2 laser irradiation (wavelength 9.6µm) and quantifying early detection of occlusal enamel demineralization by Quantitative Light-induced Fluorescence.

The hypothesis to be tested is that specific wavelength carbon dioxide (CO_2) laser treatment to occlusal tooth surfaces results in changes in enamel crystal composition and structure which increase the resistance of dental mineral to dissolution by acid. We expect this process to prevent dental caries progression in the occlusal surface of vital teeth *in vivo* by decreasing the amount of demineralization.

Aim 1: To compare the relative mineral loss (ΔZ) in the occlusal pits and fissures of untreated molars with relative mineral loss (ΔZ) in the occlusal pits and fissures of laser treated molars.
Aim 2: To compare the efficacy of early caries detection in occlusal surfaces by 1) ICDAS visual inspection and 2) Quantitative light-induced fluorescence (QLF).

C. Background (Summarize previous studies. Explain the rationale for the proposed investigation and if applicable how this research differs from local standard of care.)

Caries Prevalence in US children

Dental caries continues to be a problem for our nation's school-aged children. It is the most common chronic childhood disease and is the most prevalent unmet health need of children in the United States.[1] More than 50% of children have detectable caries by mid-childhood [2] and approximately 80% of late adolescents have dental caries.[3] This preventable infectious disease poses significant medical and financial consequences. In its earliest stages, dental caries is a reversible process via remineralization. One of the most effective means of caries prevention is water fluoridation.[1] However, the effect of topical fluoride preferentially targets smooth surfaces and does not protect the occlusal pits and fissures as successfully.[4] This leads to a significant problem as occlusal pit and fissure caries account for over 80% of all caries in young permanent teeth.[5] Because pits and fissures are the earliest and most prevalent surfaces for decay, new methods of caries prevention focus on these susceptible areas.[5]

Methods of occlusal caries prevention

Historically, many creative approaches have been taken in attempts to reduce the risk of occlusal caries.[5] Currently, the most effective way to prevent occlusal caries is the use of dental sealants.[4] Sealants are polymers that are applied to the occlusal surface of the tooth, adhering to it, and providing a barrier against bacterial acids that dissolve the enamel and dentin during the dental caries process. However, according to Feigal "even under the best of circumstances, sealants fail." [6] The 5-10% per year failure rate of sealants account for the need for meticulous recall and maintenance procedures which are vital for long term success.[6] Since the ideal solution for pit and fissure caries prevention has yet to be discovered, creative efforts against pit and fissure caries continue as new technology and materials are developed each year.

One novel approach to occlusal pit and fissure caries prevention is the treatment with a carbon dioxide (CO_2) dental laser with specifically designed parameters of wavelength, pulse duration, repletion rate and fluence (energy/surface area). To safely heat dental enamel to alter its composition and solubility, laser light must be strongly absorbed and efficiently converted to heat without damaging the underlying and surrounding tissues.[7] Enamel, dentin and cementum are composed of carbonated hydroxyapatite that has high absorption bands in the infrared radiation (IR) region of 9.0-11.0μm.[8]

When considering caries prevention, focus is placed on altering the solubility characteristics of enamel, which has an extremely high absorption coefficient at 9.6μm.[7] Thus, low-energy, pulsed radiation CO_2 lasers operating at the wavelength 9.6μm are efficiently absorbed within the outer 1μm surface region and have been shown to be the system of choice in preventing demineralization in laboratory experiments.[7,9]

Previous laboratory, in situ and in vivo safety studies

When pulsed CO_2 laser irradiation interacts with the phosphate groups in enamel, it is absorbed and transformed into heat that can briefly raise temperatures to greater than 400°C, which are capable of driving off carbonate from the carbonated apatite mineral of the tooth.[7,9] This leaves a hydroxyapatite-like mineral that is less soluble than carbonated apatite.[7] The pulse duration is so short and the absorption coefficient so high that the heat is essentially confined to the outer surface of the tooth and the transfer to the pulp is minimal. Studies in our laboratories have shown that the CO_2 laser treatment of enamel can inhibit caries-like progression by up to 85% in a laboratory setting.[10, 11]

In a safety study in humans, which enrolled only adult subjects, Goodis et al. showed that "the 9.6μm wavelength laser, with irradiation conditions comparable to those proposed in the present study, causes no permanent/serious pulpal damage at the energy levels used and can be used safely for caries prevention treatments in humans."[12]

A recent study by Rodrigues et al. showed that CO_2 laser treatment with 9.6μm wavelength and 5μs pulse duration can inhibit enamel mineral loss in an *in situ* high caries challenge situation on smooth surfaces of enamel in the mouth.[13] Enamel samples, cut from extracted teeth, were irradiated externally with laser conditions comparable to those proposed in the present study. The irradiated and control (non-irradiated) slabs were worn in the mouths of subjects in a "caries challenge" environment and the amount of demineralization measured after the test period. These very promising results are expected to be found in the present study in the occlusal surfaces of vital teeth.

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.[14] 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.[7] By the time an occlusal caries lesion is detectable radiographically, it is too large to be remineralized.[7] 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 inhibit the caries process.[7]

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.[15] ICDAS assigns scores to lesions in occlusal surfaces based on apparent caries status and lesion severity of plaque-free teeth with the use of a ball-ended probe.[15] Of particular interest to this study are the coronal caries detection criteria.[15]

Laser fluorescence is a new method for early caries detection. By analyzing the 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.[7] When compared to conventional visual examination or other caries detection instruments, QLF can detect twice as many precavitated demineralized enamel areas.[17] Its ability to detect and quantify changes in clinically visible white spot lesions allows QLF to determine the impact of preventative measures on inhibition of demineralization.[17] QLF has been proven to be an effective method of detecting smooth surface demineralization,[7] and a recent in vitro study demonstrated that it can be used for occlusal surfaces.[18]

Conclusion

In conclusion, high caries prevalence especially in occlusal pits and fissures warrants novel caries prevention methods. Sealants are effective in caries prevention but have limited lifespan and require multiple repairs. CO_2 lasers with the correct wavelength (9.3 or 9.6 μm) and pulse characteristics (pulse duration 2-100 μs) can offer a novel alternative for early caries prevention in pits and fissures. The use of an appropriate CO_2 laser in combination with QLF caries detection and caries risk assessment is potentially an ideal method of caries prevention in the future.

D. Design

1. (Check all that apply):

[X]Phase I [ ]Phase II [ ]Phase III [ ]Phase IV [X]Randomized [X]Blinded

[ ]Open Label Extension: If so, specify CHR Approval Number for original study: __

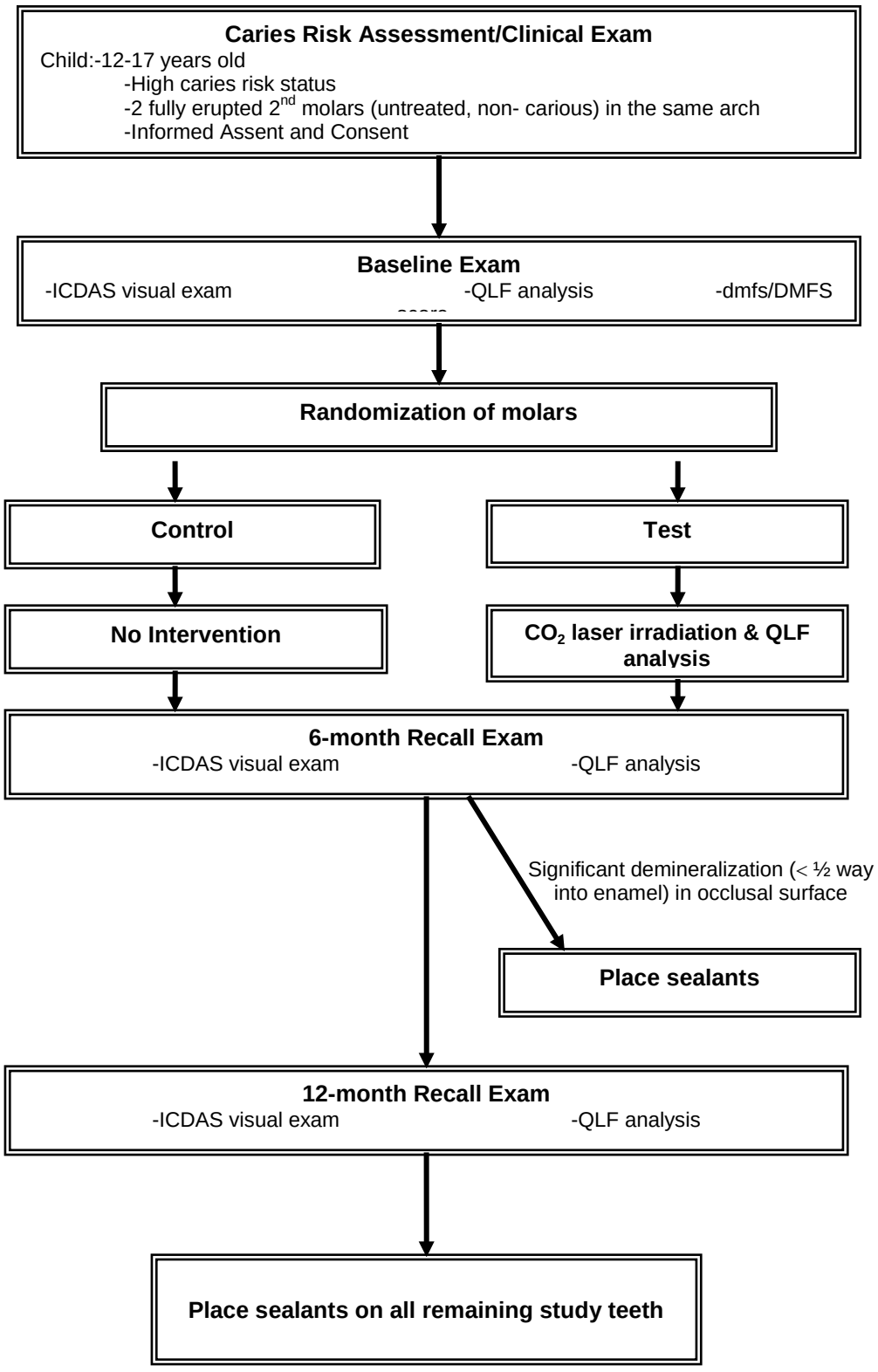
[ ]Behavioral

[ ] Multicenter: If so, is UCSF the coordinating center or the prime grant holder? If yes, please complete Section III of the IRB Approval Certification Supplement	[ ]Yes [X]No

2. Additional description of *general* study design. Attach flow diagram if appropriate.

This pilot project will be a randomized, clinical split-mouth study. Patients meeting the inclusion criteria from the UCSF Predoctoral and Postgraduate Pediatric Dental clinic will have the study explained to them and be invited to participate. The Caries Management By Risk Assessment (CAMBRA) questionnaire currently used in the predoctoral dental clinics at UCSF will be

conducted at baseline to assess the caries risk status. If the patient is determined to be at high caries risk, then the patient will be asked if he/she wishes to provide assent and the parent or guardian to provide informed consent. The molar on their dominant side (right or left) will be randomly assigned to either the test or the control group with the non-dominant side assigned to the other group. This is because dominant side determined by handedness may relate to oral hygiene. The study molars will be brushed and digital intraoral photos, a baseline visual inspection, Decayed, missing, filled, surfaces (dmfs/DMFS) scores for primary and permanent teeth will be obtained, and QLF assessment will be made by a dentist blinded to intervention assignment. The control tooth will receive no intervention and the test tooth will subsequently be treated with CO_2 laser irradiation followed by QLF analysis. The patient will be asked to return for a 6-month and a 12-month follow up exam, at which time the study molars will be brushed, digital intraoral photos taken, visual inspection and QLF assessment will be conducted by the same dentist who originally completed the baseline exam. Data collected will be coded and blinded to the dentist. The endpoint of the study for each participant will be when either the control or test tooth is found to have significant demineralization (less than halfway into enamel) by QLF assessment or at the 12 month exam, whichever comes first. This will constitute a result. The control and test teeth will be sealed with Helioseal (dental sealant) at the end of the study.


Caries Risk Assessment/Clinical Exam
Child:-12-17 years old
-High caries risk status
-2 fully erupted 2nd molars (untreated, non- carious) in the same arch
-Informed Assent and Consent
Baseline Exam
-ICDAS visual exam
-QLF analysis
-dmfs/DMFS
Randomization of molars
Control
Test
No Intervention
CO2 laser irradiation & QLF analysis
6-month Recall Exam
-ICDAS visual exam
-QLF analysis
Significant demineralization (< ½ way into enamel) in occlusal surface
Place sealants
12-month Recall Exam
-ICDAS visual exam
-QLF analysis
Place sealants on all remaining study teeth

E. Data Analysis (How and by whom will data be analyzed?)

Sample size:

A conservative sample size was calculated using the parallel groups trial data from our previous CO_2 laser study[20], that examined demineralization in vivo using tooth blocks that had been treated extra-orally. In the present study fifteen teeth for each group (control and test) will be needed for 80% power, two-sided, paired t-test. That is we will need a minimum of 15 study participants who will have test and control teeth in the same mouth. To allow for drop-out and non-compliance, 20 participants will be enrolled in the study, providing a total of 20 pairs of teeth.

Statistical Analyses:

The relative mineral loss (ΔZ) values for the test teeth versus the control teeth will be compared. The data will be analyzed using a 2-sided paired t-test with a significance level of $p \leq 0.05$. Person-level effects such as gender, age, and other conditions will be controlled in the within-person treatment estimate. Success will be measured by the level of inhibition of demineralization based on QLF data which we anticipate will be in the range of 70-100%. Dr. Stuart Gansky, an experienced biostatistician in the PRDS department, will assist Dr. Hsu to perform all statistical analyses.

PART 3: PROCEDURES

A. Check all that apply.

[ ] Human Biological Specimen Banking **Attach - Banking Supplement**
[ ] Genetic Testing [ ] HIV Testing
[ ] Human Gene Transfer/Recombinant DNA Research **Attach - Human Gene Transfer / Recombinant DNA Research Supplement**

B. Please list, in sequence, all study procedures, tests, and treatments required for the study. Indicate which would be done even if a subject does not enroll in the study. Include a detailed explanation of any experimental procedures. Attach table if available.

Caries Management By Risk Assessment (CAMBRA) Questionnaire:
Administer CAMBRA (Caries Risk Assessment) Questionnaire from UCSF Axium (electronic patient record) clinical form, as is the current procedure in the predoctoral dental clinics.

Informed Consent:
Obtain written informed consent from parent/guardian and verbal assent from patient.

Study Procedures:

Baseline exam:
Prior to randomization of molars, a blinded, trained dental examiner will brush the study molars, take digital intraoral photos, and conduct a clinical exam to assess caries status via visual inspection based on the International Caries Detection and Analysis System (ICDAS) clinical visual criteria. Codes for coronal caries (0-6) will be assigned based on severity of caries. DMFS/dmfs

scores will be determined by exam and radiographs according to World Health Organization (WHO) standards.
(http://www.whocollab.od.mah.se/expl/orhdmft.html) This will be followed by QLF analysis.

The molars will be randomized into test and control groups. The test molar will receive intervention with CO_2 laser irradiation and the control molar will initially remain unsealed. Under normal conditions, it is the standard of care at UCSF for molars in patients with high caries risk to receive sealants. According to Vanderas et. al., the progression rate of a carious lesion through enamel of permanent teeth takes an average of four years.[19] Thus, waiting six months to seal a permanent molar will not cause a significant increase in risk for the patient.

Treatment with CO_2 laser irradiation:

Following baseline exam, an independent dental provider, trained in this technique, will apply CO_2 laser irradiation to the test molar.

CO_2 laser irradiation (test tooth):

A Pulse Systems (New Mexico) 9.6 μm wavelength clinical laser will be used at a fluence (energy/surface area) of 2.0 J/cm^2 per pulse, and a pulse duration of 20 μs, which is sub-ablative and likely effective for caries inhibition. Laser treatment will be applied in overlapping spots, approximately 1 mm in diameter, 20 pulses per spot, along the occlusal pits and fissures. Because the conditions are sub-ablative, no water spray will be necessary. The total energy delivered is far below proven safe conditions, no water cooling will be needed at all.

This will be followed by QLF analysis of the test molar.

Six-month recall:

The original dental examiner will brush the study molars, take digital intraoral photos, and conduct a clinical exam to assess caries status via visual inspection based on the International Caries Detection and Analysis System (ICDAS) clinical visual criteria. Codes for coronal caries (0-6) will be assigned based on severity of caries. This will be followed by QLF analysis.

If significant demineralization (less than halfway into enamel) is found per QLF analysis in the occlusal surface of one or more of the two test teeth, then a sealant will be placed, and the patient will have come to the end point of the study.

Sealant (demineralized tooth):

Sealants will be placed according to standard clinical procedures. Cotton roll and dri-angle isolation will be used. The occlusal surface of the tooth will be cleaned with pumice, rinsed, etched with 35% phosphoric acid for 40 seconds, rinsed and dried thoroughly. The surface will be primed and bonded with 3M Multipurpose adhesive, air dried gently, and light cured for 20 seconds. Helioseal will be applied, wait 15 seconds and light cured for 20 seconds.

12-month recall:

The same dental examiner will brush the study molars, take digital

intraoral photos, and conduct a clinical exam to assess caries status via visual inspection based on the International Caries Detection and Analysis System (ICDAS) clinical visual criteria. Codes for coronal caries (0-6) will be assigned based on severity of caries. This will be followed by QLF analysis.

All control and test teeth not previously sealed will have a sealant placed.

C. List the clinics and/or other specific locations where study procedures will be performed. Indicate how much time will be required of the subjects, per visit and in total for the study.

The procedures will be performed at the UCSF Dental School. The facilities of the Pediatric Dental and Preventive and Restorative Dental Sciences Departments will be used. Additional study visits besides the regular visits for sealant placement and necessary baseline/recall exams are not expected. The amount of time for the subjects in addition to their normal dental treatment is the time necessary for:

1) informed consent/assent procedure (20 minutes)
2) QLF assessment (20 minutes)
3) Laser irradiation of the occlusal surface of test tooth (20 minutes)

This adds up to a total of about one hour.

D. Will any interviews, questionnaires, surveys or focus groups be conducted for the study? If "Yes," please list any standard instruments used for this study and attach any non-standard instruments.	[X]Yes [ ]No

Caries Management By Risk Assessment (CAMBRA) Questionnaire:
CAMBRA (Caries Risk Assessment) Questionnaire from UCSF Axium (electronic patient record) clinical form will be used as a screening tool, as is the current procedure in the predoctoral dental clinics.
*see attachment

E. Will subjects undergo any study procedures or tests off-site by non-UCSF personnel? If "Yes," please explain.	[ ]Yes [X]No

F. Will subjects or their health care provider be given the results of any experimental tests that are performed for the study? If "Yes," please describe the tests, provide a rationale for providing subjects with the experimental test results and explain what, how and by whom subjects and their health care provider will be told about the meaning, reliability, and applicability of the test results for health care decisions.	[ ]Yes [X]No

PART 4: ALTERNATIVES

A. Describe the alternatives to study participation that are available to prospective subjects.
The alternative is not to be part of the study and to receive the same dental treatment, including sealants as previously planned.

B. Is study drug or treatment available off-study? If "Yes," discuss this in the consent form.	[ ]Yes [X]No [ ]N/A

PART 5: RISKS AND BENEFITS

A. Risks and Discomforts: **1.** Describe the risks and discomforts of any investigational or approved drugs, devices and procedures being used or assigned for study purposes. Describe the expected frequency of particular side effects. If subjects are restricted from receiving standard therapies during the study, please also describe the risks of those restrictions.
Risks and discomforts will be negligible. One minor risk is damage to the gingiva by the etching gel prior to sealant placement, leading to small gingival erosion. This is part of the normal standard of care. This is similar to getting hurt from a standard dental instrument. Another minor risk is gingival irritation with the irradiation laser. The laser treatment will be done on the occlusal surfaces of the teeth. However, it is possible that soft tissue nearby could be accidentally irradiated. Due to the low energy used in comparison to typical laser use in dentistry for the ablation of hard tissues, only a minor burn of the gingiva is expected if the laser accidentally hits the tissue. The superficial lesions of the gingiva will heal spontaneously in 1-3 days without any intervention. There will be no laser interaction with the adjacent teeth as the laser beam can readily be contained to the occlusal surface of the treated tooth. Potential risk of accidental irradiation of the eyes could cause potential injuries. This has not been reported during laser use in dentistry to date. Safety glasses for patient, doctors, and everyone else in the nominal hazard zone to protect eyes from irradiation will be used during the entire laser procedure. Another potential risk is that the laser irradiation may affect the vitality of the tooth because the laser treatment heats the outer surface for a few microseconds. Laboratory and clinical safety studies in our laboratories (funded by an NIH grant) have shown this risk to be minimal. In a safety study in humans Goodis et al. showed that "the 9.6μm wavelength laser, with irradiation conditions comparable to those proposed in the present study, causes no permanent/serious pulpal damage at the energy levels used and can be used safely for caries prevention treatments in humans."[12]
2. Describe the steps you have taken to minimize the risks/discomforts to subjects (e.g., supportive interventions or special monitoring):
-Danger of small etching damage to the gingiva can be minimized by using a high speed suction to remove bulk etching gel from tooth surface prior to

rinsing off with water.
-The use of laser safety eyewear for everyone in the nominal hazard zone will minimize the risk of injuries to the eye.

B. Data and Safety Monitoring Plan (DSMP): ***All interventional studies involving more than minimal risk must include a DSMP.*** A DSMP is a plan established to assure that each research study has a system for appropriate oversight and monitoring of the conduct of the study to ensure the safety of participants and the validity and integrity of the data. The DSMP should indicate specifically whether or not there will be a formal Data Safety Monitoring Board (DSMB) or Data Monitoring Committee (DMC).

Note: Most, but not all studies (i.e., non-interventional studies) undergoing full committee review will require a DSMP.

For *what to include in a DSMP* see DSMP Information Sheet for Principal Investigators.

The guidelines for a Data and Safety Monitoring Plan state that the degree of monitoring should be commensurate with the risk. Because the risk of adverse events related to the study is minimal and because we will take appropriate measures to ensure confidentiality, we do not require a Data Safety Monitoring Board. This study is a small scale pilot study and is not a Phase III clinical trial. However, we will conduct our own monitoring according to recognized procedures to prepare for and respond to any adverse events.

C. Adequacy of Resources:

Principal Investigators must have the necessary resources required to conduct the proposed research in a way that assures the rights and welfare of participants are adequately protected. Depending on the nature of the research study, investigators should consider the proximity or availability of critical resources that may be essential to the safety and welfare of participants. For example, the proximity of an emergency facility for care of participant injury, or availability of psychological support after participation, or resources for participant communication, such as language translation services.

Have you or will you undergo formal resource review (e.g., VAMC, SFGH, GCRC) prior to study implementation?	*[ ] Yes [X] No*

If yes, please specify entity providing review:

If no, please describe below the resources you have in place to conduct this study in a way that assures protection of the rights and welfare of participants:

The study will be conducted on the UCSF campus at 707 Parnassus Ave, where full emergency services are available within the school of dentistry and the nearby hospital. Administrative procedures are in place to ensure the welfare of the patients. This is a non-significant risk study.

D. Confidentiality and Privacy: Privacy concerns people, whereas confidentiality concerns data. Specifically, confidentiality refers to the researcher's plan to handle, manage

and disseminate the participant's identifiable private information. Privacy refers to a person's wish to control the access of others to themselves. **Address each of the following privacy issues questions 1-3 below:**

1. How will the investigator access information from or about participants?

Information from or about participants will be accessed via the participant's paper and electronic chart at UCSF Pediatric Dental Clinic and via participant responses during questionnaire.

2. How will the investigator maintain privacy in the research setting(s)?

Privacy will be maintained in the research setting by removing all identifiers from the patients' records when transferring data to study database.

3. What are the consequences to participants of a loss of privacy (e.g., risks to reputation, insurability, other social risks)?

There are no obvious consequences to subjects in case of a loss of privacy. Sealant placement is a typical treatment in this age group. Thus, being a part of this study inflicts no negative effect on the subjects.

The following questions address confidentiality issues:

4. Identifiers: Please indicate all identifiers that may be included in the research records for the study. Check all that apply.

[X] Names	[ ] Social Security Numbers*	[ ] Device identifiers/Serial numbers
[X] Dates	[ ] Medical record numbers	[ ] Web URLs
[X] Postal address	[ ] Health plan numbers	[ ] IP address numbers
[X] Phone numbers	[ ] Account numbers	[ ] Biometric identifiers
[ ] Fax numbers	[ ] License/Certificate numbers	[ ] Facial Photos/Images
[ ] Email address	[ ] Vehicle id numbers	[ ] Any other unique identifier
[ ] None of the 18 identifiers listed above		*Required for studies conducted at the VA

5. Determining Whether HIPAA Regulations Apply to This Study: Please answer the questions below for the identifiers marked in the above section. Check all that apply:

Are study data: [X] Derived from a medical record? ***Please identify source***: UCSF dental record [ ] Added to the hospital or clinical medical record? [ ] Created or collected as part of health care? [ ] Used to make health care decisions?	**HIPAA regulations apply.** The identifiers marked in section C.1 are PHI.
[X] Obtained from the subject, including interviews, questionnaires? [ ] Obtained from a foreign country or countries only? [ ] Obtained from records open to the public? [ ] Obtained from existing research records? [ ] None of the above.	**HIPAA regulations do not apply.** The identifiers marked section C.1 are not PHI.

If HIPAA regulations apply, you are required to obtain individual subject authorization or a CHR-approved waiver of authorization, or both, to be allowed access to medical records. For the VA, use the SFVAMC authorization. (The one exception to these requirements is the use of a Limited Data Set along with a Data Use Agreement.)	
6. Use and Disclosure of Personal Health Information: Please indicate to whom or where you may disclose any of the identifiers listed above as part of the study process. Check all that apply: [ ] We do not plan to share any of the personally identifying information listed above outside the research team. [ ] The subject's medical record [ ] The study sponsor: ***please indicate***: [ ] The US Food & Drug Administration (FDA) [ ] Others: ***please indicate***: [ ] A Foreign Country or Countries	
7. Data Security: Identifiable data should not be stored on laptops, PDA's or other portable devices. Please indicate how study data are kept secure. Check all that apply:	
[ ] Data are coded; data key is destroyed at end of study or ***provide date***: [X] Data are coded; data key is kept separately and securely [X] Data are kept in locked file cabinet [ ] Electronic data are protected with a password [X] Data are kept in locked office or suite [ ] Data are stored on a secure network	
8. Describe any additional steps taken to assure that identities of subjects and any of their health information which is protected under the law is kept confidential. If video or audio recordings will be made as part of the study, disposition of these recordings should be addressed here and in the consent form.	
Data will be kept in locked files which are in locked offices accessible only for the PI and authorized study personnel. For clinical procedures and for statistical analyses data will be coded.	
9. Reportable Information: Is it reasonably foreseeable that the study will collect information that State or Federal law requires to be reported to other officials (e.g., child or elder abuse) or ethically requires action (e.g., suicidal ideation)? If "Yes," please explain below and include a discussion of the reporting requirements in the consent form.	[ ]Yes [X]No

E. Benefits: **1.** Are there potential direct benefits to study subjects? If "Yes," please describe below.	[X]Yes [ ]No
If the CO_2 laser irradiation proves to be effective in preventing caries in vivo and this effect is long lasting, then the patient would directly benefit from the long lasting effects of caries prevention.	
2. What are the potential benefits to society?	

If the specially designed CO_2 laser irradiation used in this study proves to be effective in preventing caries in occlusal surfaces of vital teeth, then this procedure will provide a novel and rapid means of caries prevention for pediatric dentistry.

F. Risk/Benefit Analysis: How do the benefits of the study outweigh the risks to subjects?
Since the risk related to being in this study is very small, the risk to benefit ratio is reasonable.

PART 6: SUBJECT INFORMATION

A. Number of Subjects:	
1. How many subjects will be enrolled at UCSF and affiliated institutions?	20
2. How many subjects will be enrolled at all sites (i.e., if multicenter study)?	20
3. How many people do you estimate you will need to consent and screen here (but not necessarily enroll) to get the needed subjects?	30

B. Types of Subjects: Check all that apply. Click on links for additional instructions.	
[X]	Minors **Attach - Inclusion of Minors Supplement**
[ ]	Subjects unable to consent **Attach - Surrogate Consent or Emergency Waiver of Consent Supplement**
[ ]	Subjects with Diminished Capacity to Consent
[ ]	Subjects Unable to Read, Speak, or Understand English – **Complete Part 8.D of this application**
[ ]	Pregnant Women – **Complete Part 6.G of this application**
[ ]	Fetuses
[ ]	Neonates
[ ]	Prisoners **Attach - Inclusion of Prisoners Supplement**
[ ]	Inpatients
[ ]	Outpatients
[X]	Healthy Volunteers
[ ]	Staff of UCSF/affiliated institution

C. Eligibility Criteria: **1.** General description of subject population(s):
The study will be conducted in permanent teeth in high caries risk children aged 12-17 years. The study uses fully erupted second molars because they are the teeth in need for caries prevention treatment in this age group. Children in this age group will be used because their dietary habits and their teeth make them well suited for fast caries initiation and progression of caries during the time period of the study. Further, this age group is most likely to have newly erupted second molars requiring sealants. Consequently, adults are inappropriate for the study. There will be no discrimination as regards to

gender or race/ethnicity.

Subjects will be drawn from the patient pool at the UCSF School of Dentistry. The demographics of the UCSF population are as follows: Of the 10,166 patients of record in the Comprehensive Care Clinic, 50.7% are male and 49.3% are female. The age distribution in the comprehensive care general dentistry clinic is 1.5% 1-17 years old, 9.1% 18-24 years old, 24.3% 25-34 year old, 19.7% 35-44 year old, 17.3% 45-54 year old, 10.5% 55-64 year old, and 16.2% 65+ year old. Ethnicity is varied with 48.8% White, 11.6% Black, 10.0% Asian, 11.0% Hispanic, and 18.6% other, no disclosed or not specified. Demographics in the pediatric clinic are similar, but the age range is exclusively children, as required in the proposed study. Our target enrollment will reflect this distribution.

2. Inclusion Criteria:

- 12 – 17 years old
- high caries risk status determined using CAMBRA
- two fully erupted second molars with untreated, non-carious occlusal surfaces in the same arch
- healthy and able to cooperate for treatment in dental chair
- parent/guardian able to provide written informed consent in English
- patient provide verbal assent
- residing in San Francisco or other nearby locales with community water fluoridation
- willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form

3. Exclusion Criteria:

- low or moderate caries risk status determined using CAMBRA
- no pair of untreated or non-carious occlusal surfaces on second molars in the same arch
- under active orthodontic treatment involving 2nd molar bands
- has limited range of opening
- will leave the area and will not be available for recall visits
- has underlying systemic disease which could alter enamel composition or formation
- significant medical history with conditions that may affect oral health or flora (i.e. diabetes, HIV, heart conditions that require antibiotic prophylaxis)

D. How (chart review, additional tests/exams for study purposes), when and by whom will eligibility be determined?

Patients meeting the inclusion criteria from the UCSF Predoctoral and Postgraduate Pediatric Dental clinic will have the study explained to them by the Clinical Investigator (Dr. Tiffany Hsu) and be invited to participate. The Caries Management By Risk Assessment (CAMBRA) questionnaire currently

used in the predoctoral dental clinics at UCSF will be conducted at baseline to assess the caries risk status. If the patient is determined to be at high caries risk, then the patient will be asked if he/she wishes to provide assent and the parent or guardian to provide informed consent.

E. Are there any inclusion or exclusion criteria based on *gender*, *race* or *ethnicity*? If "Yes," please explain the nature and rationale for the restrictions below.	[ ]Yes [X]No

F. Populations Likely to be Vulnerable to Coercion or Undue Influence:
1. List subject groups who are likely to be vulnerable to coercion or undue influence, such as mentally disabled persons, economically or educationally disadvantaged persons, or investigators' staff or students. **Omit *minors, those unable to consent for themselves,* and *prisoners*** (who are covered by separate Supplements); **for *pregnant women, fetuses, and neonates,* see section G below**):
Economically disadvantaged persons
2. Explain why it is appropriate to include the groups listed above in this particular study:
It is important to not discriminate based on socioeconomic status of the patient.
3. Describe **additional** safeguards that have been included in the study to protect the rights and welfare of these subjects and minimize coercion or undue influence. For example, you might provide competence evaluations (specify) for the mentally disabled, payment amounts calibrated to be noncoercive for the financially disadvantaged, extra-careful evaluations of subjects' understanding of the study, advocates to be involved in the consent process, or use flyers to recruit subjects instead of directly approaching staff or students:
Payment amounts are calibrated to be noncoercive for the financially disadvantaged.

G. Pregnant Women, Human Fetuses, and Neonates:
Identify all sections of 45 CFR 46 Subpart B (see Chart) under which you believe the research falls and provide study-specific information showing why the research falls within those sections:
N/A

PART 7: RECRUITMENT

A. Please review CHR Recruitment Guidelines for more information about acceptable recruitment methods. Note that all advertisements, whether posted or broadcast, and all correspondence used for purposes of recruitment require CHR review and approval before they are used. Check all that apply:	
[X]	Study investigators recruit their own patients directly and/or nurses or staff working with researchers approach patients. ***Please explain in Section B.***

[ ]	Study investigators send a CHR-approved letter to colleagues asking for referrals of eligible patients interested in the study. The investigators may provide the referring physicians a CHR-approved Information Sheet about the study to give to the patients. If interested, the patient will contact the PI. Or, with documented permission from the patient, the PI may be allowed to talk directly with patients about enrollment. ***Attach letter for review.***
[ ]	Study investigators provide their colleagues with a "Dear Patient" letter describing the study. This letter can be signed by the treating physicians and would inform the patients how to contact the study investigators. The study investigators may not have access to patient names and addresses for mailing. ***Attach letter for review.***
[X]	Advertisements, notices, and/or media used to recruit subjects. The CHR must first approve the text of these, and interested subjects will initiate contact with study investigators. ***Attach ads, notices, or media text for review. In Section B, please explain where ads will be posted.***
[ ]	Study investigators request a Waiver of Consent/Authorization for recruitment purposes. This waiver is an exception to the policy but may be requested in circumstances such as:
[ ] [ ] [ ]	Minimal risk studies in which subjects will not be contacted (i.e., chart review only); Review of charts is needed to identify prospective subjects who will then be contacted. (Explain in Waiver form); Large-scale epidemiological studies and/or other population-based studies when subjects may be contacted by someone other than personal physician. (Explain in Waiver form.)
[ ]	Direct contact of potential subjects who have previously given consent to be contacted for participation in research. Clinic or program develops a CHR-approved recruitment protocol that asks patients if they agree to be contacted for research (a recruitment database) or consent for future contact was documented using the consent form for another CHR-approved study. ***Please explain in Section B.***
[ ]	Study investigators list the study on the UCSF Clinical Trials Seeking Volunteers web page or a similarly managed web site. Interested subjects initiate contact with investigators.
[ ]	Study investigators recruit potential subjects who are unknown to them. Examples include snowball sampling, use of social networks, direct approach in public situations, random digit dialing. ***Please explain in Section B.***

B. Provide detail in the space below (*i.e., how, when, where and by whom are potential subjects approached?*).
At the UCSF School of Dentistry in the Predoctoral and Postgraduate Pediatric Dental Clinics after the independent examiner has determined the caries risk and decided that second molars have to be sealed due to caries prevention reasons, the Clinical Investigator will be informed by the examiner about this potential subject. Advertisement flyers will be posted throughout the UCSF campus.

PART 8: INFORMED CONSENT PROCESS

A. Check all that apply:	
[X]	Signed consent will be obtained from subjects and/or parents (if subjects are minors),

[ ]	Verbal consent will be obtained from subjects, using an:	
	[ ]	Information sheet (attach)
	[ ]	Script (attach)
[ ]	Signed consent will be obtained from surrogates **Attach - Surrogate Consent Supplement**	
[ ]	Informed consent will not be obtained. **Attach - either the Waiver of Consent/Authorization or the Emergency Waiver of Consent Supplement as appropriate**.	

B. In the space below, describe *how*, *where*, *when* and *by whom* informed consent will be obtained. How much time will prospective subjects be given to consider study participation? If special subject populations will be included, be sure to describe any additional plans for obtaining consent from particular populations. Justify any plans to use verbal consent instead of signed consent.

Subjects will first be verbally informed about the study including purpose and aim, procedure, their task and especially all risks. They will be urged to ask questions whenever they want. Then they will be asked to read the consent/assent forms and ask whenever they need more explanations. After they have read the form they will be asked to explain in their own words what will be done, what we ask them to do and what risks they understand are involved. This procedure will take about 20 minutes; it will take place in an office setting outside the treatment area and will be performed by the Clinical Investigator. This procedure will be performed after the independent examiner has determined the patient's treatment needs. The subjects will be given all the time they need to decide whether they want to participate or not.

In case that the Clinical Investigator experiences the feeling that there are doubts to participate she will suggest to the subjects to go home and call, if they are interested and have an informative meeting again on another day or she will decide that the subject should not be enrolled.

C. How will you make sure subjects understand the information provided to them?

As mentioned above, we will make sure the subjects understand the information provided to them by letting them describe in their own words what will be the procedure, what are their risks and what we want them to do.

D. Subjects Who Do Not Read, Speak, or Understand English.

1. If you will enroll subjects who are unable to Read, Speak or Understand English, what method will you use to obtain consent? *Preferred Method* should be used if a substantial number of prospective subjects are expected to be non-English speakers. See **Those Who Do Not Read, Speak or Understand English** for details of methods.

[ ]	*Preferred Method*—Consent form and other study documents will be available in the subject's primary language. Personnel able to discuss participation in the patient's language will be present for the consent process.
[ ]	*Short-Form*—A qualified interpreter will translate the consent form verbally, and subjects will be given the Experimental Subject's Bill of Rights in their primary language, following instructions in **Those Who Do Not Read, Speak or Understand English** for required witnessing and signatures.

2. How will you maintain the ability to communicate with non-English speakers throughout their participation in the study?

PART 9: FINANCIAL CONSIDERATIONS

A. Payments to Subjects: **1.** Will subjects receive payments or gifts for study participation? If "Yes," please review CHR Subject Payment Guidelines and complete the following:	[X]Yes [ ]No
2. Payments will be (check all that apply):	[ ] Cash [ ] Check [X] Other (describe below)
3. Please describe the schedule and amounts of payments, including the total subjects can receive for completing the study. If deviating from recommendations in Subject Payment Guidelines, include specific justification below.	
Subjects will not be charged for the additional study treatments or procedures. Subjects who participate in the study will receive a $10 gift card for the child and a $10 gift card for the parent at baseline. A $20 gift card will be given to the child at the 6-month recall exam and at the 12-month recall exam. A $20 cash bonus will be given to the child upon completion of the study at either the 6-month recall or 12-month recall exam.	

B. Costs to Subjects: Will subjects or their insurance be charged for any study procedures? If "Yes," describe those costs below, and compare subjects' costs to the costs associated with alternative care off-study. Finally, explain why it is appropriate to charge those costs to the subjects.	[ ]Yes [X]No

C. Treatment and Compensation for Injury: The investigators are familiar with and will follow the University of California policy and (if applicable) Veteran's Affairs policy regarding treatment and compensation for injury. If subjects are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, by the Department of Veteran's Affairs (for subjects eligible for veteran's benefits, if the SF VAMC is a study site), or by the study sponsor, if any, depending on a number of factors. The University does not normally provide any other form of compensation for injury.

PART 10: BIBLIOGRAPHY

1. Mouradian, WE, Wehr E, Crall JJ. Disparities in children's oral health and access to dental care. JAMA 2000 Nov;284(20):2625-31.

2. US Public Health Service. Healthy People 2000 Progress Report on Oral Health. Washington, DC: US Dept of Health and Human Services; 1995.

3. National Institute of Dental Research. Oral Health of the United States Children: The National Survey of Dental Caries in School Children, 1986-86; National and Regional Findings. Bethesda, Md: National Institute of Dental Research; 1989. DHHS publication PHS 89-2247.

4. American Society of Dentistry for Children and American Academy of Pedodontics. Rationale and guidelines for pit and fissure sealants. Pediatr Dent 1983;5(1):89-90.

5. Feigal RJ, Donly KJ. The use of pit and fissure sealants. Pediatr Dent 2006;28:143-50.

6. Feigal RJ. Sealants and preventive restorations: review of effectiveness and clinical changes for improvement. Pediatr Dent 1998;20(2):85-92.

7. Featherstone JDB. Caries detection and prevention with laser energy. Dent Clin North Am 2000 Oct; 44(4):955-69.

8. Wigdor HA, Walsh JT, Featherstone JDB, Visuri SR, Fried D, Waldvogel JL. Lasers in Dentistry. Lasers in Surgery and Medicine. 1995;16:103-33.

9. Fried D, Ragadio J, Akrivou M, Featherstone JDB, Murray MW, Dickenson KM. Dental hard tissue modification and removal using sealed transverse excited atmospheric-pressure lasers operating at λ=9.6μm and 10.6μm. J Biomed Opt 2001 Apr;6(2):231-8.

10. Featherstone JDB, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO_2 laser inhibition of artificial caries-like lesion progression in dental enamel. J Dent Res 1998 June;77(6):1397-403.

11. Kantorowitz Z, Featherstone JDB, Fried D. Caries prevention by CO_2 laser treatment: dependency on the number of pulses used. J Am Dent Assoc 1998 May;129:585-91.

12. Goodis HE, Fried D, Gansky S, Rechmann P, Featherstone JDB.

Pulpal safety of 9.6μm TEA CO_2 laser used for caries prevention. Laser Surg Med 2004;35:104-10.

13. Rodrigues LKA, Nombre dos Santos M, Featherstone JDB. *In situ* mineral loss inhibition by CO_2 laser and fluoride. J Dent Res 2006;85(7):617-21.

14. Lussi A. Validity of diagnostic and treatment decisions of fissure caries. Caries Res 1991;25:296-303.

15. International Caries Detection and Assessment System Coordinating Committee. Available at "http://www.icdas.org". Accessed December 2006.

16. Hsu CYS, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. J Dent Res 2000;79(9):1725-30.

17. Stookey GK. Quantitative light fluorescence: a technology for early monitoring of the caries process. Dent Clin North Am 2005 Oct;49(4):753-70, vi.

18. Kuhnisch J, Ifland S, Tranaeus S, et al. Establishing quantitative light-induced fluorescence cut-offs for the detection of occlusal dentine lesions. Eur J Oral Sci 2006 Dec;114(6):483-8.

19. Vanderas A, Manetas C, Koulatzidou M, Papagiannoulis L. Progression of proximal caries in the mixed dentition: a 4-year prospective study. Ped Dent 2003;25(3):229-34.

20. Featherstone JDB, Fried D, Gansky SA, Stookey GS, Dunipae A. Effect of carbon dioxide laser treatment on lesion progression in an intra-oral model. Lasers in dentistry VII 2001;4249:87-91.

21. Amaechi BT, Podoleanu A, Higham SM, Jackson DA. Correlation of quatitative light-induced fluorescence and optical coherence tomography applied for detection and quantification of early dental caries. J Biomed Opt 2003 Oct;8(4):642-7.

22. Featherstone JDB, Nelson DGA. Laser effects on dental hard tissues. Adv Dent Res 1987 Oct;1(1):21-6.

23. Gorton J, Featherstone JDB. *In Vivo* Inhibition of Demineralization Around Orthodontic Brackets. Am J Orthod Dentofac Orthop 2003;123:10-4.

24. Pretty IA, Edgar WM, Higham SM. Detection of in vitro demineralization of primary teeth using quantitative light-induced fluorescence (QLF). Int J

Paediatr Dent 2002;12:158-67.

25. Rodrigues LKA, Nombre dos Santos M, Pereira D, Assaf AV, Pardi V. Carbon dioxide laser in dental caries prevention. J Dent 2004;32:531-40.

26. Walsh LJ, Perham SJ. Enamel fusion using a carbon dioxide laser: a technique for sealing pits and fissures. Clinical Preventive Dentistry 1991;13(3):16-20.

PART 11: ATTACHMENTS

Please list Attachments, Supplements and Appendices	Version number(s) or date(s)
1. Inclusion of Minors	6/6/07
2. Non-significant Risk Determination for an Investigational Device	4/17/07 6/6/07 8/3/07
3. Advertisement flyer	4/3/07
4. Parental Informed Consent/12-17 year old Minor Assent	6/6/07 4/3/07
5. Permission to Use Personal Health Information for Research form	8/3/07 4/3/07 8/3/07
6. Experimental Subject's Bill of Rights	4/3/07 4/3/07 4/3/07
7. CAMBRA caries risk assessment form	
8. Data Collection Forms (A1-A6) • A1: Enrollment Form • A2: Subject's Contact Information and Ethnicity • A3: Subject Oral Care Survey • A4: Baseline dmfs/DMFS Record Sheet • A5: Mailing Address Form • A6: Phone Record Sheet	

Appendix 2

CARIES RISK ASSESSMENT FORM FOR CHILDREN 6 YEARS AND OLDER/ADULTS

Instructions on reverse

Patient Name:_____ **I.D. #** _____ **Age** ____ **Date** _____

Initial/baseline exam date_____ **Recall/POE date**_____

Respond to <u>each</u> question in sections 1, 2, and 3 with a check mark in the yes or no column			**Notes**
	Yes	**No**	
1. High Risk Factors**			
(a) Visible cavitation (carious) or caries into dentin by radiograph			
(b) Caries restored in past three years			
(c) Readily visible heavy plaque on teeth			
(d) Frequent (greater than three times daily) between meal snacks of sugars/cooked starch			
(e) **Saliva-reducing factors:**			
1. Hyposalivatory medications			
2. Radiation to head and neck			
3. Systemic reasons, e.g. Sjögren's			
(f*) Visually inadequate saliva flow. (If yes, measure) less than 0.7 ml/min by test= low salivary flow or dry mouth			Amount: _____ ml/min
(g) Appliances present, fixed or removable, e.g. orthodontic brackets/bands/retainer or removable partial denture(s)			
2. Moderate Risk Factors			
(a) Exposed roots			
(b) Deep pits & fissures/developmental defects			
(c) Interproximal enamel lesions/radiolucencies			
(d) Other white spot lesions or occlusal discoloration			
(e) Uses recreational drugs			
3. Protective Factors			
(a) Lives /works/school in fluoridated community			
(b) Uses fluoride toothpaste daily			type _____
(c) Uses fluoride mouthwash/rinse/gel daily			type _____
(d*) Salivary flow visually adequate >1 ml/min by test			
(e) Uses xylitol gum or mints 4 x day			Type _____ and % xylitol_____
(f) Mother/caregiver has no caries activity			Brand _____ Frequency_____

****If yes to 1 (a) or any two of 1 (b)-(g), perform bacterial culture***	**High Count Date:** _____	**Moderate Count Date:** _____	**Low Count Date:** _____	
(a) Mutans streptococci				(Place a check in the box below the count)
(b) Lactobacillus				(Place a check in the box below the count)
Caries risk overall* (see over)	**High**	**Moderate**	**Low**	**Circle High, Moderate or Low**

Recommendations given: yes_____ **no:** _____ Date given:_____ or Date follow up:_____

*Indicates that test descriptions for these procedures are on the following pages

Appendix 3

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
ASSENT/CONSENT TO BE A RESEARCH SUBJECT

In vivo occlusal caries prevention by pulsed CO_2 laser treatment quantified by QLF

Dr. John Featherstone, MS, PhD together with his clinical investigator Dr. Tiffany Hsu, DDS from the Departments of Preventive and Restorative Dental Sciences and Orofacial Sciences here at the University of California, San Francisco (UCSF) are conducting a study to learn more about how well a new laser can prevent tooth decay.

What is this study about?

The purpose of this research project is to see how well an experimental laser works to prevent tooth decay in comparison to the regular oral hygiene homecare

This study will be done on children ages 12 to 17 years who are patients in the Predoctoral or Postgraduate Pediatric Dental Clinic.

This study is being sponsored by the NIH (National Institutes of Health)

You are being asked to participate in this study because you are a patient of the Pediatric Dental Clinic, two of your second molars (back teeth) have fully erupted (grown into your mouth), and you are at high risk for cavities.

How many people will take part in the study?

If you agree to participate, you will be one of approximately 20 subjects in this research study.

What will happen if you decide you might want to be in this research study?

First, your parents will be asked if they give their permission for you to be in this study. If your parents don't agree, you cannot be in the study.

If your parents do agree, and you agree too, here's what will happen next:

1. A dentist will complete a baseline clinical exam of your mouth, review of your dental x-rays, caries risk assessment, medical history and your dental history to date. Two fully grown second molars will be selected for the study. If more than two second molars are fully grown, we will only use two in one jaw for the study, that is, the two from the top or the two from the bottom. The two second molars will be randomly (by chance, like flipping a coin) assigned to get the left

or right second molar laser treated. One second molar will receive the laser treatment, while the other second molar will remain unsealed for the first six months. We will brush your teeth and check these teeth with a highly sensitive laser. We will take pictures of your teeth. Then, we will treat the chewing surface of one of those two second molars with a specially designed laser for several seconds.

2. You will be asked to brush twice daily with your regular fluoride toothpaste. You will be asked to use only a pea-sized amount of toothpaste on your toothbrush. You will start brushing two times daily, morning and evening, for a total of six or twelve months.
3. At 6 months, you will return to clinic for your regular dental exam, cleaning, and fluoride treatment. At this appointment, the dentist will brush your second molars, examine them, take pictures of your teeth, and check them again with a highly sensitive laser. If your second molar(s) have lost significant amount of mineral content (that is still not large enough to be considered a cavity), then both second molars will be sealed with a dental sealant (a smooth plastic lining to protect the chewing surface of your tooth). You will have completed the study.
4. If your molar(s) have not lost significant mineral content, you will be asked to return in another 6 months for your regular dental exam, cleaning, and fluoride treatment. At this appointment, the dentist will again brush your teeth, take pictures of your teeth, and check your second molars with a highly sensitive laser. Both second molars will be sealed with a dental sealant, and you will have completed the study.
5. You may be withdrawn from the study without your agreement if the researchers believe it is in your best interest for safety concerns or if you cannot follow study procedures.

This study will not require you to have an additional examination visit. The information in this consent form will be explained to you, and you will have the opportunity to ask questions and to decide if you would like to participate in this study.

All study procedures (dental exam, tooth mineral analysis, laser treatment, and sealants) will be done at the Dental School, the Predoctoral or the Postgraduate Pediatric Dental Clinics at the University of California, San Francisco.

How long will I be in this study?

Taking part in the study will take a total of about 1 hour 20 minutes over a period of 6 months or 1 hour 40 minutes over a period of 12 months in addition to your regularly scheduled dental exams.

Will any parts of this study hurt or have other risks?

1. Risks and discomforts are expected to be small.

An occasional (1% to 10%) minor risk is damage to your gums, near the tooth by the cleaning gel that is used to prepare the teeth prior to placing the sealants – this leads to a small sore on your gums which is similar to a sore by getting hurt from a standard dental instrument. This heals rapidly. This danger will be made even smaller by using a high speed vacuum to remove the cleaning gel prior to rinsing with water.

Another occasional minor risk is to irritate the gums by contact with the laser beam. Those superficial lesions of the gums will heal on their own without any intervention in a short period of time (1 – 3 days).

The teeth that will be studied are scheduled for dental sealants regardless of whether or not you participate in the study. The laser treatment will not affect the neighboring teeth.

The use of lasers requires safety glasses for the patient as well as the doctors and everyone else in the treatment area to protect the eyes from accidental exposure to the laser beam. Laser safety eyewear will be used during the whole procedure.

2. Randomization: Your second molars will be assigned to either receive laser intervention or no intervention by chance (like flipping a coin). One tooth will go untreated for either 6 months or 12 months even though you are at high risk for cavities, but as dental decay in teeth like yours normally takes an average of four years, waiting six months will not significantly increase the risk.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, Dr. Tiffany Hsu, if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call her or her staff at (415) 476-3276, Mon-Fri from 8:30am-5:00pm.

Treatment and Compensation for Injury: If you are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at (415) 476-1814.

Will you benefit from being in this study?

There will be a potential direct benefit to you from participating in this study if the laser is proven to have long-lasting, protective effects. The teeth in the study will be protected from developing cavities with sealants. It is hoped that the information

gained from the study will help the researchers learn more about how well the laser can be used to prevent dental decay (cavities).

What are your choices?

If your parents agree, you can be in this study if you want to. But you don't have to be in it if you don't want to. Nobody will get mad at you if you don't want to do this.

If you don't want to be in the study, you will receive the same dental treatment, including the dental sealants, as it was already planned, but without having to undergo the procedures (tooth mineral content analysis and laser treatment) involved in the study.

Will my medical information be kept private?

Participation in research may involve a loss of privacy, but information about you will be handled as confidentially as possible. Representatives from the Food and Drug Administration (FDA) may review information about you to check on the study. If you sign this consent form, you are allowing the FDA to review your medical and dental records. Your name will not be used in any published reports about this study. Your name, telephone number and address will be kept on record at UCSF in the event you need to be contacted in case follow-up is needed for any reason, but we will take measures to keep this information private.

Can I stop being in the study?

If you decide to be in the study now and you change your mind later, that's okay, too.
You just have to tell the study doctor or the study staff as soon as you change your mind, and you will be taken out of the study.

Will the study cost you money?

If you choose to participate in the study you will not be charged for any of the <u>additional</u> study treatments or procedures. We will cover all costs concerning the placement of dental sealants for the two teeth used in this study.

Will you get any payment for being in the study?

In addition to the free placement of sealants, in return for your time, effort and travel expenses, you will receive a $10 gift card for the child/adolescent and $10 gift card for the parent at baseline exam. A $20 gift card will be given to the child at the 6 month recall exam and 12 month recall exam. A $20 cash bonus will be received by

the child upon completion of the study at either the 6 month recall exam or 12 month recall exam.

What if you have questions?

This study has been explained to you by Dr. Tiffany Hsu or the person who signed below and your questions were answered. If you have any other questions about the study you may call Dr. Tiffany Hsu or her associate at (415) 476-3276 between 8:00 in the morning to 5:00 in the afternoon. The principal investigator for the study is Dr. John Featherstone, telephone 415-476-0456.

If you have any comments or concerns about participation in this study, you should first talk with the clinical investigator (Dr. Hsu). If for some reason you do not wish to do this, you may contact the Committee on Human Research, which is concerned with the protection of volunteers in research projects. You may reach the committee office between 8:00 and 5:00, Monday through Friday, by calling (415) 476-1814, or by writing to the Committee on Human Research, Box 0962, University of California San Francisco, San Francisco, CA 94143.

What if you want to be in the study?

You have been given copies of this assent/consent form and the Experimental Subject's Bill of Rights to keep.

You will be asked to sign a separate HIPAA Authorization Form authorizing access, use, creation, or disclosure of health information about you.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You may stop participating in this study for any reason and at any point without jeopardy to your medical care status or being in a future study. You may do this by telling the study Investigator (Dr. Hsu) or other study staff that you wish to stop your participation in the study.

Your participation may end before the study is completed if the investigator feels it is in your best interest for safety concerns or if he feels you are not properly following the study instructions. Your second molars will still receive dental sealants according to your dental treatment.

If you wish to participate in this study, you should sign on Page 6.

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Please read the sentence below and think about your choice. Please put your initials in the YES or NO Box.

Someone may contact me in the future to ask me or my child to take part in more research.

YES	*NO*

Subject's Assent and Parental/Legal Guardian Consent

Based on what I have read on the first five pages, I give my consent to participate in this research study. I may drop out of or be withdrawn from the study without jeopardizing my participation in future studies. I acknowledge getting a copy of this informed consent statement.

_____

Name of Child Participating in Study (Please Print)

_____

Date Subject's Signature for Assent

The person being considered for this study is unable to consent for himself/herself because he or she is a minor. You have been asked to give your permission to include your child in this study.

_____

Date Parent's or Legal Guardian's Signature

_____

Date Person Obtaining Consent

Appendix 4: Recruitment Flyer

UCSF Pediatric Dental Research Study

ATTENTION PATIENTS!!!

A study to look at a new way to protect you from getting cavities!!

- **If you are 12-17 years old**
- **If you have 2 permanent 2nd molars**

You can participate in a laser study to test prevention of tooth decay!

What it involves:

1. FREE dental exam
2. FREE dental sealants (worth over $40)
3. A dental exam of your teeth will be conducted at the start, middle, and end of the study
4. up to $80 when you complete the study
5. FREE laser analysis and treatment of your tooth

Cavity Protection Study
Dr. Tiffany Hsu (415) 476-3276

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Author Signature

6/9/2009

Date