Title
Enhancing Caries Resistance in Occlusal Fissures with a Short-Pulsed 9.6µm Carbon Dioxide Laser and Fluoride

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Enhancing Caries Resistance in Occlusal Fissures with a Short-Pulsed 9.6 μm CO_2-Laser and Fluoride.

by

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THESIS

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DEDICATION AND ACKNOWLEDMENTS

To my loving wife Pamela, and my entire family, especially my parents (Ilene and Louis Charland).

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ABSTRACT

Background and Objectives: High caries prevalence in occlusal pits and fissures warrants novel prevention methods. Treatment of occlusal surfaces with a short-pulsed CO_2 9.6 µm wavelength laser has previously been proposed as a method for caries prevention. The objective of the *in vitro* study part was to determine which non-destructive diagnostic method is clinically applicable and reliable at resolving early enamel changes in occlusal fissure caries created in the laboratory.
The objective of the *in vivo* study part was to conduct a single-blinded, randomized clinical trial of occlusal caries inhibition using short-pulsed 9.6µm CO_2-laser irradiation and fluoride varnish (FV) compared to FV alone, quantified by ICDAS II visual inspection, DIAGNOdent, quantitative light-induced fluorescence (QLF), optical coherence tomography (OCT), and SoproLife.
In Vitro Methods and Results*:* A sample of 20 extracted human molars were measured before and after demineralization-remineralization pH-cycling with ICDAS II visual inspection, DIAGNOdent (Kavo, Charlotte, NC), QLF (Inspektor Research Systems, Inspektor Pro, Amsterdam, Holland), SoproLife white daylight and blue light-induced fluorescence modes (Acteon Imaging, SoproLife, Marseille, France), OCT (NRC-IBD, Winnipeg, MB) and polarized Raman spectroscopy (PRS). Per tooth, one part of the fissure was subjected to laser treatment using a short-pulsed CO_2 laser at 9.6 µm wavelength with a fluence of 3.5 J/cm^2, 20 Hz pulse repetition rate, 20 µs pulse duration, angulated handpiece, and focus diameter of 600 µm, while the other fissure part was left untreated as control. The teeth were subjected to a demineralization-remineralization pH-cycling for 9 days. Cross-sectional micro-hardness testing was done as a gold standard to compare results with findings from the other detection methods used. Due to the small sample size reported, the trend observed was that laser treated fissures demonstrated a smaller relative mineral loss ΔZ than the controls. QLF findings followed a similar trend.
Using a rotary catheter probe, OCT measurements were acquired from the various fissures to generate circularly mapped OCT depth images. PRS measurements of parallel- and cross-polarized spectra were acquired with a Raman microscope system. Preliminary OCT images showed differences in the initial air-tooth interface, with PRS results indicating a change in the surface property along with biochemical alterations after pH-cycling. Also following pH-cycling, an increase in the OCT subsurface light backscattering intensity in the control fissures was observed compared to the laser test fissures. Porphyrin based fluorescence methods like DIAGNOdent and SoproLife, respectively demonstrated only additional light scattering due to the demineralization process.
In Vivo Methods and Results: Twenty high caries risk participants, ages 10-17, were recruited from UCSF Dentistry clinics. Untreated, non-cavitated cross-arch permanent second molars were randomly assigned to test or control groups. A baseline assessment included: QLF, visual inspection (ICDAS-II), SoproLife, DIAGNOdent, and OCT. Test teeth were irradiated with the same parameters as described in the *in vitro* study part, and then reassessed with all methods. FV was then applied to all molars. At 6-months, all clinical assessments performed at baseline were repeated. In the *in vivo* study, ICDAS II visual examination was the only detection method to demonstrate with statistical significance ($P<0.05$) that 9.6 µm short-pulsed CO_2 laser inhibited early caries after 6-months in high caries risk subjects. A strong tendency supporting this result was found with QLF and SoproLife images captured under both white and blue light sources. The author hopes that OCT data may add power to the current findings and analysis of future 12-month data may show current trends becoming statistically significant.
Conclusion: ICDAS II visual detection demonstrated that short-pulsed 9.6 µm CO_2 laser inhibits occlusal caries and that at this time ICDAS II appears to be the most accessible and relevant method of assessing early carious changes of the pit and fissure system.

Keywords:
CO_2 laser, 9.6 µm wavelength, microsecond pulsed, in vitro caries prevention, optical coherence tomography, polarized Rama spectroscopy, micro-hardness, quantitative light-induced fluorescence, SoproLife light-induced fluorescence evaluator

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1. LITERATURE REVIEW AND INTRODUCTION

1.1. Dental Caries: Sizing-Up the Disease

Dental caries is a diet influenced bacterial disease, which results in acid dissolution of the inorganic components of teeth. Over the last two decades caries prevalence in the permanent dentition has declined nearly ten percent in American children aged 9-16[1]. While this remarkable decrease in caries is significant, 42% of the population in this age range is still infected with dental caries[1]. Nearly 80% of all carious lesions in adolescences involve the occlusal pits and fissures[2]. The disease is known to be preventable in non-inoculated individuals and can be arrested in individuals with active disease.

1.2. Background: Pit and Fissure Caries Prevention

Currently, there are two common professionally delivered techniques towards caries prevention: pit and fissure sealants, and topical fluoride. Other interventions such as diet counseling or motivational interviewing for oral hygiene improvement will not be discussed due to their high dependence on daily patient compliance. The high proportion of occlusal decay suggests that current preventive practices are inadequate whether it is due to the method, technique or patient access to preventive care. This background section will review the current prevention techniques for early (non-cavitated) pit and fissure caries.

Fluoride application and sealants

At the earliest stage, a carious lesion is defined as the loss of mineral from the enamel surface due to the acidic products of oral bacteria. Enamel can be protected from acid demineralization by means of topical fluoride[3]. In a review of the topical benefits of fluoride, Featherstone (2004) discusses how enamel's carbonated hydroxyapatite can be made more resistant to acid dissolution with the replacement of hydroxyl groups for fluoride ions. Another benefit of topical fluoride is

its capacity to strengthen already demineralized enamel, thus inhibiting the caries progression[4]. Unfortunately, the application of topical fluoride appears to be more effective in the prevention of smooth surface caries then in preventing occlusal fissure caries[5].

In a 2006 Cochrane review, a systematic review of the literature comparing fluoride use to sealants in the prevention of pit and fissure caries was performed. Hiiri et al (2006) as primary author of the Cochran review concluded that although there is insufficient literature to accurately quantify the difference, sealants are more successful in reducing pit and fissure caries than fluoride varnish[6]. Beauchamp et al (2008) made clinical recommendations for the use sealants in the areas of: caries prevention, placement over non-cavitated carious lesions, the use of glass ionomer versus composite resin sealants and placement techniques.[7]

One of the problems with pit and fissure sealants is their limited time of retention and need for regular maintenance. According to Feigal (1998), 5-10% of pit and fissure sealants will require annual repair or replacement[8]. US data collected between 1999 and 2002 shows that 30.5% of American children ages 6-11 have sealants on permanent teeth[1]. Sealants are most effective when the appropriate risk analysis was performed, and vigilant recalls are followed [9]. This suggests that sealants are contra-indicated in patients who do not have routine periodic oral exams.

LASERS in Dentistry

The capability of lasers to modify enamel surface properties has promised a new type of caries prevention therapy. Popular lasers used in dentistry are from the Erbium family: the Er:YAG laser with an emission wavelength of 2970 nm and the ErCr:YSGG laser (wavelength 2780 nm)[10]. These lasers are efficient at cutting hard and soft tissue, and have even been shown to produce acid resistance in vitro[11]. Currently, there is no information available showing the

clinical uses of Er,Cr:YSGG, 2780 nm lasers for caries prevention. Specific Carbon dioxide (CO_2) lasers have been better studied for caries prevention.

In-Vitro Caries Prevention with Pulsed CO_2 LASERS

McCormack et al. (1995) found that the surface and subsurface enamel crystals could be fused with a pulsed CO_2 laser of 9.3 µm or 9.6 µm, and 10.3 µm however warned that adverse surface effects are seen outside of specific parameters[12]. The enamel subsurface can be made appreciably more resistant to acid dissolution by being treated with transverse excited atmospheric pressure (TEA) pulsed CO_2 lasers[13]. This was further refined by the same group of researchers who uncovered that a CO_2 laser with a wavelength of 9.3 µm and 9.6 µm, applied for 25 pulses at 1 to 3 J/cm^2 inhibited 70% of artificial caries[14]. Remarkably, although the subsurface heats to greater then 800 ºC, less than a 1 ºC temperature change was observed at a 2 mm depth[14]. TEA CO_2 laser therapy heats the enamel surface and subsurface to a depth of 10 microns, causing the release of carbonate from the carbonated apatite, resulting in a more resistant hydroxyapatite tooth surface[15]. The use of a TEA CO_2 laser of 9.6 µm wavelength pulsed delivering a total of 2.4 J or 4.8 J had no lasting pulpal adverse side effects reported by the patients or histological signs of inflammation[16], suggesting this type of therapy is safe for clinical use.

Nobre dos Santos et al.[17] found that a TEA CO_2 laser at 25 pulses per spot, 5-8 µs pulse duration, followed by fluoride application significantly inhibited and reversed occlusal caries progression in vitro. To test caries reduction, they used micro-radiography as the gold standard. Considering this technique requires sectioning teeth, it is not useful for in vivo analysis of caries progression.

In-Vivo Caries Prevention with Pulsed CO_2 LASERS

The clinical application of CO_2 laser therapy has been examined in a clinical prospective trial. Rechmann et al[18] took a group of 24 subjects (mean age: 14.5 years) with premolars scheduled for extraction for orthodontic reasons: 12 each for a) a 4-week arm and b) a 12-week arm. Orthodontic brackets were placed on those premolars with a conventional composite resin and an area next to the bracket was irradiated with a pulsed CO_2 laser, wavelength 9.6 μm, pulse duration 20 μs, pulse repetition rate 20 Hz, beam diameter 1,100μm, average fluence 4.1 ± 0.3 J/cm^2, 20 laser pulses per spot. An adjacent non-irradiated area was used as the control surface. Premolars were extracted after 4 and 12 weeks respectively for a quantitative assessment of demineralization by cross-sectional micro-hardness testing. The laser treatment produced a 46% demineralization inhibition after 4 weeks ($p<0.04$) and a marked 87% inhibition for the 12-week arm ($p< 0.002$). This study shows, for the first time in vivo, that the specific CO_2 pulsed laser irradiation can be used successfully for the inhibition of dental caries in enamel in human mouths.

The *in vivo* application of pulsed CO_2 laser irradiation for the prevention of occlusal caries has been studied in our group[19]. Hsu et al demonstrated a significant reduction in relative mineral loss as shown with QLF ($p=0.01$). The use of a straight laser handpiece, which made delivering laser pulses to the occlusal pits and fissures of 2nd permanent molars very difficult, was identified as possible source for confounding results.

In an *in vitro* study comparing sodium fluoride treatment followed by CO_2 laser treatment to CO_2 laser treatment alone, the addition of sodium fluoride to CO_2 laser treatment significantly reduced Ca^{+2} solubility ($p<0.01$)[20]. This exciting result should be pilot tested *in vivo* and may offer an alternative option to placing occlusal sealants.

1.3. Background: Methods of Early Caries Detection

Explorer and radiographs

Currently, commonly used caries detection methods in the United States include visual inspection, tactile use of a dental explorer, and radiographs. Studies in Europe have shown that the explorer is only correct less than 50% of the time[21]. 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[22]. By the time an occlusal caries lesion is detectable radiographically, it is too large to be remineralized[22]. 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 stop or reverse the caries process[22].

ICDAS II

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 II (ICDAS II) provides a standardized method of lesion detection and assessment, leading to caries diagnosis[23]. ICDAS II assigns scores to lesions based on apparent caries status and lesion severity of plaque-free teeth[23]. Of particular interest to this study are the coronal primary caries detection criteria, specifically for the occlusal sites. The ICDAS II detection codes for coronal pits and fissures caries ranges from 0 to 6 as seen in Figure 1 (code 0 to 3) and described in Table 1[23]:

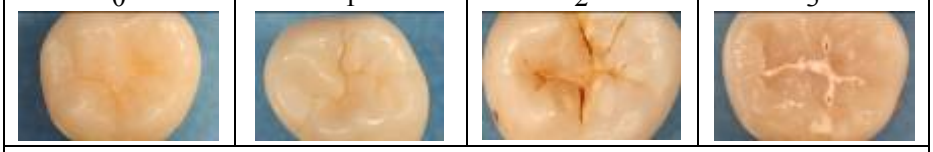

Figure 1: ICDAS II system scores 0-3; score 0 – sound enamel; score 1 - first visual change in enamel; score 2 - demineralization extending from the base of the pit and fissure areas onto the slopes; score 3 – first loss of enamel.

Table 1: ICDAS II Codes for Coronal Caries

Code	Description
0	Sound
1	After prolonged air drying, the first visual change in enamel demineralization is seen limited to part or all of the base of the pit and fissure system
2	After prolonged air drying, a distinct demineralization change in enamel from part or all the base of the pit and fissure system extending up the slopes (walls) towards the cusps
3	After prolonged air drying, the first visualized breakdown of enamel, often localized to the base of the pit and fissure system, without visual signs of dentinal involvement
4	Underlying dark shadow from dentin
5	Distinct cavity with visible dentin
6	Extensive distinct cavity with visible dentin

*See Appendix i for more thorough description of ICDAS Codes

Laser Fluorescence

Laser fluorescence detects early caries by analyzing the fluorescence emission spectrum of carious regions versus sound tissues. Studies have shown that laser fluorescence is useful as a quantitative measure distinguishing carious from non-carious surfaces[22]. Two main methods of laser fluorescence have been studied: KaVo DIAGNOdent and quantitative light-induced fluorescence (QLF).

DIAGNOdent (Kavo, Charlotte, NC) is a laser (665 nm) caries detection system with an audible signal and digital readout. The system emits light that causes fluorescence of bacterial porphyrins; after filtering out emitted light, sensors capture and quantify fluorescence in real-time. DIAGNOdent's tip design allows for assessment of occlusal pits and fissures.

QLF (Inspektor Research Systems, Inspektor Pro, Amsterdam, Netherlands) is a technology based on the fluorescence of tooth structures. Blue light (mean λ=390nm) is sent to the tooth and green light is fluoresced from the tooth, whereby the fluorescence of a tooth is related to the

mineral content. When compared to conventional visual examination for caries detection, QLF can detect twice as many early non-cavitated demineralized enamel areas[24]. Its ability to detect and quantify changes in clinically visible white spot lesions allows QLF to determine the impact of preventive measures on inhibition of demineralization[24]. While QLF has been proven to be an effective method of detecting smooth surface demineralization, its effectiveness in detecting occlusal caries has yet to be proven[22].

Intra-Oral Digital Photography

Intra-oral digital photography has been shown to improve caries diagnosis for restorative treatment decisions of the occlusal surfaces on posterior teeth compared to visual exam and surgical microscopes[25]. SoproLife (Acteon Imaging, SoproLife, Marseille, France) is an intra-oral dental imaging system, which allows imaging of teeth at high magnification in a white daylight mode and illuminated with a blue (450 nm) light source in a fluorescence mode. Surface and subsurface composition determine the auto-fluorescence of the tooth; healthy dental structures appear green, demineralized (porous) enamel appears grey, while more complex lesions return varying intensity reds or dark signals[26]. In combination with clinical judgment, SoproLife's camera may be helpful in detecting early caries.

Optical Coherence Tomography

Optical Coherence Tomography (OCT) is a non-ionizing imaging technique, which can produce cross section images of biologic tissues such as ocular, intravascular, gastrointestinal, epidermal, soft oral tissues and teeth [27-31]. Fried et al (2002) demonstrated that polarized sensitive OCT (PS-OCT) can be correlated with the degree of demineralization and lesion severity[32]. They proposed a potential utility for the system as monitoring *in vivo* caries lesion changes. Jones et al (2006) showed the advantages of a PS-OCT system in an artificial caries model by correlating OCT

images lesion depth to those from sectioned micro-radiography, as well as quantifying lesions severity [32]. The same group more recently applied PS-OCT to an *in vitro* study which concluded that the system is a good tool to monitor caries inhibition in enamel from CO_2 laser treatment in addition to fluoride[33].

Polarized Raman Spectroscopy

Polarized Raman spectroscopy (PRS) yields biochemical information about the molecular composition and molecular orientation/structure of tissues. Biochemical changes due to demineralization and remineralization can be followed using PRS by analyzing changes to a prominent spectroscopic band derived from the phosphate of hydroxyapatite, localized at ~959 cm^{-1} in sound tissues. Ko et al. have previously shown that carious and sound enamel can be differentiated by PRS through the depolarization ratio that provides information on changes in the orientation and/or structure of the enamel rods found within the enamel matrix[34].

1.4. Summary of Literature Review and Conclusion Of Introduction:

Nearly 80% of all of carious lesions in adolescence involve the occlusal pits and fissures[2]. Currently, there are two common professionally delivered techniques towards caries prevention: pit and fissure sealants, and topical fluoride. Long term maintenance requirements and need for frequent re-application respectively make fissure sealants and topical fluoride non-ideal solutions to prevent occlusal caries[7]. Thus there is a need for a novel approach to occlusal pit and fissure caries prevention.

Carbon dioxide lasers with at a specific wavelength (9.3 or 9.6 μm) and pulse characteristics (pulse duration 2-100 μs) in addition to fluoride treatment can offer a novel alternative to caries

prevention in pits and fissures. There is no ideal non-destructive clinical method of monitoring changes identified in the current scientific literature.

1.5. Significance

If the CO_2 laser irradiation combined with fluoride treatment proves to be effective in preventing caries in vital teeth, then this could lead to an improved method of treating pit and fissure caries, the most prevalent site for caries today. If proved to be just as effective as sealants, then CO_2 laser treatment could potentially be standardized to treat other tooth surfaces of high caries risk patients in the future.

2. PURPOSES, HYPOTHESES AND RESEARCH AIMS

This paper describes an *in vitro* study and an *in vivo* study in two parts:

2.1. *In Vitro* Study Purpose, Hypothesis and Research Aims

The purpose of the *in vitro* study part was to investigate the utility of optical coherence tomography in identifying early carious changes in occlusal pits and fissures of extracted human teeth treated with specific laser compared to a control. This investigation was done utilizing CO_2 laser irradiation (wavelength 9.6μm) for demineralization prevention prior to a laboratory demineralization-remineralization pH-cycling simulating approximately 6 months of high caries challenge (demin-remin). Fissures were examined at three time points: baseline, post-laser treatment and post-demin-remin by ICDAS II visual exam, DIAGNOdent, QLF, SoproLife imaging, OCT and PRS. After demineralization-remineralization cycling, the teeth were examined using the same examination methods and then sectioned and subjected to cross-sectional micro-hardness tests.

The hypothesis was that there exists a non-destructive clinically applicable method of reliably resolving early enamel changes in occlusal fissure caries *in vitro*.

Aim 1: To compare data collected from ICDAS II visual inspection, DIAGNOdent, QLF, SoproLife imaging, OCT and PRS, from before and after laboratory demineralization-remineralization pH-cycling with the relative mineral loss (ΔZ), as measured by cross sectional micro-hardness tests in the occlusal fissures of laser treated molars and non-treated fissure parts as control.

2.2. *In Vivo* Study Purpose, Hypothesis and Research Aims

The purpose of this *in vivo* study part was to conduct a 12 month examiner-blinded pilot scale prospective randomized clinical study of caries inhibition in humans on occlusal pit and fissure tooth surfaces following pulsed CO_2 laser irradiation (wavelength 9.6μm) in addition to fluoride therapy and quantifying early occlusal enamel demineralization by ICDAS II visual inspection, DIAGNOdent, QLF and OCT, as well as qualifying changes with data from SoproLife images.

The hypothesis tested was that CO_2 laser treatment in addition to fluoride therapy results in changes in crystal composition and structure which increase the resistance of dental enamel mineral to dissolution by acid and will work to better prevent dental caries in the occlusal surface of vital teeth when compared to fluoride therapy alone.

Aim 1: To compare the relative mineral loss (ΔF), as measured by QLF, in the occlusal pits and fissures of CO_2 laser treated molars with the relative mineral loss (ΔF) in the occlusal pits and fissures of control molars, *in vivo*.

Aim 2: To compare the efficacy of early caries detection in occlusal surfaces by 1) ICDAS II visual inspection, 2) DIAGNOdent 3) QLF, 4) SoproLife, and 5) OCT *in vivo*.

3. METHODS & MATERIALS

Introduction to Methods:

In vitro and *in vivo* treatment and detection methods were very similar. Treatment groups in both studies were irradiated with short-pulsed CO_2 9.6 μm laser with the same specifications. All diagnostic methods were performed in both *in vitro* and *in vivo* studies with the exception of polarized Raman spectroscopy and micro-hardness testing being limited to the *in vitro* study. For simplicity, the methods for the *in vitro* and *in vivo* studies are presented as fit to the study parts in section 3.1 and 3.2 respectively.

3.1. *In Vitro* Study Methods:

Twenty extracted molars were selected and the test and control part of one fissure per each tooth were marked. Baseline measurements were taken from control and test fissures. The test fissures were then irradiated with a short-pulsed CO_2 9.6 μm laser and re-assessed with all diagnostic techniques. Subsequently the teeth were subjected to demineralization-remineralization pH-cycling to simulate a high cariogenic challenge over a 2-week period of time. The teeth were measured again utilizing all techniques, and finally sectioned and tested with cross-sectional micro-hardness.

Tooth Selection

Twenty unfilled extracted human molars with a continuous fissure greater than 8mm in length, or two parallel fissures of minimum 3 mm length each, were selected from a bank stored in 0.1% thymol solution. There were 5 teeth for each of ICDAS II codes 0, 1, 2 and 3. Using a thin carbide dental bur (169L), perpendicular marks were created to clearly delineate the test and

control fissures of each molar (figure 3a). As well, an "X" was marked at the proximal end of the tooth nearest the test fissures.

Baseline Measurements

Each fissure was analyzed with DIAGNOdent, QLF, and SoproLife in daylight and fluorescence modes. Teeth were re-assessed following laser treatment with ICDAS II, DIAGNOdent, QLF, SoproLife, OCT and PRS. All measurements were recorded with the occlusal surface of the teeth dried with compressed air for a minimum of 5 seconds.

QLF

Using QLF Inspektor Research Systems' software "White Spot Wizard", a circle or ellipse shape was drawn surrounding the area to be used by the software which calculated the loss of fluorescence radiance in the area of the caries (area within the ellipse) compared to the reference area (black line is on "healthy, reference" enamel) (figure 2a and 2b) and is expressed as a percentage fluorescence loss (ΔF in %)[35]. The reference line was manipulated to "de-activate" segments of the line that were believed by the user to not lie on sound enamel and thus not be used as reference areas.

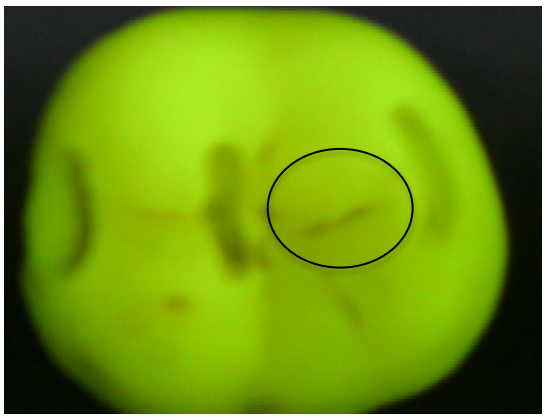

Figure 2a: Baseline QLF image after short-pulsed CO_2 laser treatment (left), with relative mineral loss (ΔF) analysis ellipse over control fissure. This image was taken before demineralization-remineralization pH-cycling.

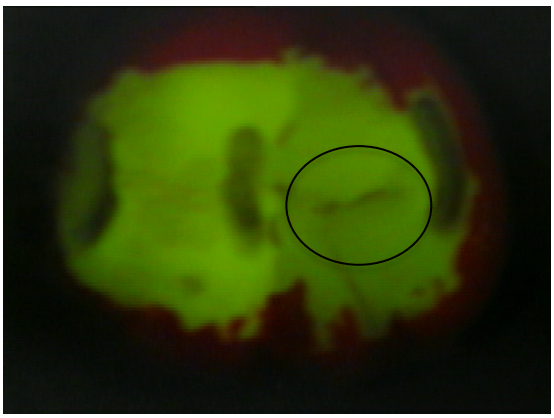

Figure 2b: QLF image after short-pulsed CO_2 laser treatment (left), with relative mineral loss (ΔF) analysis ellipse over control fissure. This image was taken after demineralization-remineralization pH-cycling (same tooth as figure 2a, nail varnish covering outer tooth surface).

SoproLife

In order to facilitate analysis, we developed scoring systems for the SoproLife images; the white light images were scored applying the ICDAS II code system, the blue light images were coded: green (1), grey/white (2), orange/red (3), dark/brown (4). All teeth were analyzed with SoproLife after cleaning and thorough air-drying.

Optical Coherence Tomography

OCT (NRC-IBD, Winnipeg, MB) images were acquired with a custom developed rotating and linear pullback catheter probe tip. The swept-source OCT (SS-OCT) system (Novacam Technologies, Pointe Claire, QC) was configured as a Mach-Zehnder interferometer with balance detection. The system operated with a Santec source (Aichi, Japan) operating at a sweep rate of 30 kHz and sweeping at half maximum of over 98 nm around 1326 nm. The theoretical OCT resolution was 8 μm. Typically, 4 second pullback scans at 1.5 mm/sec were used to measure

along the ~6 mm region of interest incorporating the fissure and adjacent burr marks used as reference points resulting in 50 μm step sizes per image frame. Image acquisition was at 30 frames/sec with 30,000 A-scans/sec. Data was collected from matched tomograms before and after demineralization-remineralization pH-cycling.

Polarized Raman Spectroscopy

Parallel-polarized (p1) and cross-polarized (p2) Raman spectra were acquired with a LabRamHR confocal Raman microspectrometer (Horiba Jobin-Yvon, Edison, NJ) with 830 nm laser excitation, as described previously[34]. Each Raman spectrum was collected for 15 seconds with 3 co-additions for a total acquisition time of 45 seconds. The analytic laser was focused to a spot through a Nikon 10x microscope objective with a power of 125 mW. At each fissure, PRS spectra were acquired from 5 locations to match regions of interest as revealed by OCT imaging. For spectral comparisons, spectra were normalized relative to the p1 intensity of the ~959 cm^{-1} Raman band.

Laser Treatment Protocol

A short-pulsed CO_2 laser, (Pulse System Inc. Model #LPS-500, Los Alamos, NM), wavelength 9.6 μm, pulse duration 20 μs, pulse repetition rate 20 Hz, beam diameter at focus roughly 0.6 mm delivered through a contra-angle handpiece was used in conjunction with air cooling. The test fissure length was measured and the number of laser pulses and corresponding irradiation time respectively was calculated; the goal was to irradiate each spot of the test fissure with 20 laser pulses. The laser fluence used in this study was an average of 3.5 J/cm^2. Fluence was checked after every fifth treatment, the laser energy output was verified with an energy meter (Energymax 400, Molectron Detector, Inc., Portland, OR).

Demineralization-Remineralization pH-Cycling Protocol for Simulation of High Cariogenic Challenge

A high caries challenge was simulated via demineralization–remineralization pH-cycling as described by Featherstone et al[14]. An etch resistant varnish (Revlon nail polish) was applied to all areas of the teeth sparing the fissures in order to protect the remainder of the tooth from exposure to buffer solution.

In each 24-hour period, each tooth was bathed in 40 ml of acetate/calcium/phosphate demineralizing buffer (calcium and phosphate at 2.0 mmol/L, 0.075 mmol/L acetate) at pH 5.0 for 8 hours at 37° Celsius. The teeth were then rinsed with de-ionized water and placed in a calcium phosphate remineralizing solution (0.8 mmol/L calcium, 2.4 mmol/L phosphate,

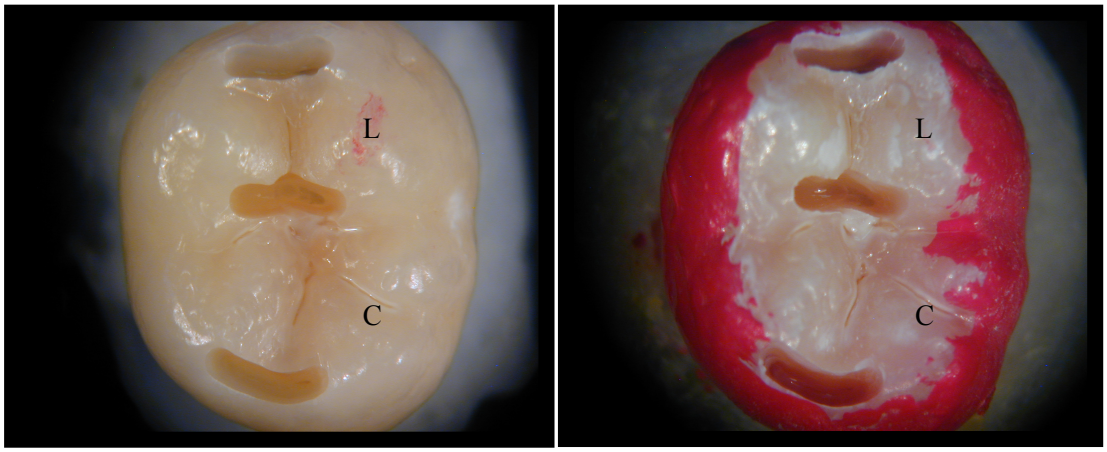

Figure 3a (left): Clinical picture of the occlusal surface of a molar: the cross cuts of the central fissure divide the tooth in half horizontally. The upper half of the tooth represents the laser treated fissure (L) of the extracted molar, with the lower half of the tooth showing the untreated control fissure (C).

Figure 3b (right): The occlusal surface of the same molar which has been subjected to 9 days of demineralization (8 hours) and remineralization (16 hours) cycling to simulate 4-6 months of high caries challenge. The upper half of the tooth contains the laser treated fissure (L) of an extracted molar and the lower half of the tooth contains an untreated control fissure (C) (red nail varnish enclose the occlusal surface of interest).

cacodylate 20 mmol/L and fluoride as sodium fluoride at 0.1 ppm F) at a pH of 7 overnight (16 hours) at 37° Celsius. The cycle was repeated for a total of 9 days and nights (with one

intervening weekend), which is estimated to be the equivalent of 4-6 months of high caries challenge (Figure 3b).

Post Demineralization-Remineralization pH-Cycling Measurements

After the pH-cycling, each fissure was assigned an ICDAS II score and re-analyzed with DIAGNOdent, QLF, SoproLife, OCT, and PRS. The teeth were then processed for cross-sectional micro-hardness testing.

Knoop Cross-Sectional Micro-Hardness

The molars were sectioned, embedded in epoxy resin with the cut surface exposed, serially polished and analyzed by Knoop cross-sectional micro-hardness testing. For each of the control and test fissures, the respective enamel nearer to the DEJ (left unaffected by the demineralization-remineralization pH-cycle) is used as the internal control (Figure 4).

The overall relative mineral loss, ΔZ, for each sample was calculated by creating a hardness profile curve by plotting normalized volume percent mineral against distance from the enamel surface. The area under the curve that represents ΔZ (vol% mineral x μm) was calculated using Simpson's integration rule[36, 37]. The individual ΔZ values for each lesion in each group were combined to give a mean ΔZ and standard error.

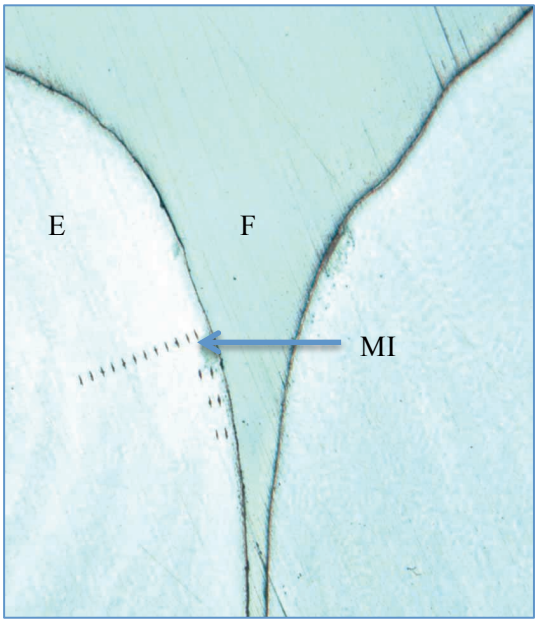

Figure 4: Photomicrograph of a cross-section of a molar fissure. After demineralization and re-mineralization pH-cycling, the tooth was sectioned vertical to the fissure of interest, embedded in epoxy resin, and serial polished. Shown is an area of the occlusal central fissure, with rows of Knoop cross-section micro-hardness indentations (MI) in the enamel (E) following a defined scatter pattern adjacent to the occlusal fissure (F).

3.2. *In Vivo* Study Methods:

Twenty high caries risk subjects were recruited and pairs of second molars were randomized as test or control teeth. Baseline measurements with ICDAS II, DIAGNOdent, QLF, SoproLife images and OCT were taken from occlusal pit and fissure surfaces of the control and test teeth. The occlusal pits and fissures of the test teeth were irradiated with a short-pulsed CO_2 9.6 μm laser and re-assessed with all diagnostic techniques, followed by fluoride varnish application. Following 6 months of clinical caries challenge, the teeth were measured again utilizing all techniques. A flowchart summary of the *in vivo* study is presented in figure 5.

The University of California San Francisco Human Research Protection Program, Institutional Review Board's Committee on Human Research approved this study (IRB study number 10-03431) (Complete CHR document can be viewed in Appendix ii).

Participant Selection

Subjects for the study were recruited from the UCSF Predoctoral, Postgraduate Pediatric Dental and Postgraduate Orthodontic clinics. The adult (>5 years old) Caries Management By Risk Assessment (CAMBRA) questionnaire used at UCSF School of Dentistry was applied to assess the caries risk status of each subject. Only subjects who were considered high caries risk were included in the study. The complete inclusion and exclusion criteria were:

Inclusion Criteria:

- 10 -17 years old at time of baseline exam
- Healthy and able to cooperate for treatment in dental chair
- High caries risk assessment (CAMBRA)

- Two fully erupted second molars with untreated, non-cavitated occlusal surfaces in the same arch (less than ICDAS II code 3)
- Residing in San Francisco or other nearby locales with community water fluoridation
- Parent/guardian able to provide written informed consent in English
- Patient able to provide written assent
- Willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form

Exclusion criteria:

- Low or moderate caries risk assessment
- No untreated or non-cavitated occlusal surfaces
- Subject is planning to leave the area and will not be available for recall visits
- Known systemic disease which could alter enamel composition or formation
- Significant medical history which may affect oral health or flora (i.e. diabetes, HIV, heart conditions that require antibiotic prophylaxis)

Written informed consent was obtained from a parent/guardian of each subject and written assent was obtained from each subject (for consent form see Appendix iii). Each subject was furnished a copy of the consent form and subject's bill of rights.

Study Procedures:

Intra-Examiner and Inter-Examiner Reliability

Both dental examiners (DC and PR) were trained with ICDAS e-learning software[38]. Each examiner (DC and PR) evaluated all posterior teeth on 7 volunteer subjects on two separate dates 1-week apart.

Baseline Exam:

Cleaning and Field Isolation

The subjects' study and control molars were brushed with a rotary prophy brush, followed by thorough rinsing. Cotton rolls were used as necessary to maintain a dry visual field. The study and test molars were air dried with compressed air from a dental air/water syringe prior to visual exam and each diagnostic technique.

Two experienced dentists (DC and PR) independently conducted visual exams using 2.5x magnification loupes and assigned ICDAS II scores to the occlusal pits and fissures of the study molars. The dentists then discussed their scoring until consensus was reached; the consensus ICDAS II codes were recorded. The area with the greatest ICDAS II code on each tooth became the 'area of interest' for all other diagnostic methods regardless of baseline or recall visits.

Study molars were scanned with DIAGNOdent, and then imaged with QLF, SoproLife (white and blue light sources) and OCT. For more details on these procedures, refer to the respective descriptions earlier. All data was recorded on respective subject's case report forms.

Randomization

Subjects' teeth were randomly assigned to test or treat by using a random number generator scheme.

Treatment with CO_2 Laser Irradiation:

The randomization assignment was then given to laser operating dentist (PR) and the test tooth was irradiated with a CO_2 laser under the same conditions as the *in vitro* treatment. The second examiner (DC) was blinded as to which tooth was treatment or control.

The teeth were re-analyzed with all diagnostics techniques (ICDAS II, DIAGNOdent, QLF, SoproLife, OCT).

Treatment with Fluoride Varnish:

Study and control teeth were air-dried and DuraShield 5% sodium fluoride varnish (Sultan Healthcare, Hackensack, NJ) was applied with a micro-brush. The subjects received instructions to not eat or drink for 30 minutes.

Three-Month Recall:

The teeth were cleaned and isolated as described for the baseline exam. The same two dentists (PR and DC) repeated the ICDAS II visual examination, DIAGNOdent, QLF and SoproLife assessments of both the test tooth and control teeth. All data was recorded on respective subject's case report forms.

Six-Month Recall:

The teeth were cleaned and isolated as described for the baseline exam. The same two dentists (PR and DC) repeated the baseline diagnostic techniques (ICDAS II visual examination, DIAGNOdent, QLF, SoproLife, and OCT) with the same method as described.

If significant demineralization was found, defined as an ICDAS II score of greater than 2 in the occlusal surface of one tooth involved in the study, a sealant would have been placed, and the subject would have come to the end point of the study.

Twelve-Month Recall:

The above-described study will extend to a twelve-month recall under the supervision of Dr. Peter Rechmann, principal investigator. The twelve-month protocol will repeat the six-month recall appointment. This will be considered the end point of the study and all teeth will be prescribed sealants.

3.3. Clinical Protocol

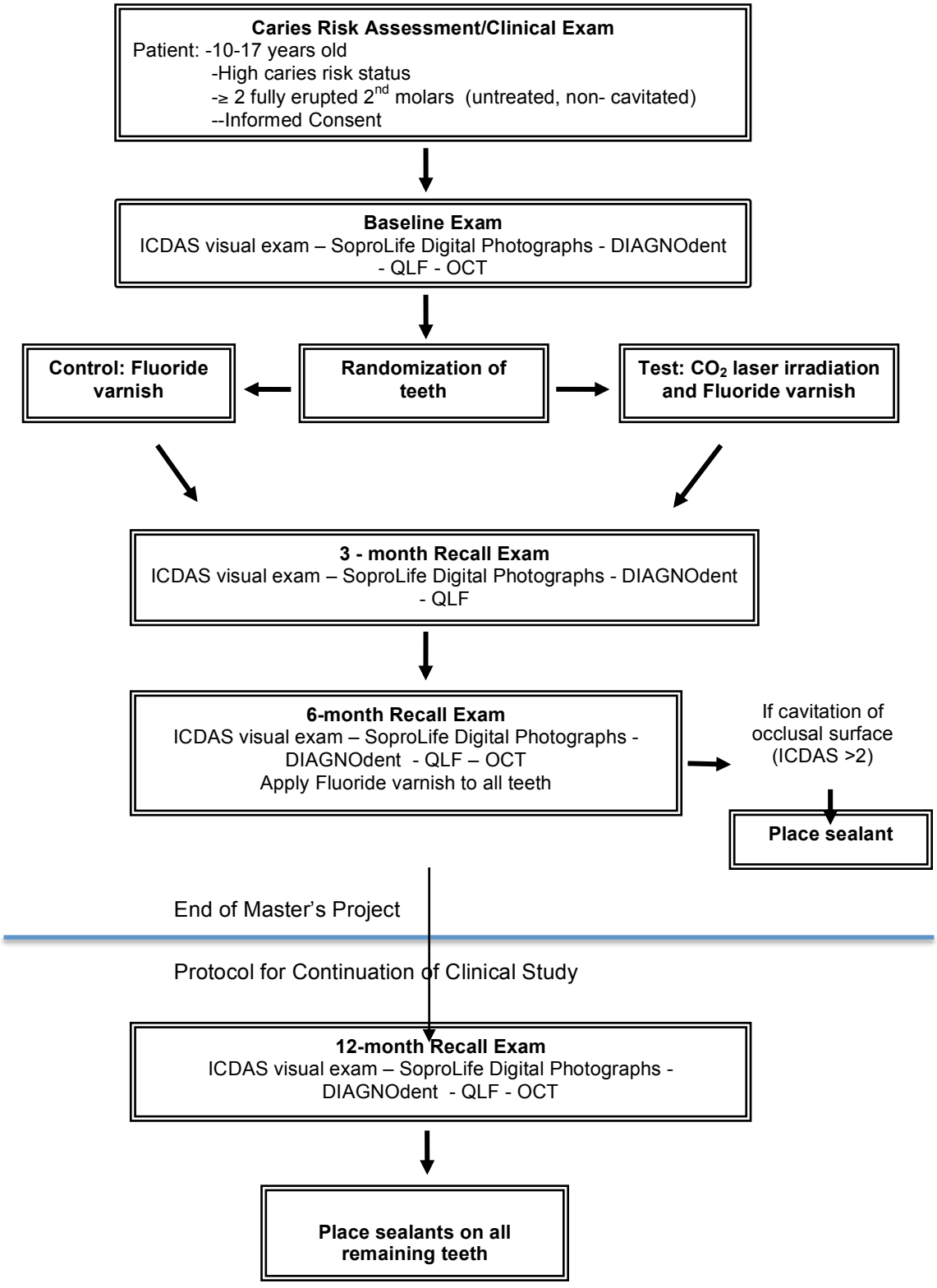

Figure 5: Summary of *in-vivo* study

Methods of Evaluating Data

All SoproLife images were evaluated by one examiner (PR), who was blinded from all image manipulation. The SoproLife white light source images were evaluated by two different methods: 1) Caries assessment coding and 2) "Better, Same or Worse". For method 1, the images were first imported into Microsoft PowerPoint, and placed one image per slide, with no subject or visit information visible on the screen. The slides were then randomized and caries assessment coding was applied according to the criteria as described in ICDAS II. In the second method, for each the control and test groups, comparable size and magnification baseline and 6-month images were placed side by side on PowerPoint slides. An ellipse was drawn surrounding the fissure corresponding to that of the highest clinical ICDAS II score at baseline exam and duplicated on the 6-month follow-up SoproLife image. The blinded examiner (PR) was unaware of the order of the teeth on any given slide (baseline or 6-month), and blinded to whether they belonged to the control or test groups. The examiner was instructed to record whether the image on the right appeared "Better, Same or Worse" in comparison to the image on the left. Criteria judged for determining "Better, Same or Worse" were: darkening of area, growth of lesion, or any indication of caries progression.

The SoproLife images captured with a blue light source were evaluated with two methods: 1) they were coded according to colors seen, as described in the *in vitro* study and 2) the images were manipulated as per the white light source images and judged "Better, Same or Worse".

The optical coherence tomography data was analyzed with the assistance of Dr. Lin-P'ing Choo-Smith at the CNRC.

3.4. Statistical Analyses:

Sample Size:

A conservative sample size was calculated in the same way as in our previous CO_2 laser studies[19, 39], using the parallel groups trial data from Gorton and Featherstone which examined demineralization surrounding orthodontic brackets on teeth scheduled for extraction[40]. That study had an effect size of 2.02; based on a 2-tailed paired t-test (alpha=.05), 80% power and a more conservative effect size of 1.0 (i.e. less than half the effect in the parallel groups study). Hsu et al's study estimated the inhibition of demineralization seen with QLF to be between 70-100%[19], based on their results, this estimation remains applicable. On this basis, a minimum of 10 study participants, each with one test and control, thus 10 teeth for each group (control and test), were needed. In an attempt to increase statistical power and to allow for dropouts and other non-compliances, 20 participants were enrolled in the study, providing a total of 20 pairs of teeth.

Statistical Analyses:

Control and intervention groups are independent. Data from any dropout subjects was discarded from the statistical analysis.

ICDAS II codes, as well as the coding for SoproLife white light and coding for blue light images are considered to be ordinal variables. These data sets are assumed to have a Gaussian distribution. The mean differences between scores from baseline to 6-month recall were analyzed using a 2-sided unpaired t-test with a significance level of $p \leq 0.05$.

Data from the "Better, Same or Worse" SoproLife analysis was categorical (nominal). This data was entered into a contingency table with the control data set as the "expected" and laser treated data set as the "observed". A Pearson Chi-squared test was applied with the H_0: control and

treated distribution of caries are not different. These data were also converted into a numerical scale (better=1, same =0 and worse= -1) and the difference between the means for control versus treatment groups was analyzed using a 2-sided unpaired t-test with a significance level of $p \leq 0.05$.

Changes in the means from baseline to 6-month recall for QLF relative mineral loss (ΔF), DIAGNOdent and cross sectional micro-hardness data comparing test teeth versus the control teeth were calculated. These data are considered numerical/interval variables. The difference in the means of control versus test teeth for each these data sets was analyzed using a 2-sided unpaired t-test with a significance level of $p \leq 0.05$.

Prism 5 for Mac OSX by GraphPad Software Inc.[41] was used for all above statistical analysis.

Inter-examiner and intra-examiner reliability testing was calculated as a weighted Kappa statistic using GraphPad's online QuickCalcs[42]. The weighted Kappa statistic is a good choice due to the ordinal nature of the data; it reflects how far apart two different data points are from another in contrast to the standard kappa statistic.

4. *IN VITRO* RESULTS

4.1. Cross-Sectional Micro-Hardness Testing

Cross-sectional micro-hardness indentation testing was done on the control fissure of 5 teeth and the test fissure of 6 teeth, which had been assigned ICDAS II codes 0 and 1 at baseline measurement. Figure 6 represents a plot of the volume percentage mineral of enamel versus depth from the outer surface to give a mineral loss profile. Each symbol on each curve represents the mean volume % mineral at each depth from 15 to 100 µm. The error bars represent standard error. At a depth of 15 µm the control teeth (square dots) show an average volume % mineral of only 58%, which increases to an average of 85% at a depth of 100 µm. In contrast for the laser treated enamel (triangular symbols) the average volume % mineral at the 15 µm depth is still 70% and increases to the typical volume % mineral content of enamel at the depth of 100 µm of 85%[43].

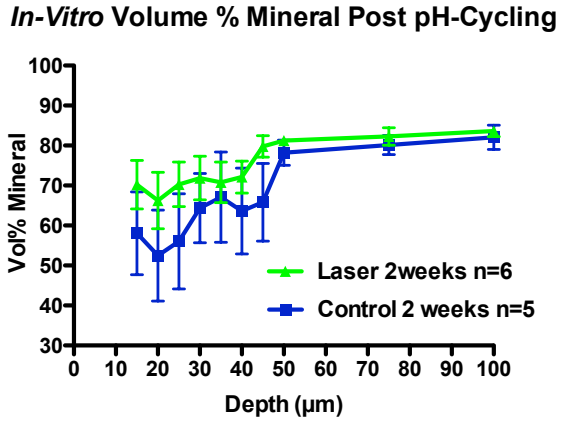

Figure 6: Depth profile of volume % mineral for the controls (square symbols) in comparison to the laser treated areas (triangular symbols) from the molars after 9 days of demineralization-remineralization pH-cycling (error bars represent standard error).

As seen in figure 7, the mean relative mineral loss, ΔZ (vol% x µm) for the laser treated enamel was 580.4 ± 156.1 n=6 (SE), while the control area showed a much higher relative mineral loss of 981 ± 298.1 n=5 (SE) following 9 days of demineralization-remineralization pH-cycling. The differences between the means of each group were not statistically significant as analyzed with an

unpaired t-test value of P=0.2. Nevertheless the laser treatment reduced demineralization by 40% in comparison to the non-laser treated control group.

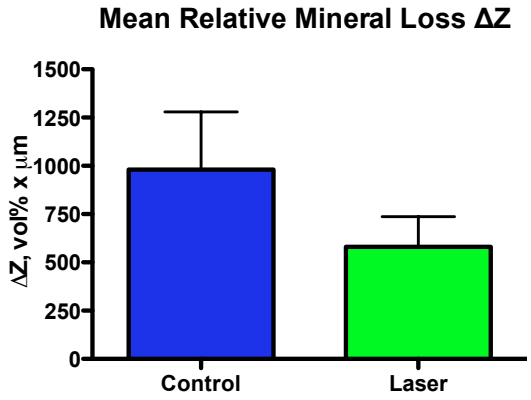

Figure 7: Relative mineral loss ΔZ (vol% x μm) for the short-pulsed CO_2 laser treated enamel (n=6) and for the controls (n=5, SE) following 9 days of demineralization-remineralization pH-cycling. The laser treated group showed a 40% reduction in relative mineral loss (P=0.2)

4.2. Quantitative Light-induced Fluorescence

When comparing the changes in mean relative mineral loss, ΔF, determined by QLF, between baseline and post pH-cycling, the mean change for the laser treated enamel was 0.13 ± 0.55 (SE) n=20, while the control area showed a higher mean change in relative mineral loss of 0.83 ± 0.59 (SE) n=20 (Figure 8). The change in mineral loss in the non-laser treated group was 6.4 times higher than in the laser treated group but the difference was not statistically significant (P=0.2).

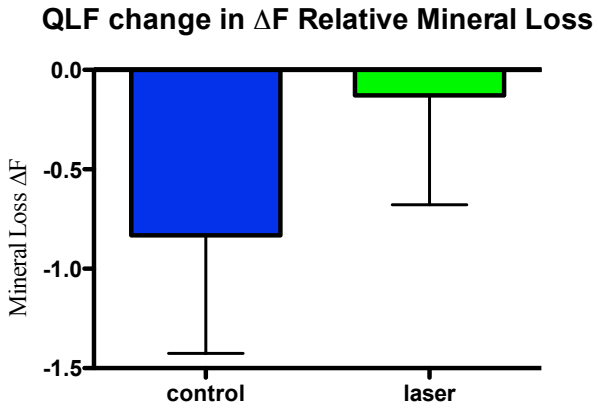

Figure 8: *In-vitro* relative mineral loss ΔF as seen with QLF for the short-pulsed CO_2laser treated enamel and for the controls (n=20, Mean ± SE) following 9 days of demineralization-remineralization pH-cycling. Reduction of relative mineral loss ΔF from 0.83±0.59 (control) to 0.13±0.55 (laser treated) is not statistically significant (P=0.2).

4.3. Optical Coherence Tomography

Preliminary results from optical coherence tomography showed an increase in surface reflection intensity at the laser treated fissure compared to the control fissures for the images captured at baseline (figure 9a, 9b). Following demineralization-remineralization pH-cycling an increase in subsurface light backscattering in the control fissure (figure 9c) was observed compared to the laser test fissure was observed (figure 9d).

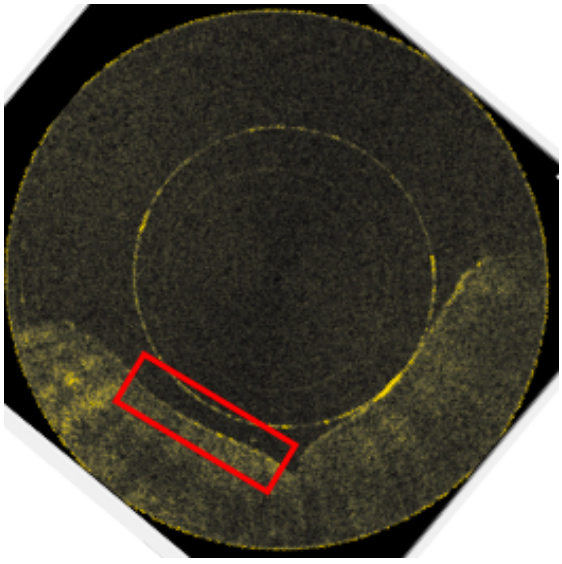

Figure 9a: OCT image of control fissure at baseline.

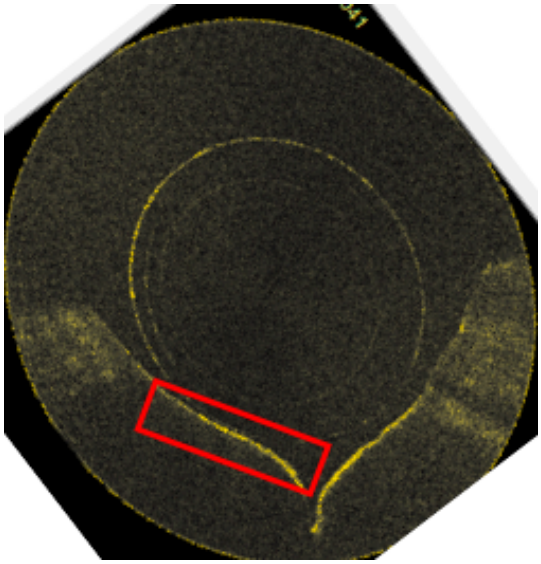

Figure 9b: OCT image of CO_2 laser treated fissure at baseline: increased surface intensity is observed.

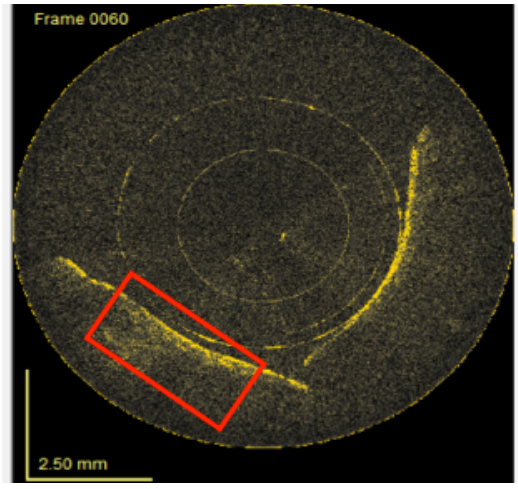

Figure 9c: OCT image of control fissure post-demineralization-remineralization pH-cycling: increased subsurface backscattering is observed.

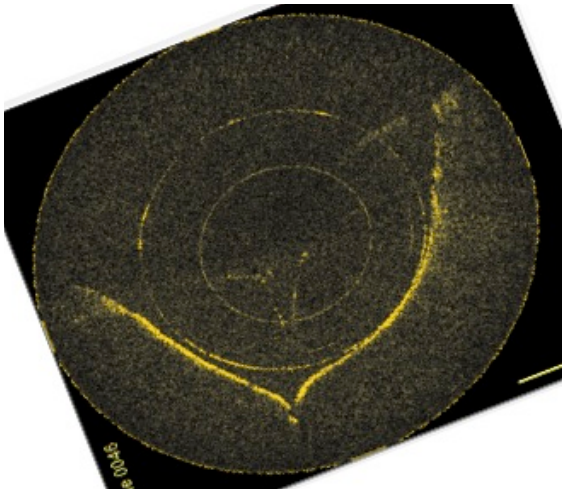

Figure 9d: OCT image of CO_2 laser treated fissure post-demineralization-remineralization pH-cycling: maintained surface intensity and minimal subsurface backscattering is observed.

4.4. Polarized Raman Spectroscopy

Figure 10a indicates that for control untreated regions, post-demineralization-remineralization pH-cycling results in a characteristic increase in the p2 cross-polarization Raman intensity. In contrast, regions that have been CO_2 laser treated show that the p2 cross-polarization is unchanged after demineralization-remineralization pH-cycling (Figure 10b). This suggests that CO_2 laser treatment offers protection against demineralization, as there is no alteration in the intensity after demineralization. Figure 10c indicates that laser treated regions (at baseline and post-demineralization-remineralization pH-cycling) exhibited consistent increased sloping baseline in the spectra. This finding suggests that laser treatment induces a change in the surface properties of the tooth surfaces.

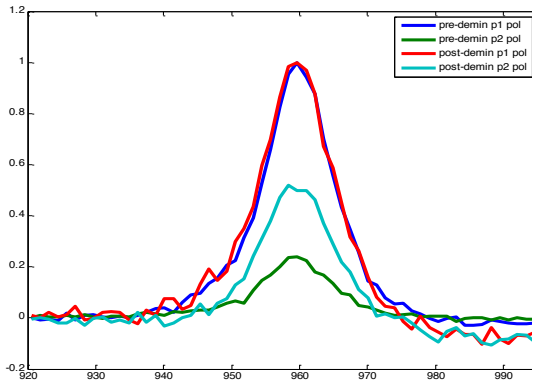

Figure 10a: Sample polarized Raman spectra for a control tooth at baseline measurement and after demineralization-remineralization pH-cycling.

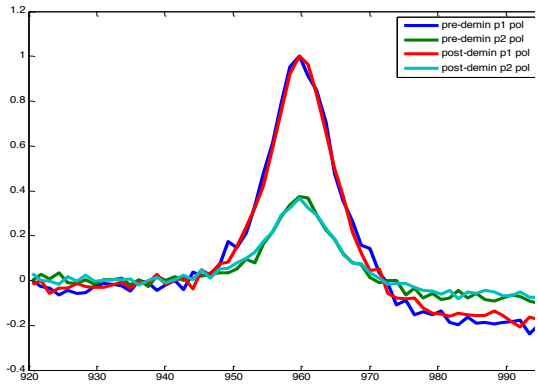

Figure 10b: Sample polarized Raman spectra for a CO_2 laser treated tooth at baseline measurement and after demineralization-remineralization pH-cycling.

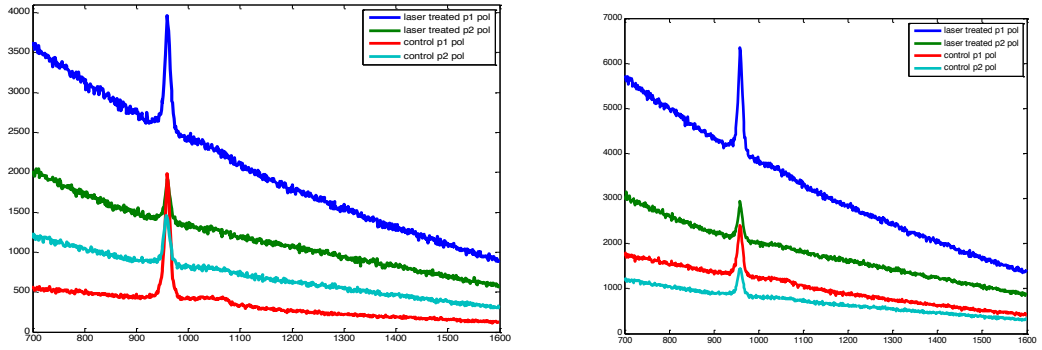

Figure 10c: Polarized Raman spectra on left are at baseline. The polarized Raman spectra on the right are after demineralization-remineralization pH-cycling.

4.5. SoproLife

Baseline SoproLife images provide a magnified view of *in vitro* control and CO_2 laser treated (test) fissures in both daylight and fluorescence mode (figures 11a, 11b). Areas treated with the CO_2 laser appear whiter and have a slight stippled texture. Following 9 days of demineralization-remineralization pH-cycling, the entire occlusal surface appears greyish white, in both daylight and fluorescence modes (only fluorescence mode shown in figures 11c, 11d).

Following the demineralization-remineralization pH-cycling all occlusal tooth surfaces have turned from a more or less green fluorescence to grey/white due to scattering in the demineralized porous enamel; only some fissure areas remained green due to a thinner layer of enamel. Orange/red or dark/brown areas were not observed.

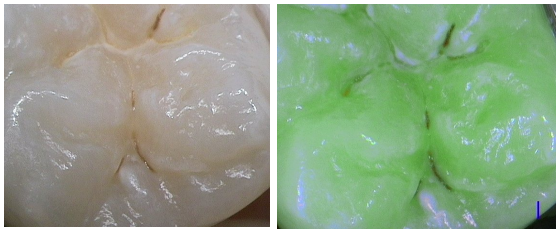

Figure 11a: A sample control fissure at baseline, in daylight (left) and fluorescence (right) modes.

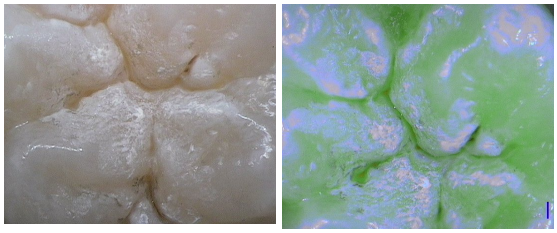

Figure 11b: A sample CO_2 laser treated fissure at baseline in daylight (left) and fluorescence (right) modes.

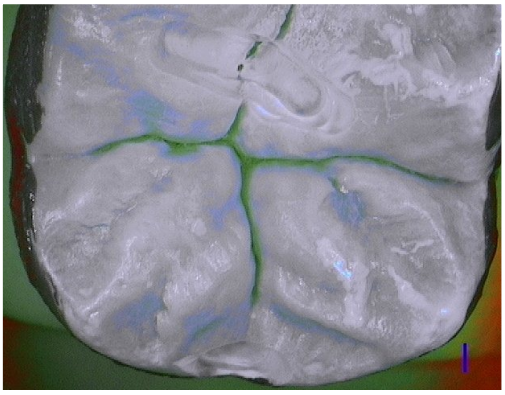

Figure 11c: A sample control fissure after demineralization-remineralization pH-cycling, in fluorescence mode.

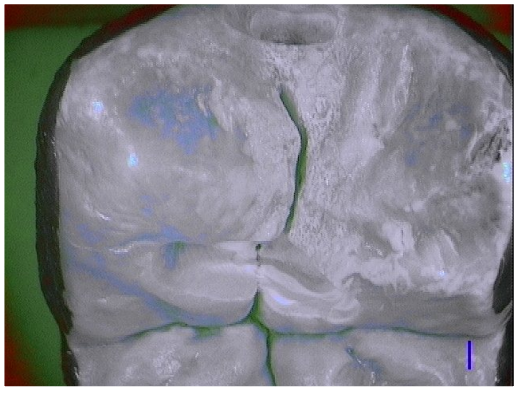

Figure 11d: A sample CO_2 laser treated fissure after demineralization-remineralization pH-cycling, in fluorescence mode.

4.6. DIAGNOdent

The mean change in DIAGNOdent readout between baseline and post demin-remin was -0.05 (± 0.69 n=20) and 6.2 (± 0.86 n=20) for control and test groups respectively (figure 12). The change in mean DIAGNOdent readout between control and test groups was statistically different ($P<0.0001$).

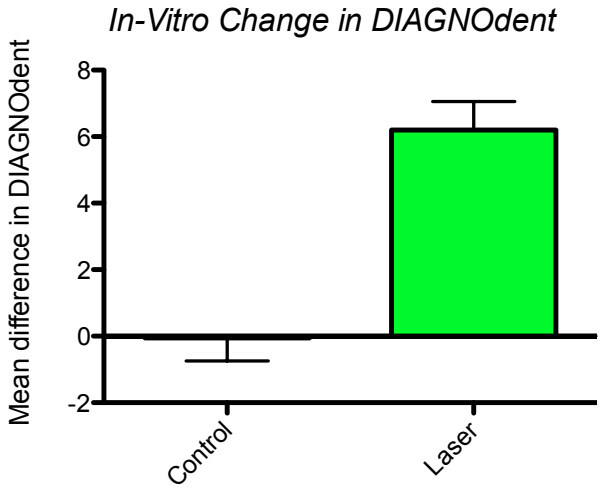

Figure 12: *In vitro* change in mean DIAGNOdent from baseline to post demin-remin for control (-0.05) and test (6.2) groups.

4.7. ICDAS II Visual Inspection

ICDAS II visual inspection did not contribute to the analysis of the *in vitro* study. The initial treatment with the short-pulsed CO_2 laser changed the fissures surfaces to a more white/opaque appearance. This made coding ICDAS difficult as white typical identifies demineralization, which would give an ICDAS II code of 2 if found on the fissure slopes (the area treated with the CO_2 laser). The differences between baseline and following demineralization-remineralization pH-cycling were also difficult to interpret using the ICDAS II coding system due to the generalized occlusal surface demineralization appearing again as grey-whitish.

5. *IN VIVO* RESULTS

5.1. Subject Demographics

Twenty subjects (14 males, 6 females) aged 11-15 (mean 13.6, SD 1.2) were enrolled in the clinical study. The ratio of maxillary molars to mandibular molars was 12 to 28. The average CO_2 laser irradiation time for maxillary molars was 80 seconds (SD 44) and 102 seconds (SD 19) for mandibular molars, with an average fluence of 3.4 mJ/cm^2. Nineteen subjects returned for the 6-month follow up exams, one subject dropped out due to a new medical diagnosis. No subject exceeded the maximum ICDAS II score >2 at the 6-month exam. All subjects were seen within a two-week time frame for each set of baseline and treatment appointments, 3-month recall appointments and 6-month recall appointments.

5.2. Intra-Examiner and Inter-Examiner Reliability for ICDAS II scoring

Intra-examiner reliability for DC was fair: weighted Kappa= 0.36 (SE of Kappa = 0.07), 95% confidence interval 0.10 to 0.37. Intra-examiner reliability for PR was good: weighted Kappa= 0.66 (SE of Kappa = 0.06), 95% confidence interval 0.39 to 0.63. Inter-examiner reliability was fair: weighted Kappa= 0.38 (SE of Kappa = 0.04), 95% confidence interval 0.13 to 0.28. See appendix iv for comprehensive details including all contingency tables.

5.3. ICDAS II Visual Inspection

At baseline examination, the control group had a mean ICDAS II code of 1.16 (SEM 0.17) and the laser treatment group had a mean of 1.21 (SEM 0.14) (figure13). After 6 months, the mean ICDAS II code for the control group had increased to 1.68 (SEM 0.13), and for the laser group to 1.32 (SEM 0.15) as seen in figure 14.

The mean change in ICDAS II over 6 months for the laser teeth was 0.11 (± 0.15), while the control teeth showed a mean change of 0.53 (± 0.14), as seen in figure 15. The increase in

ICDAS II seen in the control group was greater than the change seen in the laser group and was statistically significant (P<0.05).

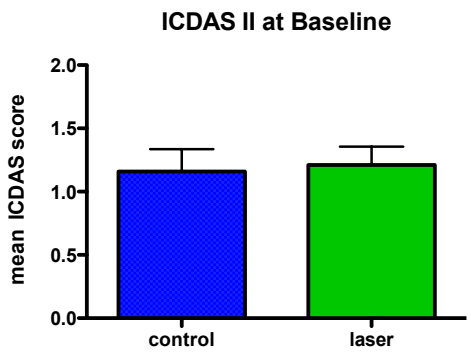

Figure 13: *In vivo* ICDAS II scores for control and laser groups at baseline.

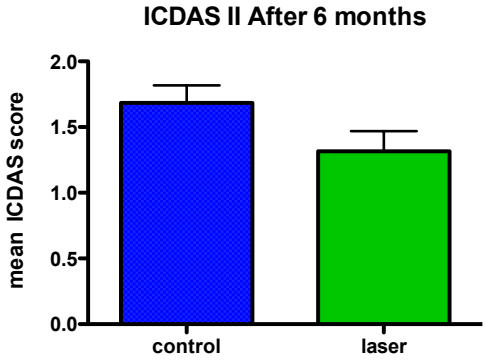

Figure 14: *In vivo* mean ICDAS scores for control and laser groups after 6 months of high caries challenge.

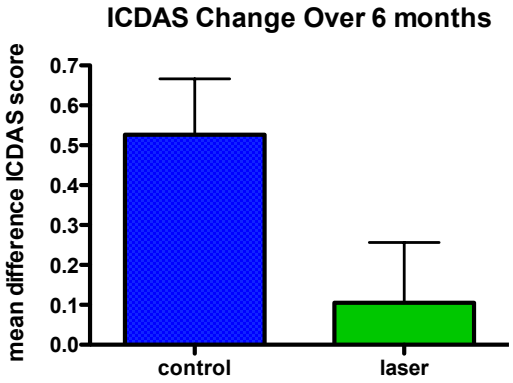

Figure 15: *In vivo* change in ICDAS II recording between baseline and 6-month follow-up for control and short-pulsed CO_2laser treated. Differences in mean change between groups is statistically significant (P<0.05).

5.4. DIAGNOdent

In the *in vivo* study, at baseline, the mean (± SD) reading for the control was 24.4 (± 15.4), compared to 24.2 (± 11.4) for the test group. After 6 months clinical caries challenge, these readings were 30.2 (± 17.64) for the control and 30.9±15.6) in the test group. The mean (± SEM) change in DIAGNOdent recording for the laser teeth over 6 months was 5.95 (± 4.56), while the control teeth showed a change in recording of 5.47 (± 3.97). The difference was not statistically significant with an unpaired t-test value of P=0.93 (figure 16).

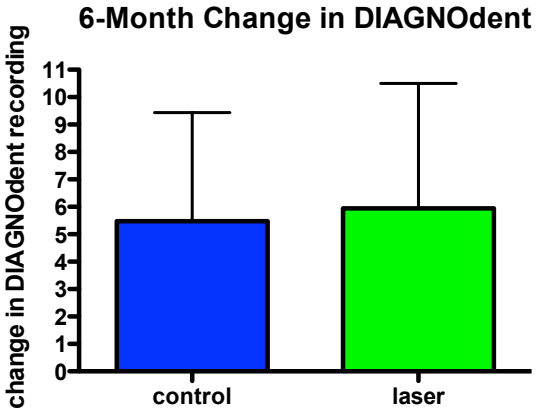

Figure 16: *In vivo* change in DIAGNOdent recording between baseline and 6-month follow-up for control and and short-pulsed CO_2 laser treated teeth.

5.5. Quantitative Light-induced Fluorescence

One QLF image from each group (test and control) was discarded from the analysis due to very poor image resolution. As seen in figure 17, the mean 6 month change in relative mineral loss, ΔF, as determined by the QLF, for the laser treated teeth was -2.10 ± 1.14 (SEM) n=18, while the control teeth showed a relative mineral loss of -0.06 ± 0.47 (SEM) n=18. The differences were not statistically significant with an unpaired t-test P=0.11. The data indicates that the laser treated teeth gained some mineral (negative-negative mineral loss) after 6 months, while the control remained fairly mineral content neutral.

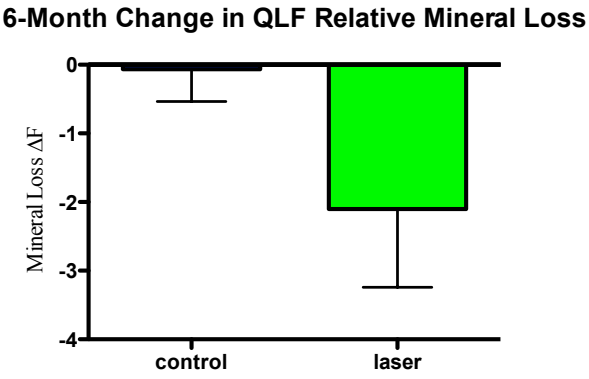

Figure 17: *In vivo* relative mineral loss ΔF as seen with QLF for short-pulsed CO_2laser treated teeth and for control teeth following 6-months of clinical caries challenge (n=18 Mean ± SEM). Reduction of relative mineral loss ΔF is not statistically significant (P=0.11).

5.6. Optical Coherence Tomography

OCT images were acquired for control and test fissures at baseline and 6 month recalls, however no analysis of the *in vivo* OCT data was performed.

5.7. SoproLife

In the *in vivo* study, the high-power magnification of the SoproLife images show detailed occlusal pit and fissure anatomy (figures 18a and 18b). In the examples of figures 18a and 18b, at baseline in both the control and laser treated teeth, demineralization and loss of enamel is seen at the base of the fissure in the distal pit (top of image) as well as extending towards the buccal (towards left side of tooth) (control tooth only). At the 6-month recall, the lesions appear to have progressed in the control tooth (figure 18a), however in the CO_2 laser treated tooth (test), the lesions appear to have reversed (figure 18b). The result seen in this example did not repeat for every subject.

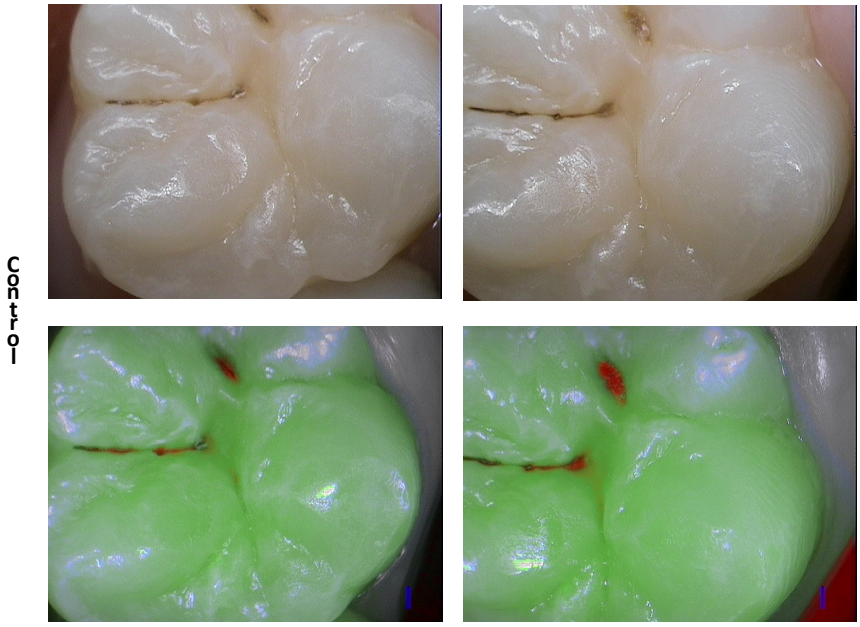

Figure 18a: ***In vivo*** **SoproLife images of a control tooth exposed with a white light source (top) and blue light source (bottom) at baseline (left) and 6-month recall (right) appointments.**

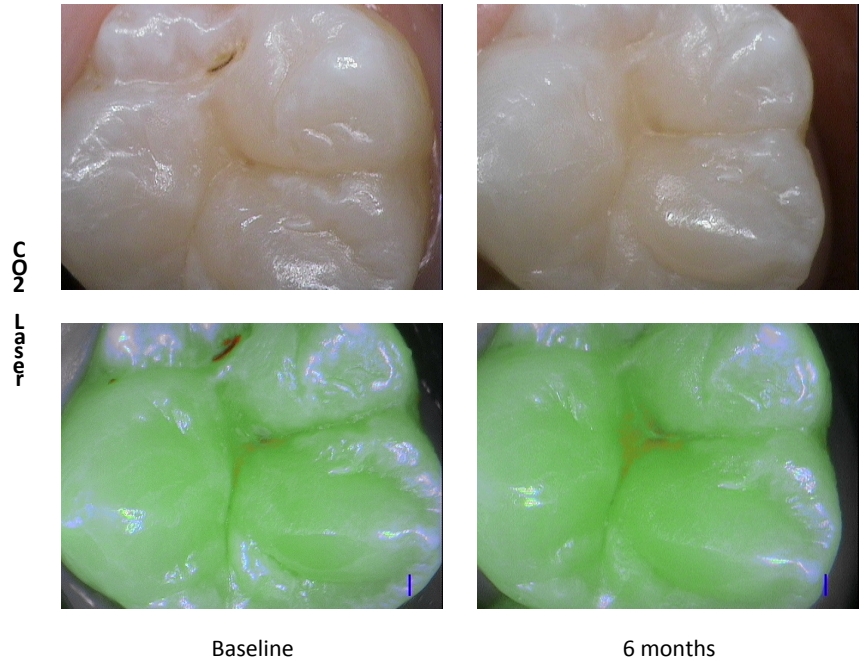

Figure 18b: ***In vivo*** **SoproLife images of a CO_2 laser treated tooth exposed with a white light source (top) and blue light source (bottom) at baseline (left) and 6-month recall (right) appointments.**

After 6 months of clinical caries challenge, the change in mean (± SEM) caries assessment code seen in the SoproLife white images (evaluated using the ICDAS II codes) for the control group was -0.10 (± 0.10 n=19), and -0.05 (± 0.14 n=19) for laser treated group. The difference between the means of the two groups was not statistically significant (P=0.77).

"Better, Same or Worse" events were tallied for control and laser treated groups in a 2x3 contingency table, seen graphically in figure 19. The distribution of events for the test group was not better than if left to chance (Chi-square = 1.95 (2 d.f.) P= 0.38). "Better, Same or Worse" Chi-squared test was applied with the H_0: control and treated distribution of caries are not different. The "Better, Same or Worse" (BWS) data were also converted into numerical values (Better=1, Same =0 and Worse= -1) and the difference between the means for control versus treatment groups was analyzed. Following 6-months of caries challenge, the converted BWS mean for the control group was 0.0 (± 0.15 n=19), and the converted BWS mean for the laser treated group was 0.26 (± 0.17 n=19) (figure 20). The small amount of "better" observances seen in the laser treated group was not statistically significant (P = 0.25).

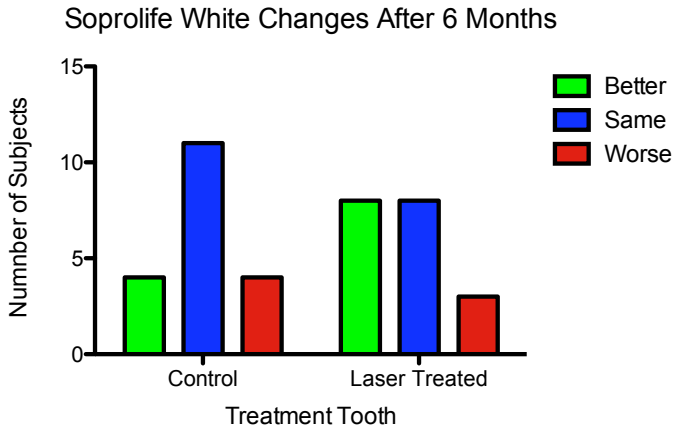

Figure 19: "Better, Same or Worse" distribution after 6 months for SoproLife white images.

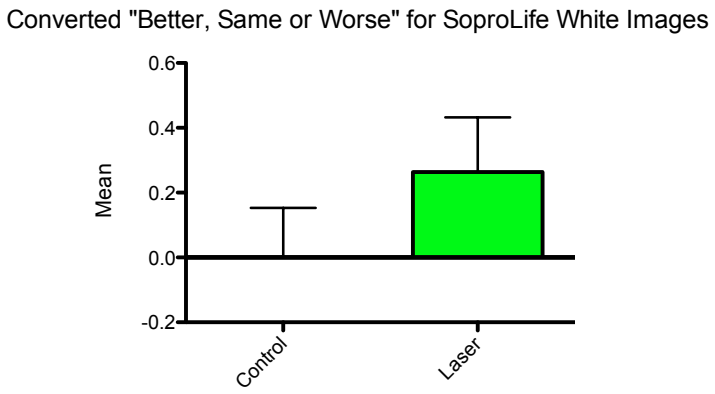

Figure 20: "Better (1), Same (0) or Worse (-1)" distribution after 6 months for SoproLife white images. Higher means represent a greater occurrence of "better" events.

Blue light source SoproLife images coded based on colors (green (1), grey/white (2), orange/red (3), dark/brown (4)), did not show a statistically significant difference in median changes from baseline to 6-month recall between control and CO_2 laser treated groups (P=0.53).

"Better, Same or Worse" changes, from baseline to 6 months, were tallied for control and laser treated groups based on SoproLife blue fluorescence images (figure 21). The number of better, same or worse changes observed in the laser treated group (compared to the control group) was not different then random probability (Chi-square = 3.38 (2 d.f.), P=0.19). Figure 22 shows the mean 6-month changes for converted data (better=1, same=0, worse=-1), where the mean for the control group was 0.11(± 0.1 n=19), and the mean for the laser treated group was 0.37 (± 0.11 n=19). While the laser treated group appeared to have a higher frequency of "better" outcomes the difference between the two means was not statistically significant (P = 0.17).

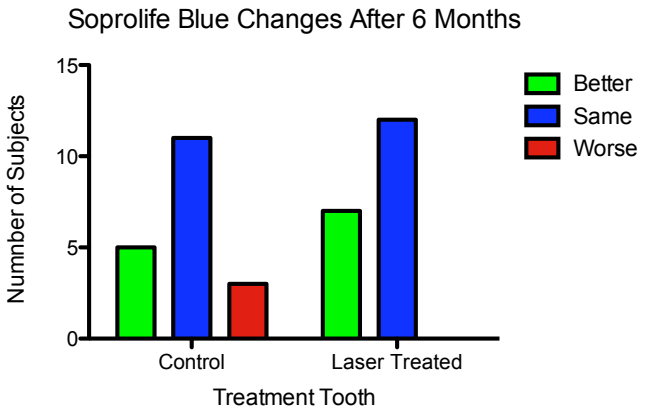

Figure 21: "Better, Same or Worse" distribution after 6 months for SoproLife blue images. The difference between the distribution for control and laser treated groups was not statistically different (P=0.19).

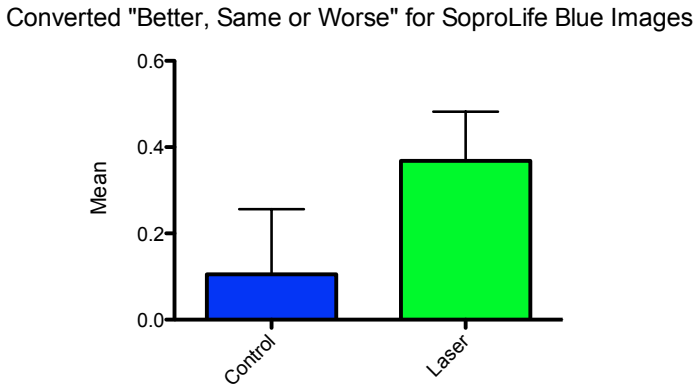

Figure 22: "Better (1), Same (0) or Worse (-1)" distribution after 6 months for SoproLife blue images. Higher means represent a greater occurrence of "better" events. The difference between the means for control and laser treated groups was not statistically different (P=0.17).

6. DISCUSSION

6.1. *In Vitro* Study Discussion

Several studies have shown that enhancing the demineralization resistance of enamel can be achieved in the laboratory by irradiation with microsecond short-pulsed CO_2 9.6 μm lasers[13, 44]. The 9.3 and 9.6 μm CO_2 laser wavelengths are the strongest absorbed wavelengths in dental enamel[45]. The loss of the carbonate phase from the enamel crystals due to the irradiation heat is reported to be responsible for the reduction in acid dissolution of enamel[46, 47].

In the *in vitro* study, having a test and control fissure on each tooth produced a more homogenous pairing of test and control fissures, controlling for anatomical tooth-level variations.

Cross-Section Micro Hardness

From cross-sectional micro-hardness testing, we found the mean relative mineral loss ΔZ (vol% x μm) for the laser treated enamel was 580.4±156.1 (SEM; n=6) while the control area showed a much higher relative mineral loss of 981±298.1 (SEM; n=5). The trend observed in the mean volume % mineral at each depth shows a similar pattern to the lesion depth profiles from Rechmann et al's *in vivo* study, which tested the CO_2 9.6 μm short-pulsed laser in an orthodontic bracket model. In Rechmann et al's study, the enamel adjacent to an orthodontic bracket showed a mean relative mineral loss ΔZ (vol%xμm) of 402±85 for the laser treated group for the 4–week cohort and a ΔZ of 135±98 (mean±SEM) for the 12-week cohort. The controls presented a much higher mineral loss ΔZ of 738±131 and 1067±254 for the 4 and 12 week cohorts, respectively[39, 48]. The results for relative mineral loss ΔZ in this study are at a similar level. The results presented are preliminary data from only one quarter of the samples. Processing and analysis of all 20 teeth may reveal a statistical significant difference in the relative mineral loss ΔZ for laser and non-laser treated fissures. This study shows that caries inhibition with 9.6 μm short-pulsed

CO_2 laser irradiation demonstrated in other studies[14, 49-51] can also be achieved on occlusal surfaces of molars. The relative mineral loss ΔZ, from cross-sectional micro-hardness test has repeatedly be shown as a reliable method of assessing the effectiveness of 9.6 μm short-pulsed CO_2 laser irradiation and should be considered as a benchmark against which other diagnostic methods can be compared.

Quantified Light-Induced Fluorescence

Using QLF also demonstrated that the short-pulsed CO_2 laser treated fissures have a trend towards less mineral loss after demineralization-remineralization pH-cycling, in comparison to the control fissures. The trend is not statistically significant (p=0.2). When the samples were pH-cycled, the entire occlusal surface had the same treatment as the fissures, resulting in demineralization of reference points used by QLF to determine the relative mineral loss ΔF (figure 5). The mean reduction in relative mineral loss ΔF of 73% in the test group may be under-estimated because of the demineralized reference points. A study design for a laboratory caries simulation of which limited caries to occur in the base of the fissure, leaving the mineral intact on the cusp slopes (reference points) would better estimate the difference in relative mineral loss ΔF. As a diagnostic method for evaluating early carious changes of the occlusal pits and fissures, relative mineral loss ΔF rates as good.

Optical Coherence Tomography

One of the interesting results of optical coherence tomography was the observation of an increase in subsurface light backscattering in the control fissure compared to the laser test fissure, following demineralization-remineralization pH-cycling. OCT imaging also showed an increase in surface backscattering for the laser treated fissures in comparison to the control areas already at baseline. The enhanced surface backscattering is likely due to an increase in mineral density

changing the optical properties of enamel. This change in optical properties of the tooth surface created from the CO_2 laser likely reduces the amount of light transmitted beyond the surface, and hence decreases the intensity of the signal beyond the tooth's surface. In effect, the CO_2 laser's effect on the tooth surface may mask demineralization from detection with OCT. Some small evidence of demineralization was observed at laser treated sites therefore indicating that the laser treatment is not necessarily homogeneous across the contours of the occlusal surface. An examination of OCT images acquired from laser treated fissures which showed demineralization in the cross-sectional micro-hardness testing revealed that subsurface backscattering can be observed despite a stronger surface backscattering. Therefore the cross-sectional micro-hardness results suggest that the CO_2 laser treated teeth contain more mineral and thus are somewhat protected from demineralization, allowing us to discount the notion of hidden subsurface demineralization in this group. OCT shows good potential to resolve early carious changes in occlusal pits and fissures, however is complicated by the physical lack of flexibility of the system, as well as would benefit from software which easily identifies the fissure of question and is capable of quantifying changes observed.

Polarized Raman Spectroscopy

Polarized Raman spectroscopy confirmed evidence of demineralization at the untreated control fissures as demonstrated by an increase in the p2 cross-polarization Raman intensity. This observation is in agreement with previous studies when comparing sound enamel from incipient caries[34]. The increased intensity confirms evidence of demineralization at untreated control sites. In contrast, regions that have been treated with the CO_2 9.6 μm laser show that the p2 cross-polarization is unchanged after demineralization-remineralization pH-cycling. This strongly suggests that the pulsed CO_2 9.6 μm laser treatment offers protection against demineralization, as there is no alteration in the peak intensity after the pH-cycle demineralization challenge. PRS

shows promise that it would be a good tool to detect early occlusal carious changes and should further investigated for clinical use.

DIAGNOdent and SoproLife

The confounding responses for tools fully or partially dependent on porphyrins' fluorescence are discountable. Due to the lack of bacterial chromophores, for instance porphyrins, in our laboratory caries simulation, additional information from fluorescence was neither expected nor observed. Our *in vitro* study results showed a mean change in DIAGNOdent (from baseline to post demin-remin) was -0.05 (± 0.69 n=20) and 6.2 (± 0.86 n=20) for control and test groups respectively. Albeit these means are statistically different ($P<0.001$), a mean increase by 6 is probably insignificant as DIAGNOdent fluctuates easily by 10 units over multiple readings. Furthermore, DIAGNOdent only functions correctly on the fluorescence of bacterial porphyrins, and thus is not an adequate device for detecting caries created through demineralization-remineralization pH-cycling. In this *in vitro* study, SoproLife showed changes in the enamel due to demineralization in both daylight and fluorescence modes. Nevertheless, no observable differences in SoproLife images were seen between control and test groups, due to the generalized demineralization of occlusal surface. The *in vitro* results from DIAGNOdent should be considered void due to absence of bacterial chromophores in this experimental model. Despite SoproLife's inability to demonstrate significant differences in the *in vitro* model, the author believes that SoproLife's high resolution and magnification power make it a good detection method for monitoring early carious changes *in vivo.*

6.2. Summary of *In Vitro* Discussion

In the *in vitro* study model, 9.6 μm short-pulsed CO_2 laser inhibited early carious changes induced by demineralization-remineralization pH-cycling as detected readily by cross-sectional

micro-hardness. Although the destructive nature of cross-sectional micro-hardness depletes its clinical applicability, it should be considered the gold standard to which all other diagnostic tests are held against. Despite the demineralization of reference points, QLF demonstrated a trend in the protective nature of short-pulsed CO_2 laser; this detection method should be applied *in vivo*. OCT and PRS were able to identify differences between control and test groups, thus show promise of future clinical use. Although SoproLife did not show differences in the *in vitro* study due to the experimental design, SoproLife's design, resolution and magnification make it an ideal instrument for monitoring occlusal surfaces. DIAGNOdent had no utility in this model, it should be tested in an *in vivo* model against the next available benchmark test.

6.3. *In Vivo* Study Discussion

Hsu et al suggested that short-pulsed CO_2 9.6 μm laser irradiation inhibited caries advancement after 12 months, as demonstrated with QLF[19]. Albeit the QLF differences between test and control group was very small, but significant at 12 months, in their study the findings at 6 months for QLF were not statistically significant. As a second diagnostic method, they used ICDAS II, however found no significant differences between control and test groups. The author challenged QLF's efficacy in resolving small changes in incipient pit and fissure caries and recommended the use of a laser delivery handpiece that can more easily access posterior molars.

Taking these recommendations into account, this study presented here was modeled from Hsu et al's study, while utilizing a contra-angle laser handpiece to facilitate the delivery of laser energy to the occlusal surface of the posterior teeth. Furthermore, multiple different caries detection methods were added in an effort to increase the resolution of small changes in early caries progression. The caries detection methods added were: DIAGNOdent, SoproLife, and OCT based

on their acceptance in the dental community, high magnification and resolution and ability to provide subsurface information, respectively.

In hope of increasing the magnitude of difference between the control and short-pulsed CO_2 9.6 μm laser group, the addition of fluoride was thought to be beneficial as it acts synergistically when applied at the time of laser irradiation. Limited by the fact that fluoride applied to one area of the mouth would carry over to all other areas, it was decided to apply fluoride to control and test teeth following laser treatment. When determining which vehicle to deliver fluoride to the patients teeth, Eakle et al's study was considered, which compared the longevity of salivary fluoride concentration between fluoride rinse and fluoride varnish. They found that the varnish elevates salivary fluoride above baseline for 24h in comparison to 2h for the rinse[52]. For this reason, fluoride varnish was the delivery modality used in this study. However, in the future different modalities of fluoride application such as rinse, gel, foam and varnish should be assessed in combination with CO_2 laser therapy.

The CAMBRA questionnaire was used to determine that each subject was high caries risk at the time of enrollment. Caries risk assessment should be reassessed at the 12-month recall visits using the CAMBRA questionnaire to determine if the study group remained high risk throughout the study.

ICDAS II

ICDAS II visual inspection is the most clinically available assessment tool for any dentist; it requires some training and ideally 2.5x magnification loupes. The mean change in ICDAS II over 6 months for the laser teeth was 0.11 (± 0.15), while the control teeth showed a mean change of 0.53 (± 0.14) ($P<0.05$). While the difference in change over 6 months was small, if the rate of

change were to remain constant over time, the difference between the two groups would be clinically very significant in just a few years. The change seen in ICDAS II from this clinical study strongly supports the efficacy of pulsed CO_2 laser treatment for prevention of occlusal pit and fissure caries. It also suggests that our eyes (with magnification) coupled with a trained examiner's mind may be the most sensitive non-destructive assessment tool currently available for early caries detection. However, because the criteria for ICDAS II codes 1-2 are not particularly different or easy to distinguish, especially considering the ease in which codes 3, 4, 5 and 6 are differentiated, ICDAS II is liable to a lesser kappa level when being applied for coding initial enamel changes.

DIAGNOdent

As expected, DIAGNOdent did not show any difference between control and treated groups clinically. At baseline, the mean (± SD) reading for the control was 24.4 (± 15.4), compared to 24.2 (± 11.4) for the test group. After 6 months clinical caries challenge, these readings were 30.2 (± 17.64) and 30 9±15.6) respectively, equating a change in DIAGNOdent (mean ± SEM) for the control of 5.47 (± 3.97) and a change for the laser teeth of 5.95 (± 4.56) (P=0.93). The small difference in change of DIAGNOdent is not significant; this may be due to the fact that in this study very early carious changes are subject of observation, which occur prior to cavitation. As a result, the DIAGNOdent scores are relatively low, and thus the small changes seen are too miniscule to be detected with DIAGNOdent. The lack of change seen clinically has also been expressed by Siva et al. who concluded that DIAGNOdent is unable to monitor clinical arrest of caries[53].

Quantified Light-Induced Fluorescence

The QLF results showed a relative mineral loss for the laser treated teeth was -2.10 ± 1.14 (SE) n=18, while the control teeth showed a relative mineral loss of -0.06 ± 0.47 (SE) n=18. The laser treated teeth showed a gain in mineral content over the 6-month time course, suggesting that the carbonated hydroxyapatite in the enamel surface layer had been changed into fluorapatite. Although not statistically significant (P=0.11), a trend in the direction of laser protecting the tooth from caries is observed. Our findings are concurrent with those of Hsu et al, who found a not statistically significant trend in caries inhibition after 6 months, however over 12 months, this difference emerged to be statistically significant[19].

SoproLife Daylight (White Light) Images

Although we used 2.5x magnification while visually assessing ICDAS II, technology with higher magnification, resulting in higher resolution, is available in dentistry. ICDAS II occlusal evaluation criteria may be able to be applied to high-resolution images. A tool, such as SoproLife, which is able to magnify the surface of the tooth, could facilitate the diagnosis of early caries. SoproLife images captured with white light were analyzed by applying the ICDAS II caries detection codes to the captured pictures. Surprisingly, no difference between mean code changes for control and laser treated groups was seen (P=0.77). After studying the images, the author noticed that although changes from baseline to 6 month recall images were obvious, they did not necessarily fall into different ICDAS II codes. As such, the "Better, Same or Worse" system was applied to quantify changes observed which were not described as a change in ICDAS II codes. The result was a strong trend of caries reversal over 6 months in the laser treated group compared to the control group (P=0.25). At this time, no standardized method for evaluating SoproLife daylight images exists; further investigation into development of evaluation criteria would be helpful to better quantify observations.

SoproLife Blue Light Images

SoproLife images captured a blue light source fluoresce green with healthy enamel and dentin. Demineralized enamel scatters light, giving a white-grey dull image. Bacterial bi-products are assumed responsible for the orange-red fluorescence, and when heavily compounded appear dark red-brown-black depending on the quantity of material. Representing this gradient, blue light images were coded by color observed: green (1), grey/white (2), orange/red (3), dark/brown (4). Results did not show a difference in mean code change over 6 months for the control group compared to treatment group (P=0.53). Similar to the white light images, observed changes were not always reflected by the suggested color coding system, and thus the "Better, Same or Worse" system was applied. The laser treated group showed a greater number of 'Better" outcomes over 6 months, albeit not statistically significant (P=0.17). The consumable information from SoproLife blue light images has not yet been defined in the literature, and thus no existing evaluation scale has been established. The development of a rigorous set of evaluation criterion would facilitate the objective analysis of what is otherwise very subjective data.

Optical Coherence Tomography

As seen *in vitro,* OCT has the ability to detect very early demineralization change, however, applying OCT in a clinical trial had many challenges. First, the physical design of the OCT probe was difficult to adapt to the occlusal surface of a molar; it frequently floated in space above the molar with its semi-rigid tip being limited by adjacent teeth. This problem resulted in a loss of depth of resolution. Second, when OCT data is reviewed, it is played as a video of cross sections, with no reference to the position of any tomograph relative to the occlusal surface. As a result, the location of a fissure of interest on any given tooth is very difficult to ascertain, and unfortunately resulted in lacking OCT data analysis. The application of software that can render a surface reference image will greatly facilitate the analysis of OCT data.

Three month recalls were initially planned primarily in hopes of showing the earliest possible carious changes, which may be demonstrated by OCT. Unfortunately, the OCT device was not available at the time of the 3 month recalls, thus no OCT data from that point was collected. To give a clear picture of the overall change seen over 6 months, mid-point data was omitted.

The author is hopeful that collected data from baseline and 6-month visits will be analyzed and compounded with the future 12-month OCT results.

6.4. Summary of *In Vivo* Discussion

In the *in vivo* study, ICDAS II visual examination was the only detection method to demonstrate with statistical significance that 9.6 μm short-pulsed CO_2 laser irradiation inhibited early caries after 6-months in high caries risk subjects. A strong tendency supporting this result was found with QLF and SoproLife images captured under both white and blue light sources. The author hopes that OCT data may add power to the current findings and analysis of future 12-month data may show current trends becoming statistically significant.

7. CONCLUSION

In a high caries challenge environment the short-pulsed CO_2 9.6 μm laser irradiation appears to protect enamel in fissure areas from mineral loss.

Quantitative light-induced fluorescence and optical coherence tomography are good assessment tools for the *in vitro* model. ICDAS II and fully or partially chromophore dependent fluorescence tools like DIAGNOdent or SoproLife, are not fully adequate tools in determining caries resistance enhancement in occlusal fissures due to irradiation with a short-pulsed CO_2 9.6 μm laser *in vitro.* No non-destructive caries detection method that reflects or replaces cross-sectional micro-hardness testing was uncovered from the *in vitro* study.

In vivo, the different methods of evaluation of early carious changes on occlusal surfaces yielded differing results. The majority of the methods tested demonstrated the trend that short-pulsed CO_2 9.6 μm laser irradiation combined with fluoride varnish inhibited occlusal caries better than fluoride varnish alone. After 6 months, ICDAS II appeared to be the best method of detection and assessment of early occlusal caries, although it is subject to human variation. The trend in caries inhibition seen in ΔF mineral loss from QLF may progress by the 12-month recall. OCT may prove to be an excellent diagnostic device to detect incipient caries, however due to insufficient data analysis, we are unable to report on this. Continued use of magnification and technology may help to eliminate examination variation.

Future research will evaluate the 12 months findings for these subjects. It would be interesting to continue the study for future years to help define how long the effect of the laser treatment lasts. Should any future data report effective prevention of caries due to the short-pulsed CO_2 9.6 μm laser irradiation, a large scale clinical trial would be indicated.

7.1. Clinical significance:

Considering that no adverse events have been reported with short-pulsed 9.6 μm CO_2 laser irradiation, as well as evidence from this study and Hsu et al's study, the author believes that CO_2 9.6 μm laser treatment is a safe and effective procedure for the prevention of occlusal pit and fissure caries. The differences reported here are small, however the effect on a larger population over a longer time course may be very significant.

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9. APPENDIX

(i) International Caries Detection and Assessment System

ICDAS II Codes: Coronal Pits and Fissures

Code 0: Sound tooth surface:
There should be no evidence of caries (either no or questionable change in enamel translucency after prolonged air drying (suggested drying time 5 seconds)). Surfaces with developmental defects such as enamel hypoplasias; fluorosis; tooth wear (attrition, abrasion and erosion), and extrinsic or intrinsic stains will be recorded as sound. The examiner should also score as sound a surface with multiple stained fissures if such a condition is seen in other pits and fissures, a condition which is consistent with non-carious habits (e.g. frequent tea drinking). Table 1 provides a useful guide for differential diagnosis for carious opacities versus other opacities.

Code 1: First visual change in enamel:
When seen wet, there is no evidence of any change in color attributable to carious activity, but after prolonged air drying (approximately 5 seconds is suggested to adequately dehydrate a carious lesion in enamel) a carious opacity or discoloration (white or brown lesion) is visible that is not consistent with the clinical appearance of sound enamel
OR
When there is a change of color due to caries which is not consistent with the clinical appearance of sound enamel and is limited to the confines of the pit and fissure area (whether seen wet or dry). The appearance of these carious areas is not consistent with that of stained pits and fissures as defined in code 0.

Code 2: Distinct visual change in enamel:
The tooth must be viewed wet. When wet there is a (a) carious opacity (white spot lesion) and/or (b) brown carious discoloration which is wider than the natural fissure/fossa that is not consistent with the clinical appearance of sound enamel (Note: the lesion must still be visible when dry).

Code 3: Localized enamel breakdown due to caries with no visible dentin or underlying shadow:
The tooth viewed wet may have a clear carious opacity (white spot lesion) and/or brown carious discoloration which is wider than the natural fissure/fossa that is not consistent with the clinical appearance of sound enamel. Once dried for approximately 5 seconds there is carious loss of tooth structure at the entrance to, or within, the pit or fissure/fossa. This will be seen visually as evidence of demineralization (opaque (white), brown or dark brown walls) at the entrance to or within the fissure or pit, and although the pit or fissure may appear substantially and unnaturally wider than normal, the dentin is NOT visible in the walls or base of the cavity/discontinuity.

If in doubt, or to confirm the visual assessment, the WHO/CPI/PSR probe can be used *gently across a tooth surface* to confirm the presence of a cavity apparently confined to the enamel. This is achieved by sliding the ball end along the suspect pit or fissure and a limited discontinuity is detected if the ball drops into the surface of the enamel cavity/discontinuity.

Code 4: Underlying dark shadow from dentin with or without localized enamel breakdown:
This lesion appears as a shadow of discolored dentin visible through an apparently intact enamel surface which may or may not show signs of localized breakdown (loss of continuity of the surface that is not showing the dentin). The shadow appearance is often seen more easily when the tooth is wet. The darkened area is an intrinsic shadow which may appear as grey, blue or brown in color. The shadow must clearly represent caries that started on the tooth surface being evaluated. If in the opinion of the examiner, the carious lesion started on an adjacent surface and there no evidence of any caries on the surface being scored then the surface should be coded "0".

Code 3 and 4, histologically may vary in depth with one being deeper than the other and vice versa. This will depend on the population and properties of the enamel. For example more translucent and thinner enamel in primary teeth may allow the undermining discoloration of the dentin to be seen before localized breakdown of enamel. However, in most cases code 4 is likely to be deeper into dentin than code 3.

Code 5 : Distinct cavity with visible dentin:
Cavitation in opaque or discolored enamel exposing the dentin beneath.

The tooth viewed wet may have darkening of the dentin visible through the enamel. Once dried for 5 seconds there is visual evidence of loss of tooth structure at the entrance to or within the pit or fissure – frank cavitation. There is visual evidence of demineralization (opaque (white), brown or dark brown walls) at the entrance to or within the pit or fissure and in the examiner judgment dentin is exposed.

The WHO/CPI/PSR probe can be used to confirm the presence of a cavity apparently in dentin. This is achieved by sliding the ball end along the suspect pit or fissure and a dentin cavity is detected if the ball enters the opening of the cavity and in the opinion of the examiner the base is in dentin. (In pits or fissures the thickness of the enamel is between 0.5 and 1.0 mm. Note the deep pulpal dentin should not be probed)

Code 6: Extensive distinct cavity with visible dentin:
Obvious loss of tooth structure, the cavity is both deep and wide and dentin is clearly visible on the walls and at the base. An extensive cavity involves at least half of a tooth surface or possibly reaching the pulp.

(ii) Committee on Human Research Approved Application

1.0 1.1 *Enter the full title of your study:
In vivo occlusal caries prevention by pulsed CO2 laser and fluoride treatment.
1.2 *Enter the study number or study alias
In vivo pulsed CO2 laser and fluoride treatment
General Information
2.0
Add Department(s) 2.1 List of Departments associated with this study:
Primary Department Name Dept?
UCSF - *008431* - RESTORATIVE DENTISTRY
3.0 Assign key study personnel(KSP) access to the study
3.1 *Please add a Principal Investigator for the study:
Peter Rechmann
Select if applicable Fellow
If the Principal Investigator is a Fellow, the name of the Faculty Advisor must be supplied below.
3.2 If applicable, please select the Protocol Staff personnel:
A) Additional Investigators
Charland, Daniel - Co-Principal Investigator Featherstone, John D - Other Investigator Weintraub, Jane A - Other Investigator
B) Research Support Staff
Rechmann, Beate M - Clinical Research Associate
3.3 * Please add a Study Contact:
Rechmann, Peter Charland, Daniel
The Study Contact(s) will receive all important system notifications along with the Principal Investigator. (e.g. The study contact(s) are typically either the Study Coordinator or the Principal Investigator themselves).
3.4 If applicable, please add a Faculty Advisor:
3.5 If applicable, please select the Designated Department Approval(s):
Bird, William - Department Chair
Add the name of the individual authorized to approve and sign off on this protocol from your Department (e.g. the Department Chair or Dean).
4.0
Qualifications of Key Study Personnel
4.1 List the study responsibilities and qualifications of any individuals who qualify as Key Study Personnel (KSP) by clicking the "Add a new row" button:
KSP Name Description of Study Responsibilities Qualifications
Principle investigator, creating research protocol, background ideas and experiences, overall coordination of the study and supervising the clinical laser aspects of the study
Co-P.I. responsible for the overall clinical aspects of this study; study design, data collection and consenting of subjects supervising research protocol
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
Data management and statistical analyses support
Experienced clinical scientist and dental laser researcher in the Preventive and Restorative Dental Sciences department (PRDS). DDS, PhD
Second year Pediatric Dentistry Resident and MS candidate (2011)
Experienced caries researcher in the, PRDS department MS, PhD
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. DDS, MPH
5.0 5.1 * This study involves human stem cells, gametes or embryos:
No Yes, and requires CHR and GESCR review Yes, and requires GESCR review, but NOT CHR review
5.2 * This application involves a Humanitarian Use Device:
No Yes, and it includes a research component Yes, and it involves clinical care ONLY
5.3 * This is a CIRB study (e.g. the NCI CIRB will be the IRB of record): Yes No
5.4 * This application includes a request to rely on another UC IRB to be the IRB of record: Yes No
Note: If this request is approved, the CHR will **NOT** review and approve this study. Another UC campus will be the IRB of record.
Rechmann, Peter
Charland, Daniel
Featherstone, John D
Weintraub, Jane A
Initial Screening Questions
Application Type 6.1 * This research involves:
6.0

Minimal risk
Greater than minimal risk
6.2 * This application is:
Full Committee Expedited Exempt
6.3 If you think this study qualifies for expedited review, select the regulatory category(ies) that the research falls under:
Category 1: A very limited number of studies of approved drugs and devices Category 2: Blood sampling Category 3: Noninvasive specimen collection Category 4: Noninvasive clinical procedures
Category 5: Research involving materials that were previously collected for either nonresearch or research purposes Category 6: Use of recordings Category 7: Low risk behavioral research Category 8: Renewal of inactive research protocols or protocols that are essentially complete
Category 9: Renewal of other minimal risk research protocols
6.4 * This study involves: Subject contact (including phone, email or web contact)
No subject contact (limited to medical records review, biological specimen analysis, and/or data analysis)
Funding 7.1 Identify all the funding sources and their roles on the project:
7.0
No Sponsors have been associated.
7.2 If you tried to add the sponsor in the question above and it was not in the list, check here:
Sponsor not in list **Only** if your sponsor is not yet in the list, type the sponsor's name:
If the sponsor is not in the system, download the C&G Add Sponsor Form from Help link and attach it to this application.
Your study will not receive CHR approval until the sponsor and funding details have been added to your application.
7.3 For Federally funded studies only, indicate which portion of your grant you will be attaching:
The Research Plan, including the Human Subjects Section of your NIH grant For other federal proposals (contracts or grants), the section of the proposal describing human subjects work The section of your progress report if it provides the most current information about your human subjects work
7.4 If this study has no sponsor, check all that apply:
Unfunded student project Unfunded (miscellaneous departmental funding) Specific departmental funding
8.0
Statement of Financial Interest
8.1 * The Principal Investigator and/or one or more of the key study personnel has financial interests related to this study:
Yes No If **Yes**, attach the **Disclosure of Investigators' Financial Interests Supplement** to this application.
9.0
Sites
9.1 Institutions (check all that apply):
UCSF Mt. Zion San Francisco General Hospital (SFGH) SF VA Medical Center (SF VAMC) Helen Diller Family Comprehensive Cancer Center Fresno (Community Medical Center) Blood Centers of the Pacific (BCP) Blood Systems Research Institute (BSRI) Gallo Gladstone Institute on Aging (IOA) SF Dept of Public Health (DPH)
9.2 Check all the other types of sites not affiliated with UCSF with which you are cooperating or collaborating on this project:
Foreign Country List:
Other UC Campus Other institution Other community-based site
9.3 * This is a multicenter study: Yes No
9.4 Check any research programs this study is associated with:
Cancer Center Center for AIDS Prevention Sciences (CAPS) Global Health Sciences Immune Tolerance Network (ITN) Osher Center Positive Health Program
Study Design
10.0 10.1 Study design:
10.2 Check all that apply:
Phase I Phase II Phase III Phase IV
Scientific Considerations
11.0 11.1 Hypothesis:
This study has a hypothesis: Yes No
If yes, state the hypothesis or hypotheses:
The hypothesis to be tested is: short pulse CO2 laser treatment at wavelength of 9.6!m in addition to fluoride varnish treatment results in enamel crystal changes which increase the
resistance of dental mineral to acid dissolution and will prevent pit and fissure caries better than fluoride varnish treatment alone in vivo.
11.2 List the specific aims:
Aim 1: To compare the relative mineral loss (DZ), as measured by QLF, in the occlusal pits and fissures of fluoride treated molars and premolars with relative mineral loss (DZ) in the occlusal pits and fissures of a

laser and fluoride treated molars and premolars.
Aim 2: To compare mineral loss of early caries in occlusal surfaces by 1) ICDAS visual inspection, 2) Quantitative light-induced fluorescence (QLF) 3)Optical Coherent Tomography (OCT), 4) Diagnodent and 5) SOPROlife fluorescence digital photography.

11.3 Statistical analysis:

The relative mineral loss (DZ) values for the test teeth versus the control teeth will be compared. The data will be analyzed using a 2-sided paired t-test and Wilcoxon signed- rank test with a significance level of p£0.05. Person-level effects such as gender, age, and other conditions will be controlled in the within-person treatment estimate. Outcome 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. OCT and ICDAS changes will be used to qualitatively describe observed changes. An experienced biostatistician in the PRDS department will be sought out to assist in performing all statistical analyses.

11.4 * This is an investigator-initiated study: Yes No

11.5 This study has received scientific or scholarly review from (check all that apply):

Cancer Center Protocol Review Committee (PRC) (Full approval or contingent PRC approval is required prior to final CHR approval for cancer-related protocols.)
CTSI Clinical Research Center (CRC) advisory committee Departmental scientific review Other:
Specify **Other**:
If applicable, attach the **Departmental Scientific Review Form** at the end of the application.

Background

12.0 12.1 Background:

Over the last two decades caries prevalence in the permanent dentition has declined nearly ten percent in American children aged 9-16 [1]. While this remarkable decrease in caries is significant, 42% of the population in this age range is infected by dental caries [1]. Nearly 80% of all of carious lesions in adolescences involve the occlusal pits and fissures [2]. Currently, there are two common professionally delivered techniques towards caries prevention: pit and fissure sealants, and topical fluoride. Other interventions such as diet counseling or oral hygiene instruction will not be discussed due to their high dependence on daily patient compliance. This background section will review the current prevention techniques for pit and fissure caries.
At the earliest stage, a carious lesion is defined as the loss of mineral from the enamel surface due to the acidic products of oral bacteria. Enamel can be protected from acid demineralization by means of topical fluoride[3]. In a review of the topical benefits of fluoride, Featherstone (2004) discusses how enamel's carbonated hydroxyapatite can be
made more resistant to acid dissolution with the replacement of hydroxyl groups for fluoride ions. Another benefit of topical fluoride is its capacity to strengthen already demineralized enamel, thus inhibiting the caries progression[4]. Unfortunately, the application of topical fluoride appears to be more effective in the prevention of smooth surface caries then in preventing occlusal fissure caries.
In a 2006 Cochrane review, a systematic review of the literature comparing fluoride use to sealants in the prevention of pit and fissure caries was performed. Hiiri et al (2006) concluded that although there is insufficient literature to accurately quantify the difference, sealants are more successful in reducing pit and fissure caries than fluoride varnish[5]. Beauchamp et al (2008) made clinical recommendations for the use sealants, in the areas of caries prevention, placement over non-cavitated carious lesions, the use of glass ionomer versus composite resin sealants and placement techniques.[6]
One of the problems with pit and fissure sealants is their limited time of retention and need for regular maintenance. According to Feigal (1998), 5-10% of pit and fissure sealants will require annual repair or replacement [7]. US data collected between 1999 and 2002 shows that 30.5% of American children aged 6-11 have sealants on permanent teeth [1]. Sealants are most effective when the appropriate risk analysis was performed, and vigilant recalls are followed [8]. This suggests that sealants are contra-indicated in patients who do not have routine periodic oral exams.
The capability of lasers to modify enamels surface properties has promised anew type of caries prevention therapy. Modern lasers used in dentistry are from the Erbium family: the Er:YAG laser with an emission wavelength of 2970 nm and the ErCr:YSGG laser (wavelength 2780 nm)[9]. These lasers are efficient at cutting hard and soft tissue, and have even been shown to produce acid resistance in vitro[10]. Other types of lasers have been better studied.
McCormack et al. (1995) found that the surface and subsurface enamel crystals could be fused with a pulsed CO2 laser of 9.6 !m [11]. The enamel subsurface can be made
appreciably more resistant to acid dissolution by being treated with those transverse excited atmospheric pressure (TEA) carbon dioxide (CO2) pulsed lasers[12]. This was
further refined by the same group of researchers who uncovered that a CO2 laser with a wavelength of 9.6 !m, applied for 25 pulses at 1 to 3 J/cm2 inhibited 70% of artificial caries[13].
Remarkably, although the subsurface heats to greater then 800 oC, less than a 1 oC temperature change was observed at a 2 mm depth[13]. TEA CO2 laser therapy
heats the enamel surface and subsurface to a depth of 10 microns, causing the release of carbonate from the carbonated apatite, resulting in a more caries resistant hydroxyapatite
toothsurface[14].TheuseofaTEACO2 laserof9.6!mwavelengthpulseddeliveringa
total of 2.4J or 4.8J had no lasting pulpal effects reported by the patients or histological signs of

inflammation[15], suggesting this type of therapy is safe for clinical use.
The clinical application of CO2 laser therapy is currently being examined in a clinical prospective trial. Rechmann et al. (2008) took a group of 24 subjects (mean age: 14.5) with premolars scheduled for extraction for orthodontic reasons; 12 each for a) a 4-week arm and b) a 12-week arm. Orthodontic brackets were placed on those premolars with a conventional composite resin and an area next to the bracket was irradiated with a CO2-
laser, wavelength 9.6mm, pulse duration 20ms, pulse repetition rate 20Hz, beam diameter 1,100mm, average fluence 4.1±0.3J/cm2, and 20 laser pulses per spot. An adjacent non-irradiated area was used as the control surface. Premolars were extracted after 4 and 12 weeks respectively for a quantitative assessment of demineralization by cross sectional micro-hardness testing. The laser treatment produced a 46% demineralization inhibition after 4 weeks and a marked 87% inhibition for the 12-week arm. This study shows, for the first time in vivo, that the specific CO2 pulsed laser
irradiation can be used successfully for the inhibition of dental caries in enamel in human mouths[16].
The *in vivo* application of CO2 pulsed laser irradiation for the prevention of occlusal caries is currently being studied in our group[17]. Some challenges were present in the study
which may confound the results. These include a straight laser handpiece, which made delivering laser pulses to the occlusal fissure system of 2nd permanent molars very difficult. The QLF analysis software has a limited capacity for detected early enamel caries. To improve on this study, a contrangle laser handpiece will be used, furthermore, a more sensitive modality of early caries detection will be employed.
In an in vitro study comparing CO2 laser therapy to CO2 laser therapy plus fluoride varnish, it appeared as there was added benefit to having fluoride available[18]. This exciting result should be pilot tested in vivo and may offer an alternative option to placing occlusal sealants.
Eakle et al. (2004) compared the longevity of salivary fluoride concentration between fluoride rinse and fluoride varnish and found that the varnish elevated salivary fluoride above baseline for 24h in comparison to 2h for the rinse[19]. In this study, fluoride varnishwillbeassessedincombinationwithCO2 lasertherapy.
Methods of early caries detection Current commonly used caries detection methods in the United States include tactile use of the explorer, radiographs and visual inspection,. Studies in Europe have shown that the explorer is only correct less than 50% of the time[20]. 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[21]. By the time an occlusal caries lesion is detectable radiographically, it is too large to be remineralized[21]. If carious lesions are detected early enough, intervention methods, such as fluoride application, sealants, preventive resin restorations, laser treatment, and antibacterial therapy, can be applied to reverse the caries process[21]. Visual inspection can be very subjective based on clinician experience and training. The International Caries Detection and Assessment System (ICDAS) provides a standardized method of lesion detection and assessment, leading to caries diagnosis[22]. lCDAS II assigns scores to lesions based on apparent caries status and lesion severity [22]. Of particular interest to this study is the coronal primary caries detection criteria. The ICDAS II detection codes for coronal pits and fissures caries ranges from 0 to 6 as follows[22]:
Code Description
(0) Sound (1) First visual change in enamel after prolonged air drying (2) Distinct visual change in enamel (3) Localized enamel breakdown without clinical visual signs of dentinal involvement (4) Underlying dark shadow from dentin (5) Distinct cavity with visible dentin (6) Extensive distinct cavity with visible dentin Laser fluorescence is a new method for early caries detection. By analyzing the fluorescence emission spectrum of carious regions versus sound tissues, studies have shown that laser fluorescence is useful as a quantitative measure distinguishing carious from non-carious surfaces[21]. Two main methods of laser fluorescence have been studied: quantitative light-induced fluorescence (blue light), and KaVo Diagnodent (red light). When compared to conventional visual examination for caries detection, QLF can detect twice as many early non-cavitated demineralized enamel areas[23]. Its ability to detect and quantify changes in clinically visible white spot lesions allows QLF to determine the impact of preventive measures on inhibition of demineralization[23]. While QLF has been proven to be an effective method of detecting smooth surface demineralization, its effectiveness in detecting occlusal caries has yet to be proven[21]. The same is true for the SOPROlife blue fluorescence digital photography (SOPRPlife, Acteon, NJ).
Optical Coherence Tomography is non-ionizing imaging technique which can produce cross section images of biologic tissues such as ocular, intravascular, gastrointestinal,
epidermal, soft oral tissues and teeth [24-28]. Fried et al (2002) demonstrated that polarized sensitive OCT (PS-OCT) can be correlated with the degree of demineralization and lesion severity[29]. They propose a potential utility for the system as monitoring *in vivo* caries lesion changes. Jones et al (2006) showed the advantages of a PS-OCT system in an artificial caries model by correlating OCT images lesion depth to those from sectioned micro-radiography, as well as quantifying lesions severity [[30]. The same group more recently applied PS-OCT to an *in vitro* study which concluded that the system is a good tool to monitor changes caries inhibition in enamel from CO2 laser treatment in
addition to fluoride[31].
Conclusion: In conclusion, high caries prevalence especially in occlusal pits and fissures warrants novel caries prevention methods. Sealants are more effective in caries prevention than fluoride treatment, but

have limited lifespan and require multiple repairs. CO2 lasers with the correct wavelength (9.6 mm) and pulse characteristics (pulse duration 2-100 ms) in addition to fluoride treatment can offer a novel alternative to caries prevention in pits and fissures. OCT is an ideal method of monitoring changes in both the study and control group.

SIGNIFICANCE

If the CO2 laser irradiation combined with fluoride treatment proves to be effective in preventing caries in vital teeth, then this could lead to an improved method of treating pit and fissure caries, the most prevalent site for caries today.

12.2

Preliminary studies:

A preliminary study using the pulsed CO2 laser on occlusal pits and fissures has been performed. The findings were statistically inconclusive, potentially as a result of the technical difficulties of irradiating the occlusal surface of 2nd molars. To help overcome this problem, a new delivery mechanism for the laser has been constructed.

12.3

References:

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If you have a separate bibliography, attach it to the submission with your other study documents.

Sample Size and Eligibility

13.0 13.1 Number of subjects that will be enrolled at UCSF and affiliated institutions:

20

13.2 Total number of subjects that will be enrolled at all sites:

20

13.3 Estimated number of people that you will need to consent and screen here (but not necessarily enroll) to get the needed subjects:

30

13.4 Sample size calculation:

A conservative sample size was calculated using the parallel groups trial data from our previous CO2 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. 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 (assuming 20%), 20 participants will be enrolled in the study, providing a total of 20 pairs of teeth. Therefore, if 20% of subjects drop-out, the data collected will still provide statistically meaningful results.

13.5 * Eligible age range(s):

0-6 years 7-12 years 13-17 years 18+ years

13.6 Inclusion criteria:

10 – 17 years old high caries risk status determined using CAMBRA two fully erupted second molars (+/- premolars) with untreated, non-cavitated 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 written consent 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

13.7 Exclusion criteria:

low or moderate caries risk status determined using CAMBRA no pair of untreated or non-carious occlusal surfaces on second molars or premolars 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)

13.8 There are inclusion or exclusion criteria based on gender, race or ethnicity:

Yes No If **yes**, please explain the nature and rationale for the restrictions:

14.0

Drugs and Devices

14.1 * Drugs or biologics will be studied under this application: Yes No

14.2 * Medical devices will be studied under this application: Yes No

14.3 Verification of IND/IDE numbers: If the sponsor's protocol does not list the IND/IDE number, you must submit documentation from the sponsor or FDA identifying the IND/IDE number for this study. Attach this documentation in the Other Study Documents section of the Initial Review Submission Packet.

Study Device Details

15.0 15.1 List the medical devices to be used:

Device List

Device Name: Pulse Systems 9.6um CO2 clinical laser Manufacturer/Supplier of Device: Pulse Systems (NM) Where will the Devices Be Stored: In a locked storage room Will Devices be supplied

at no Cost? No
Is this a HUD (HDE)? No HDE Number:: FDA Approved: No A new device or a new use of approved device: No IDE Number:
Who holds the IDE: N/A
IDE details:
Device level of risk:
No Significant Risk
15.2
*** A Non-Significant Risk (NSR) determination is being requested for the investigational device:**
Yes No
16.0
Non-Significant Risk Determination for an Investigational Device
(Note: This section replaces the old "Non-Significant Risk Determination for An Investigational Device" supplement form. Please do not attach the old form to this application.)
16.1
Explain why the use of the device in this study poses non-significant risk:
We consider the proposed treatment and use of the laser device as a non-significant risk (NSR) for the subjects. The reasons for that assumption are as followed:
A similar laser device (same wavelength CO2 9.6mm, same irradiation energies 1 J/cm2 – laser device produced by another company) was used to prove the safety of this proposed application in an earlier study. In that study conducted at UCSF and approved by the CHR (CHR approval H5362-19615-03 and CHR approval H9136-25290-01) we have shown that the use of this laser wavelength and energy settings is safe for the pulpal tissue. Due to the strong absorption of the CO2 laser wavelength in the first 10mm of enamel surface,
heat is produced which changes the existing enamel into a much more acid/caries resistant composition. Since all the applied energy is absorbed in that surface layer, laser light is not penetrating deeper into the tooth and represents no harm to the tooth.
The safety study showed that no changes in pulpal tissue occurred, no signs of inflammation or any other changes in the pulpal tissue were observed. Since the irradiation did not result in any pulpal reactions, the irradiation can be considered as safe and with low risk.
Due to the extremely low energy used in comparison to typical lasers used in dentistry, accidental irradiation of the surrounding gingival tissue may result in only a minor inflammation of the irradiated tissue. CO2 lasers are used in Oral Surgery to cut tissue or
at lower fluences to vaporize tissue. The energy applied here is extremely low and due to the extreme low penetration of that wavelength in soft tissue, a minor burn might occur.
The use of lasers requires laser safety glasses for the patient as well as the doctors and everyone else in the nominal hazard zone (10 feet around the laser) to protect the eyes from irradiation which in a worst case scenario might accidentally occur. Laser safety eyewear will be used during the whole procedure.
Finally, the laser device which we want to use **has a FDA clearance for skin resurfacing**. During skin resurfacing, much higher energies are used than during the irradiation procedure of enamel for improving caries resistance. We will use the laser with
a fluence (energy) of 1 J/cm2 while skin resurfacing uses the same device with 3 to 7 J/cm2.
In the present study we will be using the Pulse Systems (NM) 9.6 !m carbon dioxide clinical laser that is also currently in use in the approved project CHR approval H9136- 25290-03 "Laser Effects on Dental Hard Tissue".
The same device has safely been used in the study "In Vivo Occlusal Caries Prevention by Pulsed Laser treatment quantified by QLF" CHR H9136-30702-03 with PI Featherstone, and Co-PI Rechmann. No adverse affects have been encountered in the use of this device to date.
Overall, the "device" is safe for the use on patients for skin resurfacing, has proven to be safe in the aforementioned study in 12-18 year old children for occlusal tooth surface preventive treatment and we conclude that it will again be safe for the proposed intraoral use.
Attach any supporting documentation (e.g. any reports of prior investigations) at the end of the application.
Other Approvals and Registrations
17.0 17.1 This is a clinical trial:
Yes No
Clinical Trial Registration
"NCT" number for this trial:
17.2 * This study involves human gene transfer or recombinant DNA research: Yes No
17.3 This study involves other regulated materials and requires approval and/or authorization from the following regulatory committees:
Institutional Biological Safety Committee (IBC) Specify BUA #:
Institutional Animal Care and Use Committee (IACUC) Specify IACUC #:
Radiation Safety Committee Specify RUA #:
Radioactive Drug Research Committee (RDRC) Specify RDRC #:

Controlled Substances

Procedures

18.0 18.1 List all study procedures, test and treatments required for this study:

In Brief: this pilot project will be a single blind, randomized controlled clinical split mouth study. Patients meeting the inclusion criteria from the UCSF Predoctoral, Postgraduate Pediatric Dental and Postgraduate orthodontic clinics will have the study explained to them and be invited to participate. The Caries Management By Risk Assessment (CAMBRA) questionnaire currently used in the predoctoral and pediatric 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 and parent will be asked if they wish to provide informed consent. The molars or premolars will be randomly assigned to either the test or the control. A baseline visual inspection, white light and blue fluorescence SOPROlife digital photographs, DIAGNOdent, QLF and OCT assessments will be made by a blinded dentist prior to treatment. The control tooth will receive fluoride varnish treatment and the test tooth will be treated with CO2 laser irradiation and fluoride varnish. The

patient will be asked to return for a 12-week, 6-month and a 12-month follow up exam, at which time visual inspection, QLF, and OCT assessments will be conducted by the dentist who originally completed the baseline exam. The endpoint of the study for each participant will be when either the control or test tooth is found to have significant demineralization by 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. All data obtained will be analyzed for statistical significance.

If you have a procedure table, attach it to the submission with your other study documents.

Please see attached flow chart. In detail the procedures are:

Informed Consent:

Obtain written informed consent from parent/guardian and from patient.

Study Procedures:

Baseline exam: A complete medical and dental history, including CAMBRA caries risk assessment, will be obtained from the patient's dental chart. A trained dental examiner will brush the study teeth, take digital intraoral photos in white light 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 using 2.5x loupes. Codes for coronal caries (0-6) will be assigned based on severity of caries. Decayed, missing teeth (due to caries) and filled surface (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

DIAGNOdent, QLF analysis and OCT imaging and blue light fluorescence SOPROlife digital photography.

The molars and premolars will be randomized into test and control groups. The test molar and premolar will receive intervention with CO2 laser irradiation and then the test and control molars and premolars will be treated with fluoride varnish.

Treatment with CO2 laser irradiation: Following baseline exam, an independent dental provider, trained in this technique, will apply CO2 laser irradiation to the test

molar.

CO2 laser irradiation (test tooth):

A Pulse Systems (New Mexico) 9.6 mm wavelength clinical laser will be used at a fluence (energy/surface area) of 2.0 J/cm2 per pulse, and a pulse duration of 20 ms. Laser treatment will be applied in overlapping spots, approximately 1 mm in diameter, 20 pulses per spot, along the occlusal pits and fissures.

Treatment with Fluoride Varnish Following laser treatment of the test tooth, all the teeth will be dried and fluoride varnish will be applied.

12 week and Six-month recall: An exam will be completed at each recall in the same fashion as the baseline exam. This will be followed by white light

and blue fluorescence SOPROlife digital photographs, DIAGNOdent,QLF analysis and OCT imaging. Patients will receive fluoride treatment at the 6month recall as per the standard of care.

If at the 6month recall, an ICDAS score of three is found on one or more of the study teeth, then a sealant will be placed, and the patient will have come to the end point of the study.

Sealant:

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 bonded with 3M Multipurpose adhesive, air dried gently, and light cured for 20 seconds. Sealant will be applied; wait 15 seconds and light cured for 20 seconds. 12-month recall:

The same dental examiner will brush the study molars and premolars, 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 white light and blue fluorescence SOPROlife digital photographs, DIAGNOdent, QLF analysis and OCT imaging

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

18.2

Interviews, questionnaires, and/or surveys will be administered or focus groups will be

conducted:
Yes No List any standard instruments used for this study:
Attach any non-standard instruments at the end of the application.
The standard UCSF School of Dentistry "Caries management by risk assessment" (CAMBRA) questionnaire will be used. This questionnaire is asked to all UCSF dentistry patients at new patient and recall exam visits.

18.3
Conduct of study procedures or tests off-site by non-UCSF personnel:
Yes No If yes, explain:

18.4
Sharing of experimental research test results with subjects or their care providers:
Yes No If yes, explain:

18.5 * Specimen collection for future research and/or specimen repository/bank administration: Yes No

18.6 Time commitment (per visit and in total):
At the first appointment the following schedule was predicted:
1. informed consent/assent procedure (15 minutes) 2. brushing and ICDAS exam (10minutes) 3. photographs in white lightand blue fluorescence SOPROlife digital photography (5 minutes) 4. DIAGNOdent(2 minutes) 5. QLF assessment and OCT (20 minutes) 6. Laser irradiation of the occlusal surface of test tooth (10 minutes)
This adds up to a total of about one hour. In reality, initial appointments took anywhere from 35 - 60 minutes.
Each subsequent recall (12 week, 6 and 12 month) will take approximately 30-40 minutes. Therefore, the total time commitment is 60mins + 3 (30minutes) = 150minutes (2.5 hours).

18.7 Locations:
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.

18.8 Describe the resources 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.

Alternatives

19.0 19.1 Study drug or treatment is available off-study:
Yes No Not applicable

19.2 Describe the usual care or activities at UCSF (or study site) that are available to prospective subjects who do not enroll in this study:
The standard of care for patients at UCSF includes CAMBRA (caries management by risk assessment) whereby high risk patients in the age group defined would be prescribed to periodic oral exams at 6 month intervals with topical fluoride application and sealants over susceptible pits and fissures.

19.3 Describe other 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.

20.0
Risks and Benefits

20.1 Risks and discomforts:
A. Risk of caries progress without treatment. B. Risk of damage to the gingiva by the etching gel prior to sealant placement, leading to small gingival erosion. C. Risk of gingival irritation with the irradiation laser. D. Risk of accidental laser irradiation of the eyes. E. Risk of laser irradiation affecting the vitality of the tooth. F. Risk of damage from optical coherence tomography (OCT). G. Loss of privacy is potential risk for the patient.

20.2 Steps taken to minimize risks to subjects:
A. 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 [32]. Thus, waiting six to twelve months to seal a permanent molar will not cause a significant increase in risk for the patient.
B. 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. This is part of the normal standard of care.
C. 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, minimum inflammation 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.

D. 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. Such an event has not been reported during laser use in dentistry to date. The use of laser safety eyewear for everyone in the nominal hazard zone will minimize the risk of injuries to the eye.
E. The risk of tooth vitality changes is 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 9.6mm 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 [15]. Furthermore, the energy levels used are sub-ablative to enamel. 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.
F. OCT has been used in medicine for many years for imaging eyes. Its use now widespread from its roots in ophthalmology to intracoronary imaging and laparoscopic imaging of human ovaries. No adverse effects relating to the imaging technique have been published which would relate to imaging teeth.
G. Personal information will be kept in a locked cabinet or coded for patient privacy protection.

20.3 Benefits to subjects:

Yes No If yes, describe:

If the CO2 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.

20.4 Benefits to society:

If the specially designed CO2 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.

20.5 Explain why the risks to subjects are reasonable:

Since the risk related to being in this study is very small, the potential benefit of reduction of caries susceptibility outweighs any minor risks.

21.0

Data and Safety Monitoring Plan

21.1 Describe the plan for monitoring data and safety:

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. In the event of soft tissue injury, appropriate follow-up will be pursued to ensure healing. As discussed in the protocol, caries progress will be monitored throughout the study. Should caries progress from ICDAS 2 into 3 (which is the earliest marker for a routine dental exam/typical average dentist), the patient will be withdrawn and appropriate dental treatment will be provided or recommended, just as if the finding was made on a routine dental exam.

21.2 This study requires a Data and Safety Monitoring Board:

Yes No or not sure

If **yes**, press **SAVE and CONTINUE** to move to the next section of the application. **21.3 If No, provide rationale:**

Social/Behavioral research Phase I trial Treatment IND/Compassionate Use Trial Other (explain below)

If **Other,** explain:

Confidentiality and Privacy

22.0 22.1 Study data are:

Derived from the Integrated Data Repository (IDR) Derived from a medical record (identify source below) Added to the hospital or clinical medical record Created or collected as part of health care Used to make health care decisions 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

If **derived from a medical record**, identify source:

dental record at UCSF

22.2 Plans for accessing subject information while maintaining privacy:

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.

22.3 Identifiers may be included in research records:

Yes No If **yes**, check all the identifiers that may be included:

Names Dates

Postal addresses Phone numbers

Fax numbers Email addresses Social Security Numbers* Medical record numbers Health plan numbers Account numbers License or certificate numbers Vehicle ID numbers Device identifiers or serial numbers Web URLs IP address numbers Biometric identifiers Facial photos or other identifiable images Any other unique identifier

* Required for studies conducted at the VAMC

22.4 Plans for maintaining privacy in the research setting:

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

22.5 Possible consequences to subjects resulting from a loss of privacy:

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.

22.6 Identifiable information might be disclosed as part of study activities:

Yes No If **yes**, indicate where identifiable information may be released to:

The subject's medical record The study sponsor The US Food & Drug Administration (FDA) Others (Specify below) A Foreign Country or Countries

If **Others**, specify:

22.7 Indicate how data are kept secure (check all that apply):

Data are stored securely in My Research Data are coded; data key is destroyed at end of study Data are coded; data key is kept separately and securely Data are kept in a locked file cabinet Data are kept in a locked office or suite Electronic data are protected with a password Data are stored on a secure network Data are collected/stored using REDCap or REDCap Survey

22.8 Additional measures to assure confidentiality:

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.

22.9 This study may collect information that State or Federal law requires to be reported to other officials or ethically requires action:

Yes No Explain:

22.10 This study will be issued a Certificate of Confidentiality:

Yes No

Subjects

23.0 23.1 Check all types of subjects that may be enrolled:

Inpatients Outpatients Healthy volunteers Staff of UCSF or affiliated institutions

23.2 Additional vulnerable populations:

Children Subjects unable to consent for themselves Subjects unable to consent for themselves (emergency setting) Subjects with diminished capacity to consent Subjects unable to read, speak or understand English Pregnant women Fetuses Neonates Prisoners Economically or educationally disadvantaged persons Investigators' staff Students

Explain why it is appropriate to include the types of subjects checked above in this particular study:

The study will be conducted in permanent teeth in high caries risk children aged 10-17 years. The study uses fully erupted second molars and premolars 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 and oral hygiene habits 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.

Describe the additional safeguards that have been included in the study to protect the rights and welfare of these subjects and minimize coercion or undue influence:

Payment amounts are calibrated to be non-coercive for the financially disadvantaged.

24.0

Inclusion of Children in Research

(Note: This section replaces the old "Inclusion of Children and Minors in Research" supplement form. Please do not attach the old form to this application.)

24.1 This study will enroll children who can legally consent for themselves:

Yes No If **yes,** explain why they can consent for themselves in the research setting:

If you will **ONLY** be enrolling children who can legally consent for themselves, press **SAVE and CONTINUE** to skip the rest of this section.

24.2 Select all the regulatory categories that apply:

No greater than minimal risk (45 CFR 46.404, 21 CFR 50.51) Greater than minimal risk but presenting prospect of direct benefit (45 CFR 46.405, 21 CFR 50.52)

Greater than minimal risk (though only a minor increase over minimal risk) and no prospect of direct benefit but likely to yield generalizable knowledge about the subjects disorder or condition (45 CFR 46.406, 21 CFR 50.53)

Research not otherwise approvable which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children (45 CFR 46.407, 21 CFR 50.54)

Explain why the research in this study falls under the above category or categories:

24.3

Parental permission or waiver:

Parental permission will be obtained Waiver of parental permission is requested: Parental permission is not a reasonable requirement

Waiver of parental permission is requested: The waiver meets the provisions for a waiver of consent set

forth in 45 CFR 46.116, Subpart A
If you are requesting a **waiver of parental permission**, explain why the study meets the regulatory criteria for this waiver:
24.4
Assent of children or waiver:
Assent of children old enough to provide assent will be obtained
Waiver of assent is requested: Children cannot be consulted or the research has prospect of direct benefit only available in the study
Waiver of assent is requested: The waiver meets the provisions for a waiver of consent set forth in 45 CFR 46.116, Subpart A
If you are requesting a **waiver of child's assent**, explain why the study meets the regulatory criteria for this waiver:
24.5
Documentation of permission and assent (select all that will be used):
Permission form addressed to the parents Simplified assent form addressed to the child, 7-12 years old (parents get separate form) Assent form addressed to the child, 13 years and older (for subjects and parents) Assent form addressed to the child, 13 years and older (parents get separate form)
Check one: One parent's signature will be obtained Two parents' signatures will be obtained
If this study is approvable under .404 or .405 and you plan to get permission from only one parent, explain why you think one parent's permission is sufficient:
24.6
This study may enroll wards of the state:
Yes No
25.0
Recruitment
25.1
* **Methods (check all that apply):**
Study investigators (and/or affiliated nurses or staff) recruit their own patients directly in person or by phone. Study investigators recruit their own patients by letter. Attach the letter for review.
Study investigators send a "Dear Doctor" letter to colleagues asking for referrals of eligible patients. If interested, the patient will contact the PI or the PI may directly recruit the patients (with documented permission from the patient). Investigators may give the referring physicians a study information sheet for the patients.
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 Advertisements, notices, and/or media used to recruit subjects. Interested subjects initiate contact with study investigators. Attach ads, notices, or media text for review. In section below, please explain where ads will be posted.
Study investigators identify prospective subjects through chart review. (Study investigators request a Waiver of Authorization for recruitment purposes.)
Large-scale epidemiological studies and/or population-based studies: Prospective subjects are identified through a registry or medical records and contacted by someone other than their personal physician. (Study investigators request a Waiver of Authorization for recruitment purposes.)
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.
Study investigators list the study on the School of Medicine list of UCSF Clinical Trials website or a similarly managed site. Interested subjects initiate contact with investigators.
Study investigators recruit potential subjects who are unknown to them through methods such as snowball sampling, direct approach, use of social networks, and random digit dialing.
Other If **Other**, explain:
25.2 How, when, and by whom eligibility will 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 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.
25.3 How, when, where and by whom potential subjects will be 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 (+/- premolars) 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.

25.4 * Protected health information (PHI) will be accessed prior to obtaining consent: Yes
No

Informed Consent

26.0 26.1 * Methods (check all that apply):

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 or script Electronic consent will be obtained from subjects via the web or email Implied consent will be obtained via mail, the web or email
Signed consent will be obtained from surrogates Emergency waiver of consent is being requested for subjects unable to provide consent Informed consent will not be obtained

26.2 Process for obtaining informed 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 15 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 he will suggest to the subjects to go home and call, if they are interested and have an informative meeting again on another day or he will decide that the subject should not be enrolled.

26.3 How investigators will 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.

Financial Considerations

27.0 27.1 Subjects payment or compensation method (check all that apply):

Payments will be (check all that apply): Subjects will not be paid
Cash Check Gift card Other:
Specify **Other**: **27.2 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. Each of the child and parent will receive $10 at the baseline exam and at each subsequent intermediary exam, 12 week visit, and 6 month if completing at 12 month recall). Upon the completion of the study (either the 6 month recall exam or 12 month recall exam) $20 will be received by each of the child and parent. In summary, children and parents will each receive a total $50 if the participation is 6 months or $60 if the participation is 12 months.

27.3 Costs to Subjects: Will subjects or their insurance be charged for any study procedures?
Yes No

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.

28.0

CTSI Screening Questions

28.1 * This study will be carried out at one of the UCSF Clinical Research Centers (CRCs) or will utilize CRC services: Yes No

28.2 This project involves community-based research: No

28.3 This project involves practice-based research: Yes

28.4 Please check other CTSI services below that you plan to utilize to conduct your research:

Guidance and Services:

Biostatistics Study Design and Implementation Data Management Ethics Health Policy Bioinformatics Data Analysis Regulatory Knowledge
THREDS The Health Record Data Service Community-Engaged Research Collaborating with Kaiser Researchers

Clinical Research Centers:

Community Engagement (CE)

Funds to Innovate:

Strategic Opportunity Support (SOS)

Training:

Clinical & Translational Sciences Training (CTST) Career Advancement (CA)

CTSI Core Services:

Animal/Preclinical Array Bioinformatics Biostatistics
Cell Culture Clinical Services Epidemiology Flow Cytometry Human/Clinical Imaging
Immunohistochemistry Islet Production Microscopy Molecular/Genomic Monoclonal Antibody Proteomics Resale Products Tissue

(iii) Informed Consent Document

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

***In vivo* occlusal caries prevention by pulsed CO_2 laser and fluoride treatment.**

Dr. Peter Rechmann, DDS, PhD together with his clinical investigator Dr. Daniel Charland, 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 10 to 17 years who are patients in the Predoctoral or Postgraduate Pediatric Dental Clinic.

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) and/or premolars 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 30 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 and/or premolars will be selected for the study. If more than two second molars and/or premolars 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.

2. The two second molars and/or premolars will be randomly (by chance, like flipping a coin) assigned to get the left or right teeth laser treated. One tooth will receive the laser treatment, while the other tooth will be left as is for the first six months. We will brush your teeth and check these teeth with two highly sensitive lasers and an imaging device. We will take pictures of your teeth in white and blue light. Then, we will treat the chewing surface of one of those two second molars with a specially designed laser for several seconds. Both selected teeth will be treated with a fluoride varnish.
3. You will be asked to brush your teeth in the morning and evening every day with regular fluoride toothpaste. You will be asked to use only a pea-sized amount of toothpaste on your toothbrush. You will do this each day while you are in the study.
4. You will be asked to return for a 2 week and 12 week recall exam, at which time an exam, photographs in white and blue light and check them again with two highly sensitive detection lasers will be done to exam your molars and/or premolars. This will be repeated at the 6 and 12 month regular dental exams.
5. At 6 months, you will return to the clinic for your regular dental exam, cleaning, and fluoride treatment. At this appointment, the dentist will again brush your second molars and/or premolars, examine them, take pictures of your teeth in white and blue light, and. If your second molar(s) and/or premolars look like they are beginning to form a cavity, then both second molars and/or premolars 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.
6. If your molar(s) and/or premolars 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 in white and blue light, and check your second molars with two highly sensitive lasers. Both second molars and/or premolars will be sealed with a dental sealant, and you will have completed the study.
7. 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 require you to have two additional examination visits. 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 2.5 hours over a period of 6 months or 3 hours 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.

A 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 on its own in 1-3 days. This danger will be made even smaller by using a high speed vacuum to remove the cleaning gel prior to rinsing with water.

Another 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. The use of the sensitive detection laser poses no risk to you.

2. Randomization: Your second molars and/or premolars will be assigned to either receive laser intervention or no intervention by chance (like flipping a coin). All teeth will receive fluoride varnish. 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 or 12 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. Daniel Charland, if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him or his 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 additional 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, each of the child and parent will receive $10 at the baseline exam and at each subsequent intermediary exam (~~2~~ and 12 week visit, and 6 month if completing at 12 month recall). Upon the completion of the study (either the 6 month recall exam or 12 month recall exam) $20 will be received by each of the child and parent in gratitude of their commitment. In summary, children and parents will each receive a total $40 if the participation is 6 months or $50 if the participation is 12 months.

1) What if you have questions?

This study has been explained to you by Dr. Daniel Charland or the person who signed below and your questions were answered. If you have any other questions about the study you may call Dr. Daniel Charland or his 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. Peter Rechmann, telephone 415-514-3225.

If you have any comments or concerns about participation in this study, you should first talk with the clinical investigator (Dr. Charland). 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.

a. 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. Charland) 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.

****

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*

b)

c)

d) 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

(iv) Intra-Examiner and Inter-Examiner Reliability

Out of 120 observations made by Examiner DC (Table 2), the number of observed agreements was 60 (50.85% of the observations), compared to the number of agreements expected by chance: 42.0 (35.59% of the observations). In this case, the weighted Kappa= 0.361 (SE of Kappa = 0.069), with the 95% confidence interval for this statistic from 0.103 to 0.371. Assessed this way, the strength of agreement is considered to be 'fair'.

Table 2: Contingency Table of Observations Made by Examiner DC

Observations @ Time 1	Observations @ Time 2 (ICDAS II code)					
	DC 2 (0)	DC 2 (1)	DC 2 (2)	DC 2 (3)	DC 2 (4)	DC (5)
DC 1 (0)	7	18	3	0	0	0
DC 1 (1)	2	15	23	0	0	0
DC 1 (2)	2	9	36	0	0	0
DC 1 (3)	0	0	1	2	0	0
DC 1 (4)	0	0	0	0	0	1
DC 1 (5)	0	0	0	0	1	0
					TOTAL	120

Out of 118 observations made by Examiner PR (Table 3), the number of observed agreements was 84 (71.19% of the observations), compared to the number of agreements expected by chance: 48.7 (41.25% of the observations). In this case, the weighted Kappa= 0.660 (SE of Kappa = 0.063), with the 95% confidence interval for this statistic from 0.386 to 0.633. Assessed this way, the strength of agreement is considered to be 'good'.

Table 3: Contingency Table of Observations Made by Examiner PR

Observations @ Time 1	Observations @ Time 2 (ICDAS II code)					
	PR 2 (0)	PR 2 (1)	PR 2 (2)	PR 2 (3)	PR 2 (4)	PR 2 (5)
PR 1 (0)	14	0	3	1	0	0
PR 1 (1)	7	2	8	0	0	0
PR 1 (2)	0	2	61	8	0	0
PR 1 (3)	0	1	1	3	0	
PR 1 (4)	0	0	0	2	4	1
PR 1 (5)	0	0	0	0	0	0
					TOTAL	118

Scores assigned by each examiner agreed with each other. At the first examination, each examiner made 134 observations (Table 4). The number of observed agreements was 65 (48.51% of the observations), compared to the number of agreements expected by chance of 44.1 (32.88% of the observations). At the first examination, the weighted Kappa=0.384 (SE of Kappa = 0.055), with the 95% confidence interval from 0.124 to 0.341. Assessed this way, the strength of agreement is considered to be 'fair'.

Table 4: Contingency Table of Observations Made by Examiners DC and PR at Time 1

Examiner DC @ Time 1	Observations by Examiner PR @ Time 1					
	PR 1 (0)	PR 1 (1)	PR 1 (2)	PR 1 (3)	PR 1 (4)	PR 1 (5)
DC 1 (0)	12	9	6	0	0	0
DC 1 (1)	3	6	27	3	2	0
DC 1 (2)	3	2	45	6	3	0
DC 1 (3)	0	0	1	1	3	0
DC 1 (4)	0	0	0	0	1	0
DC 1 (5)	0	0	0	0	1	0
					TOTAL	134

At the second examination (7 days later), each examiner made 120 observations (Table 5). The number of observed agreements was 57 (47.50% of the observations), compared to the number of agreements expected by chance of 42.9 (35.74% of the observations). At the second examination, the weighted Kappa= 0.386 (SE of Kappa = 0.053), with the 95% confidence interval from 0.079 to 0.287. Assessed this way, the strength of agreement is considered to be 'fair'.

Table 5: Contingency Table of Observations Made by Examiners DC and PR at Time 2

Examiner DC @ Time 2	Observations by Examiner PR @ Time 2					
	PR 2 (0)	PR 2 (1)	PR 2 (2)	PR 2 (3)	PR 2 (4)	PR 2 (5)
DC 2 (0)	8	1	2	0	0	0
DC 2 (1)	11	3	26	2	0	0
DC 2 (2)	3	1	46	12	1	0
DC 2 (3)	0	0	0	0	2	0
DC 2 (4)	0	0	0	0	0	1
DC 2 (5)	0	0	0	0	1	0
					TOTAL	120

Overall, when summing the contingency tables from examination date 1 and examination date 2, each examiner made a total of 254 observations (Table 6). The number of observed agreements was 122 (48.03% of the observations), compared to the number of agreements expected by chance of 87.4 (34.43% of the observations). Combining all data, the weighted Kappa= 0.384 (SE of Kappa = 0.039), with a 95% confidence interval from 0.131 to 0.284. Assessed this way, the strength of agreement is considered to be 'fair'.

Table 6: Contingency Table of All Observations Made by Examiners DC and PR

Examiner DC	Cumulative Observations by Examiner PR					
	PR (0)	PR (1)	PR (2)	PR (3)	PR (4)	PR (5)
DC (0)	20	10	8	0	0	0
DC (1)	14	9	53	5	2	0
DC (2)	6	3	91	18	4	0
DC (3)	0	0	1	1	5	0
DC (4)	0	0	0	0	1	1
DC (5)	0	0	0	0	2	0
					TOTAL	254

(V) *In Vivo* Raw Data

Laser Treated ICDAS II

Subject #	T=0	T=3m	T3-T0	T=6m	T6-T0
1	0	0	0	0	0
2	2	2	0	2	0
3	1	2	1	1	0
4	1	1	0	0	-1
5	2	1	-1	1	-1
6	2	2	0	2	0
7	1	1	0	2	1
8	2	2	0	1	-1
9	1	1	0	1	0
10	1	2	1	1	0
11	1	1	0	1	0
12	1	1	0	2	1
13	2	2	0	2	0
14	2	1	-1	2	0
15	1	1	0	1	0
16	1	1	0	1	0
17	1	1	0	2	1
18	0	1	1	1	1
19	0	1	1		
20	1	2	1	2	1
Mean	1.15	1.30	0.15	1.32	0.11
Std Dev	0.67	0.57	0.59	0.67	0.66

Control Teeth ICDAS II

T=0	T=3m	T3-T0	T=6m	T6-T0
0	1	1	2	2
2	1	-1	2	0
2	2	0	2	0
1	1	0	2	1
2	2	0	2	0
2	2	0	2	0
1	1	0	2	1
1	1	0	1	0
0	1	1	1	1
1	2	1	1	0
1	2	1	1	0
0	1	1	1	1
2	2	0	3	1
2	2	0	2	0
1	2	1	2	1
2	2	0	2	0
1	0	-1	1	0
0	2	2	1	1
0	1	1		
1	2	1	2	1
1.10	1.50	0.40	1.68	0.53
0.79	0.61	0.75	0.58	0.61

Laser Treated DIAGNOdent

Subject #	T=0	T=3m	T3-T0	T=6m	T6-T0
1	17	12	-5	14	-3
2	44	16	-28	33	-11
3	18	9	-9	20	2
4	23	15	-8	17	-6
5	39	33	-6	41	2
6	22	12	-10	30	8
7	10	20	10	62	52
8	26	10	-16	20	-6
9	24	15	-9	40	16
10	14	15	1	17	3
11	26	21	-5	16	-10
12	13	39	26	65	52
13	27	36	9	22	-5
14	17	57	40	50	33
15	15	20	5	20	5
16	55	40	-15	33	-22
17	30	25	-5	35	5
18	10	18	8	15	5
19	26	25	-1		
20	27	18	-9	20	-7
Mean	24.15	22.80	-1.35	30.00	5.95
Std Dev	11.44	12.35	15.03	15.62	19.86

Control Teeth DIAGNOdent

T=0	T=3m	T3-T0	T=6m	T6-T0
14	12	-2	11	-3
52	16	-36	27	-25
48	38	-10	40	-8
27	23	-4	37	10
64	35	-29	60	-4
26	20	-6	20	-6
24	17	-7	18	-6
9	7	-2	6	-3
24	35	11	30	6
14	14	0	18	4
8	20	12	27	19
16	13	-3	79	63
26	30	4	25	-1
39	54	15	51	12
19	30	11	31	12
25	49	24	36	11
8	11	3	20	12
18	12	-6	18	0
15	25	10		
11	12	1	22	11
24.35	23.65	-0.70	30.32	5.47
15.41	13.16	13.94	17.64	17.29

Laser Treated QLF ΔF

Subject #	T=0	T=3m	T3-T0	T=6m	T6-T0
1	-7.93	-8.09	-0.16	-7.55	0.38
2	-22.10	-21.80	0.30	-21.40	0.70
3	-10.90	-10.40	0.50	-11.50	-0.60
4	-12.60	-10.60	2.00	-13.00	-0.40
5	-15.60	-16.20	-0.60		
6	-14.70	-11.30	3.40	-14.80	-0.10
7	9.50	-11.40	-20.90	-11.10	-20.60
8	-11.10	-13.50	-2.40	-10.70	0.40
9	-10.70	-13.30	-2.60	-11.50	-0.80
10	-10.40	-11.90	-1.50	-10.60	-0.20
11	-9.31	-11.10	-1.79	-12.40	-3.09
12	-8.12	-9.81	-1.69	-9.06	-0.94
13	-13.50	-12.80	0.70	-15.10	-1.60
14	-14.20	-12.40	1.80	-15.80	-1.60
15	-7.78	-9.38	-1.60	-9.18	-1.40
16	-10.40	-13.60	-3.20	-12.30	-1.90
17	-12.20	-15.60	-3.40	-17.10	-4.90
18	-14.50	-14.20	0.30	-16.70	-2.20
19	-13.60	-16.00	-2.40		
20	-19.60	-21.50	-1.90	-18.60	1.00
Mean	-11.49	-13.24	-1.76	-13.24	-2.10
Std Dev	6.15	3.61	4.87	3.65	4.84

Control Teeth QLF ΔF

T=0	T=3m	T3-T0	T=6m	T6-T0
-7.59	-8.14	-0.55	-8.35	-0.76
-15.40	-17.50	-2.10	-16.10	-0.70
-12.3	-12.9	-0.6	-13.4	-1.1
-10.80	-10.50	0.30	-11.40	-0.60
-27.10	-22.70	4.40		
-17.70	-15.40	2.30	-12.50	5.20
-12.90	-16.60	-3.70	-14.60	-1.70
-8.41	-7.51	0.90	-8.93	-0.52
-18.10	-20.10	-2.00	-18.10	0.00
-13.90	-12.40	1.50	-13.80	0.10
-14.20	-13.60	0.60	-12.50	1.70
-7.01	-7.28	-0.27	-7.04	-0.03
-11.80	-12.70	-0.90	-14.50	-2.70
-20.60	-19.40	1.20	-16.50	4.10
-11.40	-10.50	0.90	-12.20	-0.80
-14.80	-20.30	-5.50	-16.10	-1.30
-7.56	-7.82	-0.26	-7.80	-0.24
-10.60	-13.00	-2.40	-13.00	-2.40
-11.40	-11.40	0.00		
-13.20	-13.60	-0.40	-12.60	0.60
-13.34	-13.67	-0.33	-12.75	-0.06
4.85	4.58	2.15	3.13	2.00

SoproLife White

	"Better"	"Same"	"Worse"
Control	4	11	4
Laser Treated	8	8	3

SoproLife Blue

	"Better"	"Same"	"Worse"
Control	5	11	3
Laser Treated	7	12	0

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June 10, 2011

Date