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MI Varnish and MI Paste Plus in a Caries Prevention and Remineralization Study

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MI Varnish and MI Paste Plus in a Caries Prevention and Remineralization Study

by

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THESIS

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DEDICATION

I would like to dedicate this Master's Thesis to my loving sister, Arpi, and my wonderful parents, Marine and Nairi Bekmezian. To my parents, thank you for being so supportive of my educational pursuits when it has meant starting a new life away from home. You both are quintessentially self-less and unconditionally loving. Thank you for teaching me the importance of taking excellent care of people. To my sister, you have been the best role model and I feel so lucky to have had your guidance and encouragement along every step of my journey. You push me when I need to be pushed and you hold me when I need to be held. Thank you for helping me become true to my dreams. I love you guys so much!

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by Sona Bekmezian, DDS

ABSTRACT

Purpose: White spot lesions (WSLs) are a common complication of orthodontic therapy. It has been proposed that treatment with casein phosphopeptide-amorphous calcium phosphate, the active ingredient in MI ™ products can effectively reduce the appearance of WSLs.

The aim of this study was to investigate the effect of quarterly in-office applications of MI Varnish in combination with daily at-home application of MI Paste Plus on the prevention and regression of WSLs as compared to the use 1,100 ppm fluoride toothpaste and recommendation of fluoride mouth-rinse in orthodontic patients in a 12-month, randomized, single-blind, prospective, standard-of-care controlled clinical trial.

Methods: Forty subjects at least 11 years of age and undergoing therapy with full fixed orthodontic appliances were recruited from the UCSF Orthodontics Clinic. Subjects had at least two active WSLs on the maxillary and mandibular anterior teeth at baseline. Subjects were randomly assigned to experimental or control groups. The facial surfaces of anterior teeth were evaluated at baseline, 3, 6, and 12 months using the Enamel Decalcification Index, the International Caries Detection and Assessment System and the Nyvad criteria for caries lesion activity.

Data from the final time point will be collected after the submission of this thesis. In order to avoid unblinding the study investigators to subject group assignment, data collected over the first 6 months of the study will be presented here on the entire group of subjects as a collective.

Results: There was no difference in the number of 0s, 1s, 2s, and 3s scored for tooth surfaces in the EDI or ICDAS indices from baseline to 3 months to 6 months. There was no change in average EDI sums across time or average highest ICDAS scores across time. Gingival surfaces received higher WSL scores than mesial and distal surface scores, and occlusal surfaces scored virtually zero. Salivary fluoride levels increased significantly and plaque levels decreased significantly over time.

Conclusions: Gingival surfaces experienced greater surface coverage of WSLs and higher severity of demineralization. Plaque levels improved across subjects with quarterly oral hygiene reinforcement. Salivary fluoride levels increased over time across all subjects receiving topical fluoride.

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INTRODUCTION

Background

The white spot lesion (WSL) is an early sign of dental caries. Demineralized enamel appears white because water and air fill the porosities that have developed at the enamel surface and scatter light differentially due to their various refractive indices. The appearance of WSLs around orthodontic brackets is a common problem - fixed appliances hamper effective oral hygiene, resulting in increased plaque accumulation and increased caries risk.[1] The frequency of WSLs ranges from 2-96% and even in low risk populations with comprehensive caries prevention programs, up to 61% of patients can develop WSLs.[2, 3] The labial surface of maxillary incisors is the most commonly affected location, and over 60% of decalcification occurs in the gingival third because of low salivary clearance.[1]

A recent systematic review of available preventative and therapeutic agents for WSLs found that the use of topical fluoride and fluoride toothpaste remains the best way to avoid WSLs.[4] When WSLs do occur, reversal and esthetic improvement can be extremely difficult, often requiring microabrasion treatment.[5] De-bonding (*i.e.*, removal of orthodontic appliances) removes the major etiological factor, but some WSLs persist even 5 years after the completion of orthodontic treatment.[6] Saliva remineralizes WSLs to some degree, but the process is slow and rarely results in complete resolution of the lesions.[7] Fluoride can increase the initial rate of remineralization, arrest areas of decalcification, and prevent WSLs from progressing to cavitated lesions.[8] According to a Cochran review of the literature, daily rinsing with a sodium fluoride mouth-rinse reduces the severity of enamel decay around fixed orthodontic appliances.[9] Brushing with high fluoride toothpaste significantly reduced WSLs compared to regular fluoride

toothpaste in a recent study.[10] There is also some evidence to suggest that weekly applications of fluoride varnish during the first month after de-bonding may help reverse WSLs.[11]

However, some experts warn against the use of high concentrations of fluoride to treat WSLs for fear of remineralizing only the superficial part of the WSLs.[12] They argue that without any supplemental calcium and phosphate, the surface deposition of fluoride may produce a scarred hypermineralized lesion with a thick surface layer that restricts calcium and phosphate ions from penetrating to the deeper layers of the enamel, inhibiting the remineralization of the body of the lesion and limiting esthetic improvement (lesions continue to appear white).[13, 14] If a lesion is in a location of the mouth that does not present an esthetic concern, remineralization of surface of the lesion may be sufficient for disease arrest. However, a more ideal outcome would be incorporating as much mineral as possible, preferably fluorapatite, to help reduce the risk of future disease and reverse unsightly white spots.

Casein phosphopeptide-amorphous calcium phosphate nano-complexes (CPP-ACP), derived from the bovine milk protein casein, has been incorporated into a number of commercial products such as sugar-free chewing gums (Trident Xtra Care (Mondelēz International, Deerfield, IL) and Recaldent (Cadbury Enterprises Pte Lte, Singapore)) and dental creams (Tooth Mousse (GC America, Alsip, IL) and MI (minimal intervention) Paste and Varnish (GC America, Alsip, IL)) for the remineralization of WSLs. The investigation of CPP-ACP for enamel remineralization first began in the late 1990s. In laboratory experiments, 1.0% CPP-ACP produced a 55% reduction in smooth surface caries in rats, a result comparable to 500ppm fluoride.[15] In human *in situ* studies, CPP-ACP was found to have a significant remineralization effect.[16] CPP-ACP-containing cream was found to promote lesion regression to the same degree as Crest (Proctor and Gamble, Cincinnati, OH) 1,100-ppm-fluoride toothpaste, and an

even greater improvement when compared to fluoride mouth-rinse.[2, 17] According to a recent meta-analysis on the prevention of enamel demineralization by CPP-ACP, data from five *in situ* randomized controlled trials showed significantly increased remineralization and decreased demineralization when subjects chewed gum with added CPP-ACP compared to gum without CPP-ACP.[18]

Mechanism of action of CPP-ACP

CPP-ACP acts as a reservoir for calcium and phosphate when incorporated into dental plaque and tooth surfaces. The CPP is the carrier molecule for the ACP, localizing the highly soluble calcium-phosphate phase at the tooth surface.[19] Calcium and phosphate ions released from the CPP-ACP readily diffuse through porous WSLs and deposit on partially demineralized crystallites. Casein phosphopeptide contains a cluster of phosphoserine (Ser(P)) residues which have the ability to bind amorphous calcium phosphate, increasing its solubility and preventing mineral precipitation.[20-22] Calcium interacts with the CPP through the negatively charged residues of the peptide (–Ser(P)–Ser(P)–Ser(P)–Glu–Glu–) as well as other acidic residues of the phosphopeptide sequence.[23] Casein phosphopeptide as1-casein X-5P (f59–79) and b-casein X-4P (f1–25) can maximally bind 21 and 24 calcium ions and 14 and 16 phosphate ions per molecule, respectively, producing a metastable, colloidal solution.[23] An equilibrium between free and CPP-bound calcium and phosphate ions ultimately occurs based on the conditions of the environment, providing a reservoir of bioavailable ions for inhibition of demineralization and promotion of remineralization.

CPP-ACP inhibits demineralization in four ways. First, it acts as a protective physical barrier by binding to the apatite crystal face and blocking active sites of dissolution.[24] Second, various amino acid residues found in CPP act as acid buffers and others can be broken down to

produce ammonia, thereby increasing the pH of cariogenic plaque.[20, 25] Third, CPP-ACP maintains calcium and phosphate ion concentrations in plaque in a state of saturation with respect to tooth enamel.[19] Fourth, CPP-ACP may alter plaque microbial composition to a less cariogenic form by preventing the adherence and colonization of specific bacteria. CPP can incorporate into the pellicle in exchange for albumin; this inhibits the adherence of *S. sobrinus* and *S. mutans* by blocking specific receptors, competitively binding calcium to prevent calcium bridging of bacterial cells, and causing electrostatic repulsion by binding to the surface of bacteria.[26, 27]

The release of calcium and phosphate ions from CPP-ACP is driven by 1) equilibrium, 2) conformational changes, 3) pH, and 4) enzymatic activity. First, if the concentration of calcium and phosphate ions in solution decreases, then CPP-ACP will release more of these ions from its nano-complexes to maintain equilibrium. Second, in addition to calcium, CPP has binding affinities for apatite, pellicle, mucin, proline-rich proteins and bacteria; binding induces a conformational change in the CPP that will release calcium and phosphate ions from the nano-complex.[19, 23, 26-28] Third, as pH decreases, the phosphorylated groups of the peptide become protonated, thereby decreasing the net negative charge and causing the release of positive calcium and phosphate ions from the complexes.[29, 30] Fourth, enzymatic hydrolysis of CPP will lead to the release of the calcium and phosphate ions.[19, 31]

Interaction with Fluoride

In the presence of fluoride, CPP-ACP promotes the formation of fluorapatite deep in the subsurface lesion.[32] The combination of CPP-ACP and fluoride may have a synergistic effect on enamel remineralization due to the formation of stabilized amorphous calcium fluoride phosphate (ACFP). This may result in the increased incorporation of fluoride ions into plaque

together with increased concentrations of bioavailable calcium and phosphate ions.[32] In laboratory experiments, the anticariogenic effects of CPP-ACP and fluoride were found to be additive: animals receiving 0.5% CPP-ACP plus 500ppm fluoride had lower caries activity than those receiving CPP-ACP or fluoride alone.[15]

Some *in vivo* studies and systematic reviews have reported equivocal results for CPP-ACP in reversing WSLs as compared to traditional fluoride therapies. Most recently, in a randomized controlled trial investigating the effectiveness of MI Paste Plus and PreviDent 5% fluoride varnish (Colgate-Palmolive, New York City, NY) compared to a standard toothpaste regimen during an 8-week post-orthodontic period, there was no difference found in WSL improvement between groups.[33] Previous studies with more optimistic results had small sample sizes, lack of blinding and placebo control, and lack of endpoints of true clinical significance.[4] Other issues present in these studies were conflicts of interest (investigators were patent holders), the use of surrogate outcome measures (remineralization of enamel slabs mounted in acrylic carriers), and exclusively visual or photographic methods of measurement.[34] Finally, previous studies seldom examined the effect of chemical interventions during orthodontic treatment, when patients are most susceptible to forming WSLs, and when WSLs are most active. Therefore, our objective was to conduct a single-blind, randomized, prospective, standard-of-care controlled clinical trial assessing the effect of CPP-ACP on WSL prevention and regression for patients undergoing orthodontic treatment using visual inspection techniques such as the Enamel Decalcification Index (EDI), the International Caries Detection and Assessment System (ICDAS), the Nyvad criteria for caries lesion activity, as well as light-fluorescence techniques such as Quantitative Light-Induced Fluorescence (QLF) and the SOPROlife fluorescence system.

Hypothesis

We hypothesize that four applications of MI Varnish in combination with daily use of MI Paste Plus will show superior WSL prevention and regression in orthodontic patients as compared to the use of a standard-of-care control (Crest 1,100ppm fluoride toothpaste with fluoride mouth-rinse recommendation) over a 12 month time period.

Null Hypothesis

The null hypothesis is that four applications of MI Varnish in combination with MI Paste Plus will show the same level of caries prevention and WSL regression in orthodontic patients as the use of a standard-of-care control (Crest 1,100ppm fluoride toothpaste with fluoride mouth-rinse recommendation) over a 12 month time period.

MATERIALS AND METHODS

Institutional Review Board (IRB) approval was obtained from the UCSF Committee on Human Research (IRB approval #13-10710), and our study was registered with the US National Institutes of Health as a Phase 4 clinical trial (ClinicalTrials.gov Identifier: NCT02424097). All experiments were conducted at the UCSF School of Dentistry Clinics (707 Parnassus Avenue, 3rd and 4rd Floors, San Francisco, CA 94122). Materials and funding were provided by GC America (Alsip, IL) through the UCSF Contracts & Grants Office. Each study subject was compensated up to $125 in cash for his/her time in the study: $10 at baseline, $20 at the 3-month visit, $25 at the 6-month visit, $30 at the 9-month visit, and $40 at the last visit at 12 months.

Target Population

Subjects were recruited from the UCSF School of Dentistry Orthodontics Clinic from October 2013 to August 2014. Subjects had to have brackets bonded to the facial surfaces of the

maxillary and mandibular incisors, canines and first bicuspids and show evidence of moderate to high caries risk according to the UCSF Caries Risk Assessment.

Inclusion Criteria

To be included, participants had to fulfill the following criteria:

- age 11 years or older,* in good general health
- present with at least two active WSLs on the anterior teeth at the start of the study
- require at least 12 additional months of full fixed appliance therapy from the time they were recruited for the study
- able to cooperate for treatment in the dental chair and follow at-home instructions
- have an understanding of the study
- willing to comply with all study procedures and protocols
- able to provide written informed assent/consent in English
- willing to sign the "Authorization for Release of Personal Health Information and Use of Personally Unidentified Study Data for Research" form
- have verifiable records of bonding with non-sealant/non-fluoride releasing bonding agents/cements (*e.g.*, Transbond Plus Self-Etching Primer and Transbond Light Cure Adhesive (3M Unitek, Monrovia, CA))

**The original age limit for study participants was 13 years. However, due to large numbers of 11-and 12-year old patients being treated at the UCSF Orthodontics Clinic, the age limit was lowered to 11 years and a modification submitted to the IRB. This modification was approved.*

Exclusion Criteria

Individuals meeting the following criteria were excluded from participation:

- milk protein allergy

- untreated cavitated lesions
- underlying systemic disease(s) which could alter enamel composition or formation
- medical history significant for conditions affecting oral health or oral flora (*e.g.*, diabetes, HIV, heart conditions that require antibiotic prophylaxis, etc.)
- taking medications that cause dry mouth (extreme high caries risk)
- had any illness/condition that the investigators felt would affect the study outcome
- pregnant or lactating
- extensive restorations on the buccal surfaces of the anterior teeth, including numerous composite fillings, veneers or crowns
- intrinsic or extremely heavy extrinsic staining, including fluorosis
- any signs of morphologic/anatomical/developmental anomalies in the teeth
- previous history of in-office bleaching treatment
- in-office fluoride treatment in the preceding three months
- use of any MI product, prescription-strength fluoride products, or chlorhexidine in the preceding three months
- unwilling to stop the use of any other oral hygiene products aside from those prescribed/suggested
- unwilling to inform the study investigators about prospective visits with other dental professionals and disallowing discussion of treatment with those providers
- unwilling to refrain from any additional professional tooth cleanings or fluoride applications during the study duration.
- unavailable for recall visits

Screening

Subject enrollment required study investigator SB, a postdoctoral orthodontic resident, to screen charts of patients being treated in the UCSF Orthodontics Clinic for candidates that might fulfill the inclusion criteria. SB kept track of the schedule of orthodontic appointments of possible subjects. On the days of their orthodontic appointments, patients were approached by BR, a dental assistant and senior research associate (SRA), and informed about the study. Patients interested in participating were then screened by PR, the Principle Investigator of the study, for qualification. If patients fulfilled all criteria and agreed to participate in the study, the SRA obtained their assent and the consent of their parent/guardian (for subjects under 18). The UCSF Caries Risk Assessment Questionnaire was administered and a CariScreen ATP test (Oral Bio Tech, Albany, Oregon) performed.[35]

Approximately 110 orthodontic patients were screened for participation in this study. Chart review and screening continued until the required number of study subjects were enrolled. Visits to the orthodontic clinic were monitored for the enrolled subjects to ensure appropriate follow-up by the SRA regarding compliance with study protocols. When possible, the SRA coordinated with orthodontic resident providers to allow evaluation of study subjects before, during or after orthodontic adjustment appointments. When this was not possible, the SRA scheduled study patients on separate dates for evaluation.

Sample size calculation

To calculate the necessary number of study subjects, we looked at laboratory studies with CPP-ACP gums or lozenges, clinical studies testing the regression of WSLs after orthodontic treatment, and studies monitoring changes in WSLs during active orthodontic treatment. There is a wide range of reported efficacies regarding the use of CPP-ACP products in comparison to typical controls. There has been a superiority of up to 78% WSL remineralization in CPP-ACP

application over sugar-free lozenges reported in laboratory and *in situ* studies. In clinical studies, a success rate of up to 49% was reported. An average increase in remineralization/demineralization protection of 36.3% (+/-7.7%) across 6 different clinical studies can be estimated. Most of these studies had a short observation time and reported changes after only 3 or 6 months.

In this study, we aimed to examine at the effect of CPP-ACP treatment over a 12 month period. We applied MI Varnish every 3 months to the experimental group subjects. This resulted in a greater positive effect for the treatment group, overcoming possible compliance issues with daily MI Paste Plus use, and possibly resulting in advanced remineralization of existing lesions and demineralization protection against new lesions.

Studies state that patients undergoing orthodontic treatment with full fixed appliances develop, on average, 8-9 new WSLs. By conservative calculation and without taking into account the additional MI Varnish applications, we assumed that controls would develop 8 new WSLs (SD=8) and the treatment group would develop 35% fewer lesions (5.2 ± 5.2). The effect size was calculated as ρ =0.42. The sample size analysis was established by G*Power software, version 3.1.3 (Heinrich-Heine-University, Düsseldorf, Germany). Based on a 1:1 ratio between the groups, a sample size of 20 participants per group (40 total) would give more than 80% power to detect significant differences with a 0.42 effect size and $p<0.05$ significance level. A similar effect size of ρ=0.41 can be calculated taking a 6 month study into account, which reported WSLs for CPP-ACP versus control. Other studies allow calculating a much higher effect size than we are conservatively estimating here including a study utilizing fluorescence imaging under similar conditions (effect size of ρ=0.55). Final calculations were performed by JK, a statistician from the UCSF Clinical and Translational Science Institute.

Experimental Design

Randomization

Subjects received a random assignment to the experimental or control group. A randomization list was created by a random number generator (QuickCalcs Online Random Numbers, GraphPad Software, Inc., San Diego, CA). The randomization list was kept locked and group assignments were kept in separate, closed envelopes. Only after a subject had been enrolled would the next-in-line group assignment be revealed.

Blinding

The study investigators, SB and PR, were blinded to subjects' group assignments. The SRA was not blinded to subjects' group assignments and explained the assignment to each subject. The SRA dispensed all study products and gave subjects oral hygiene instructions and homecare instructions according to group assignment. Subjects were instructed not to discuss the treatment they were receiving with SB or PR.

Materials

MI Varnish contains CPP-ACP with 5% sodium fluoride, a concentration similar to that found in prescription fluoride dental varnishes. MI Paste Plus contains CPP-ACP with 900ppm fluoride (0.2%), a concentration similar to over-the-counter Crest 1,100 ppm fluoride toothpaste. Over-the-counter fluoride mouth-rinses contain 0.05% sodium fluoride (ACT Anticavity, Chattem Inc, Chattanooga, TN).

Treatment groups

The experimental group received in-office MI Varnish application at baseline and every 3 months (month 3, 6, and 9, for a total of 4 applications). They were instructed to brush their teeth

with Crest 1,100ppm fluoride toothpaste twice a day and apply MI Paste Plus to both dental arches after brushing with a foam application tray every evening for 3-5 minutes.

The standard-of-care control group was instructed to brush their teeth with Crest 1,100ppm fluoride toothpaste twice a day and recommended to use over-the-counter fluoride mouth rinse after brushing every evening.

Testing methods

The following tools were used: a) clinical visual evaluation of the WSLs using the Enamel Decalcification Index (EDI), the International Caries Detection and Assessment System (ICDAS) criteria for smooth surfaces caries, and the Nyvad criteria for caries lesion activity; b) digital photography using lip retractors and a Canon EOS 10D digital camera with a Canon 100mm macro lens and macro ring flash; and c) light fluorescence imaging with digital capture of fluorescence pictures using Quantitative Light-Induced Fluorescence (QLF) and the SOPROlife system. Clinical scores for EDI and ICDAS were recorded manually in case report forms for each patient at each time point. Patient identifiers were limited to subject initials for patient confidentiality.

Subjects' anterior teeth were digitally photographed by quadrant before and after cleaning using lip retractors. QLF and SOPROlife images were taken of the upper and lower anterior teeth (first bicuspid to first bicuspid) by PR and digitally captured. The study investigators SB and PR evaluated the facial surfaces of subjects' upper and lower anterior teeth for WSLs using the EDI. The investigators wore loupes (2x magnification) and used a loupe light (Ultra Light Optics, Costa Mesa, CA). Pressurized air was applied to each tooth for 5 seconds for better visualization. A wet towel was used against the facial surfaces of teeth before evaluation in order to avoid desiccation. SB and PR discussed their findings and decided on an "agreed-on

score" for each surface of each tooth. PR evaluated each tooth surface for severity of WSLs using the ICDAS criteria for smooth surface caries.

The total study duration for each subject was 12 months. Two milliliters (ml) of stimulated saliva was collected at the very start of each study visit. Subjects were asked to abstain from brushing their teeth upon arrival to their appointments so that plaque levels and salivary fluoride levels could be accurately measured. Orthodontic wires were then removed or arranged for removal by SB. All subjects received professional dental cleanings (ultrasonic scaling followed by plaque removal with a polishing brush without pumice) before evaluation.

At the end of evaluation, orthodontic wires were subsequently replaced or arranged for replacement by SB. The SRA applied or arranged for the application of MI Varnish to the facial surfaces of experimental group subjects' anterior teeth, dispensed additional study products, and reviewed oral hygiene instructions/home-care instructions according to group assignment. Subjects in the experimental group were asked to refrain from taking in any foods or liquids for one hour after varnish application. Compensation was dispensed at the end of each study visit.

Testing times

The protocol outlined above was performed four times for each study subject: baseline, 3 months, 6 months and 12 months. Each visit took approximately 60 minutes. The procedure at 9 months was slightly different and took only 10 minutes. Instead of a professional cleaning, a supervised brushing was performed by the SRA to ensure adequate plaque removal. No evaluation of WSLs was performed. The SRA applied or arranged for the application of MI Varnish to the facial surfaces of experimental group subjects' anterior teeth, dispensed additional study products, and reviewed oral hygiene instructions/home-care instructions according to group assignment. Compensation was dispensed at the end of the visit.

EDI

The EDI (Figure 1) evaluates the location and extent of white spots lesions.[36] The facial surface of each tooth is divided into 4 zones (mesial, gingival, distal, and occlusal). Zones are created with virtual diagonals connecting corners of the orthodontic bracket to the sides of each tooth. Scores are assigned as followed: 0 if there is no decalcification, 1 if there is decalcification present but it covers <50% of the surface, 2 if the decalcification covers >50% of the surface, and 3 if the decalcification covers 100% of the surface or presents with cavitation. A sum is calculated by adding the scores given to the individual zones per tooth. Sums can range from 0 to 12. Surfaces completely covered by gingiva and/or bonding material are scored as "n/a" (not available).

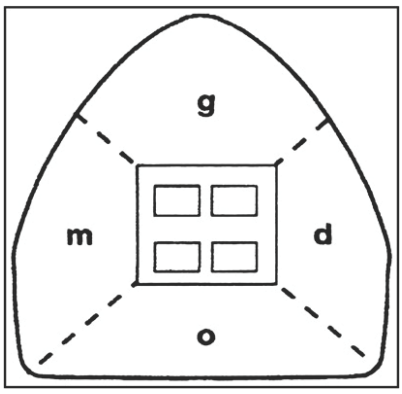


Figure 1: EDI. Division of each facial tooth surface into 4 zones around the orthodontic bracket (m=mesial, g=gingival, d=distal, o=occlusal).

ICDAS

The ICDAS provides a standardized method of lesion detection and assessment for proper caries diagnosis.[37] The ICDAS assigns severity scores to lesions based on visualization of enamel changes on plaque-free teeth when wet and air-dried.[38] An ordered score from 0 to 6 is applied to determine the level of caries: 0 signifies sound tooth surface, 1 represents the first visual change in enamel (visible only after air drying), 2 signifies distinct visual change in enamel when wet, 3 represents localized enamel breakdown due to caries without visible dentin, 4 indicates a shadowing of the underlying dentin with or without enamel breakdown, 5

represents a distinct cavity with visible dentin, and 6 signifies an extensive cavity within visible dentin. Each tooth surface (mesial, gingival, distal, and occlusal) receives an ICDAS score following the EDI scoring. Only the highest surface score per tooth is used for analysis.

Judgment of caries activity by the Nyvad criteria

Pre-cavitated caries/WSLs are judged as active or inactive according to their appearance and surface texture.[39] Lesions with opaque appearance, rough surface texture, and retention of microbial plaque even after careful removal with the side of a periodontal probe are active. White spots with a shiny appearance, smooth surface texture and no retention of microbial plaque are inactive. Patients had to have a minimum of two active lesions on the upper and/or lower anterior teeth at the start of the study to qualify for participation. White spots were recorded as active if any portion of the lesion showed active demineralization. Lesion activity was monitored at each time point. If white spots previously noted were present but inactive, a note was made regarding their change in activity (*i.e.*, the surface still appeared white but instead of chalky and sticky, it was smooth and hard).

Light Fluorescence

QLF (Inspektor Pro, Amsterdam, Netherlands) has been proven to be an effective method for detecting smooth surface demineralization (Figure 2).[40] When compared to conventional visual examination or other caries detection instruments, QLF can detect twice as many pre-cavitated demineralized enamel areas.[41] The ability to detect changes in demineralization allows the use of QLF to determine the impact of preventive measures quantitatively.[41]

QLF emits a violet-blue light (λ = 380nm on average with a range of 290-450nm). A subsurface enamel lesion occupied by water and air scatters the light as it enters the tooth or as the fluorescence is emitted, resulting in a loss of its natural fluorescence. Consequently, the

demineralized area appears dark opaque while sound tooth structure appears bright green. The QLF method can readily detect lesions to a depth of approximately 500µm on smooth and occlusal enamel surfaces according to several *in vitro*, *in situ*, and *in vivo* studies.[42-51] Fluorescent images are captured using the QLF intraoral camera hand-piece and analyzed using the White Spot Analysis wizard (Q-ray, Inspektor Research Systems BV, Amsterdam, Netherlands) (Figure 3).

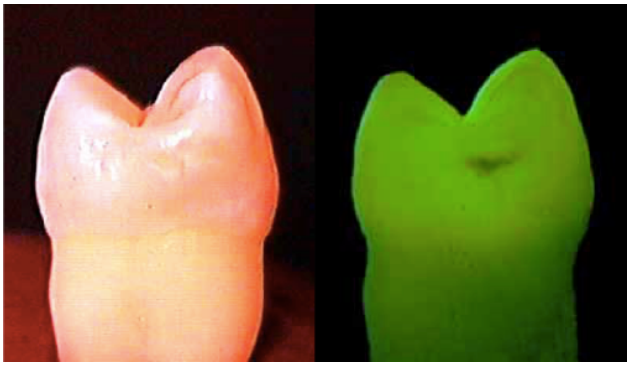


Figure 2: QLF. Decalcification on the proximal surface of a bicuspid is difficult to detect under regular light conditions (right), but clearly visible using QLF.

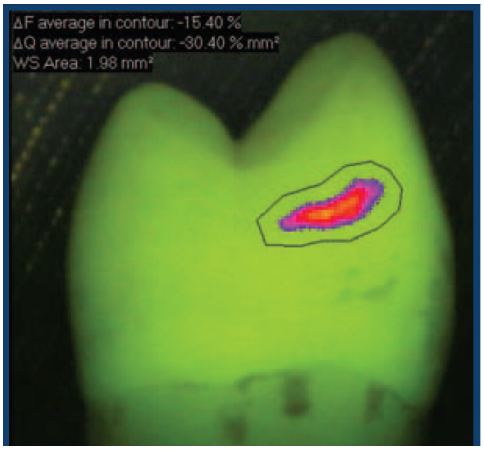


Figure 3: QLF White Spot Wizard. This analysis allows users to select areas of demineralization and compare them to sound enamel. It generates average changes in demineralization (ΔF), average changes in the volume of demineralization (ΔQ), and white spot area (WS) values for the overall tooth. The areas of most mineral change are represented with yellow; areas of moderate change are represented with red and purple, and areas of minimal mineral change are represented with blue.

The SOPROlife system (SOPRO Acteon Imaging, La Ciotat, France) combines the advantages of a high magnification oral camera (high specificity) and a laser fluorescence device (high reproducibility and discrimination).[52, 53] The "daylight mode" of the SOPROlife unit has four white light-emitting diodes (LEDs) to illuminate the tooth, while the "fluorescence

mode" has four blue LEDs that emit light at a wavelength of 450nm. The intense blue light shines through the enamel and induces a green fluorescence from the dentin core. When caries is present in occlusal pits and fissures, the light initiates a red fluorescence from the porphyrins of the oral bacteria at the caries site.[54] However, this system has not yet been tested for detecting smooth surfaces caries. Our preliminary trials suggest that in the fluorescence mode, WSLs appear as opaque white and can be scored similar to the EDI system. Digital images are captured with the SOPRO imaging software.

Additional measurements

The Turesky modification of the Quigley and Hein plaque index was utilized to assess plaque levels of the upper and lower anterior teeth at baseline, 3, 6, and 12 months before subjects' teeth were cleaned.[55] Teeth are scored as follows: 0 indicates no plaque, 1 indicates separate flecks of plaque at the cervical margin of the tooth, 2 indicates a thin continuous band of plaque (up to one mm) at the cervical margin of the tooth, 3 indicates a band of plaque wider than one mm but covering less than one third of the crown of the tooth, 4 indicates plaque covering at least one-third but less than two-thirds of the crown of the tooth, and 5 indicates plaque covering two-thirds or more of the crown of the tooth (Figure 4).

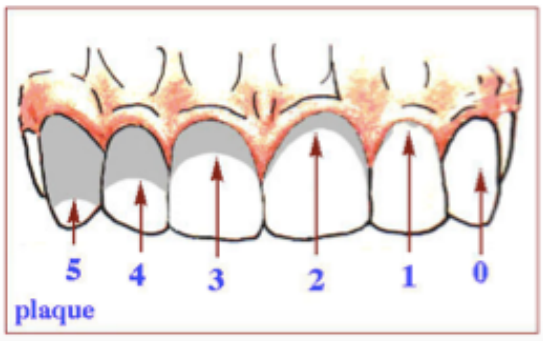


Figure 4: Turesky modification of the Quigley and Hein plaque index. Teeth are scored according to the extent of plaque covering the tooth surface starting from the gingival margin and moving down to the incisal edge.

The 2ml of stimulated saliva collected at the beginning of each study visit (baseline, 3, 6, 9, and 12 months) was used to monitor salivary fluoride levels in study subjects. Saliva samples

were coded with participant ID numbers only to ensure blinding of the laboratory investigators to group assignments. Salivary samples were analyzed by diffusion to detect concentrations ≥0.005ppm of fluoride.[56]

Compliance

To ensure compliance, the SRA called study subjects bi-weekly to remind them to use their assigned products. Subjects in the experimental group were asked to maintain a diary of their product use. They were given calendar forms and asked to fill in the time of brushing and MI Paste Plus application each day. Subject and supervisor (parent/guardian for subjects under 18) signatures were required at the end of each month. Diaries were reviewed at each study visit by the SRA.

Reliability

Intra-rater reliability of EDI scores assigned by SB and PR was assessed by replicating measurements for 5 consenting patients 1-2 weeks after their baseline, 3, 6, or 12 month visits. Subjects' orthodontic wires were removed and subjects' teeth were professionally cleaned again. SB and PR reevaluated the facial surfaces of subjects' upper and lower anterior teeth for WSLs using the EDI, discussing their findings and agreeing on one score for each surface. Measurements were compared to those made at the preceding visit and a Kappa/weighted Kappa score was generated. Orthodontic wires were subsequently replaced.

Data Analysis

The primary outcome measure was changes in the number of EDI/ICDAS 0s, 1s, 2s, and 3s scored at baseline versus at 3, 6, and 12 months between the experimental and control groups. Average EDI sums and average highest ICDAS scores were calculated and examined across time points between groups. Plaque levels and salivary fluoride levels were also evaluated between

groups across time points. QLF data was intended for evaluation of demineralization for existing or new WSLs over the course of the study between the treatment groups. SOPROlife images were intended for independent scoring of WSL location and extent at each time point.

The analysis described above required data to be collected and evaluated by the Spring 2015 term for presentation in this thesis. However, subject recruitment was not completed until August 2014; as each study subject had to be followed for one year, data from the final 12-month time point would not be available until the end of the Summer 2015 term. For this reason, the results reported below will deviate slightly from the original goals of the project described above. As all baseline, 3-month and 6-month data was collected by Spring 2015, and as the study investigators were required to remain blinded for the collection of data at the final 12-month time point, the thesis committee agreed that the analysis presented here would exclude 9- and 12-month data and be limited to an evaluation of changes in the entire subject pool as a collective, irrespective of their assignment to the experimental or control groups. A second manuscript will be presented at the completion of all data collection and subsequent unblinding of the study investigators for comparison of changes between the experimental and control groups.

The study investigators recognize the necessity of a random effects model to estimate between-subject standard deviations at each time point and a mixed effects model to estimate within-subject variability. For the preliminary analysis presented here of data up to the third time point (6 months), averages were obtained on the entire group of study subjects collectively at each time point, and weighted variances of those values used for evaluation with a Student's t-test. This presents a simplified analysis of the data; a more robust evaluation will be conducted for the second manuscript described above.

RESULTS

Subject enrollment took place from October 2013 to August 2014, for a total recruitment period of 10 months. Forty patients were recruited to participate in the study: 17 females and 23 males. The average age at the start of the study was 16y0m +/- 3y10m. The average number of days between time points 1 and 2 (baseline to 3 months) was 94.8 +/- 10 days and between time points 2 and 3 (3 months to 6 months) was 91.5 +/- 14 days. The average CariScreen value for patients at the start of the study was 8600 +/- 2409. (Appendix, Tables 1&2)

Attrition

The attrition rate was 10%: four subjects were lost. Two were female and two were male. Two were from the experimental group and two were from the control group. Subject 13 and 92 withdrew from the study after having completed only baseline measurements. Subject 13 was withdrawn from the study because she required removal of orthodontic appliances and discontinuation of orthodontic treatment to accommodate magnetic resonance imaging and medical attention for an underlying medical condition. Subject 92 withdrew from the study due to concerns about time requirements to participate in the study. These subjects' baseline measurements have been excluded from the data analysis.

The two other subjects lost from the study completed up to either a 3-month evaluation (subject 4) or a 6-month evaluation (subject 56). Subject 4 withdrew from the study because of admitted non-compliance with study product use and an expressed desire to stop participation in the study. This subject's measurements have been excluded from the data analysis. Subject 56 withdrew from the study at around 9 months because of concerns of possible adverse reactions to the milk protein in the experimental group products. No signs of allergic reaction were observed

clinically. Subject 56's clinical measurements have been included in the data analysis because of admitted compliance with daily product use up to the 6-month evaluation.

Exclusions

The decision was made by the study investigators to exclude tooth surfaces that were unavailable for evaluation at any time point from the data analysis. If a tooth surface (usually the gingival surface) was covered with gingival tissue that could not be pushed away for visual evaluation, it was scored in the EDI and ICDAS indices an "n/a." Some of these surfaces may have been scored at earlier time points (before gingival inflammation) or at later time points (if gingival inflammation improved). In order to prevent inflation of the data at time points when these surfaces were available, the decision was made to exclude all surfaces missing a score at baseline, 3 months or 6 months from the final data analysis.

Of the 40 study subjects enrolled, four presented with missing canine or anterior teeth. Subject 81 was diagnosed with a severely impacted upper right canine that required extraction. Subject 92 was congenitally missing an upper left lateral incisor. Subject 43 and subject 78 both had one lower incisor extracted for orthodontic treatment (the lower left lateral incisor and the lower right central incisor, respectively). These teeth are thus missing from the data analysis.

In cases of first premolar extractions, second premolars were substituted without any distinction. In one case (subject 65), the study investigators deemed the lower second premolars to be positioned too far distally for WSLs on these teeth to present an esthetic concern. Measurements for this subject were conducted from upper right first premolar to left first premolar and lower right canine to left canine only.

In three cases, subjects were enrolled despite not having full fixed appliances at the start of the study. In subject 21, there was originally no space in the maxillary arch for the upper right

and left canines, which had erupted ectopically. These teeth were not bracketed until space was made available, at which point they were incorporated into the appliance. This occurred after the baseline visit. In subject 117, segmental retraction was being performed in the lower arch: lower canines and premolars were bracketed but lower incisors were not incorporated into the appliance until after the baseline measurements were made. Finally, in subject 78, the lower right and left first premolars did not received brackets until after the baseline measurement. These patients were recruited because they fulfilled all other inclusion criteria and the subjects' families strongly desired for them to participate in the study. We excluded these unbracketed teeth from the analysis.

Reliability

Intra-rater reliability measurements were completed on 4 study participants at the time of the writing of the thesis. Kappa scores from these measurements are presented in Appendix, Table 3. These Kappa values consider only exact matches between observers. The weighted Kappa values included assume categories are ordered and account for how far apart the two raters are using linear weights. Combining these four sets of values, we obtain a Kappa score of 0.781, with a standard error of 0.035, 95% confidence interval (CI) of [0.711, 0.850] and a weighted Kappa of 0.806. The strength of this agreement is assessed to be "very good." One additional subject will be assessed to confirm reliability of measurements for the writing of the second manuscript.

Accuracy

To verify the accuracy of all measurements transferred from the case report forms to digital spreadsheets for analysis, a pre-doctoral dental student (KL) was recruited and trained to

review all records and compare digital recordings to paper charts for any inconsistencies. Inconsistent entries were reevaluated and corrected by SB.

Compliance

Compliance with the product diaries varied, with some patients in the experimental group providing credible proof of use of products, while others provided less credible evidence. All subjects in the experimental group received additional product supply at each time point. The only patient who admitted to not using the product was subject 4, who withdrew from the study after 3 months for this reason.

For subjects in the experimental group undergoing treatment with fixed inter-arch appliances such as Class II correcting springs, insertion of foam trays with the MI Paste Plus became impossible. Patients were encouraged to continue use of the MI Paste Plus by spreading the material on their teeth using their fingers and refraining from spitting or rinsing for 3-5 minutes.

Fluorosis

The first 15 subjects recruited to the study were evaluated for baseline measurements from October 2013 to December 2013. These subjects helped develop the learning curve for EDI scoring. These subjects also helped the investigators realize the extensive prevalence of mild fluorosis in the local population. The presence of fluorosis was one of the exclusion criteria for this study; however, while moderate to severe fluorosis was easy to identify during screening, mild cases were identified only after the professional tooth cleaning and air-drying for close examination. Many of the surfaces that were initially judged to be completely covered with decalcification (EDI score of 3 per surface) were discovered to be fluorosis instead on reexamination (Figure 5).

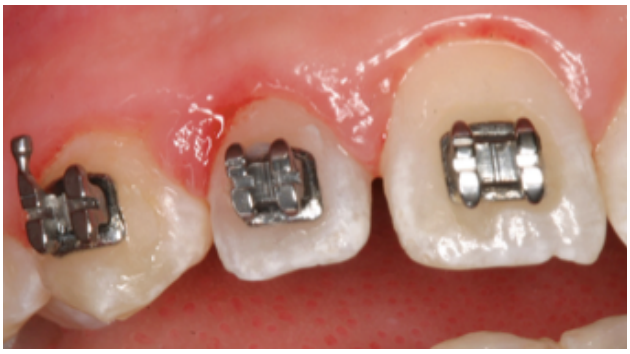


Figure 5: Fluorosis. The presence of fluorosis was previously mistaken for complete demineralization of the mesial, distal, and occlusal surfaces.

The water supply in San Francisco is supplemented with fluoride to the optimal level set by the California Department of Public Health and recommended by the US Center for Disease Control and Prevention (0.7ppm or 0.7mg/l). While demographic data regarding where participants were born and/or raised was not obtained in this study, should subjects have had formula mixed with water containing fluoride at the optimal level in their infancy, they would have an increased chance of developing dental signs of mild fluorosis.[57] The study investigators gradually learned to distinguish the appearance of fluorosis from decalcification, as both appear chalky white. Criteria used to differentiate the two included location (proximity to the gingiva was more likely to be demineralization, whereas fluorosis occurs more commonly at the occlusal edges), and symmetry (horizontal white lines running along enamel perikymata on the tooth surface were likely fluorosis; contralateral teeth with identically appearing white surfaces most likely had fluorosis). However, the study investigators learned to identify demineralization where white lines extended from the mesial and distal of the bracket slots along the path of the orthodontic wires.

The process of fine-tuning WSL versus fluorosis identification took the study investigators approximately 4 months. By the 6-month evaluation of these early subjects, the investigators had become consistent with their scoring. At the completion of the collection of all

6-month data, the study investigators returned to the baseline and 3-month measurements of the first 15 subjects to reevaluate and rescore the EDI and ICDAS for all tooth surfaces. They compared digital photographs (primarily) and fluorescence images (secondarily) from all time points to ensure consistency of measurements. Gingival surface measurements were found to be the most consistent, as fluorosis rarely occurs in this area of the tooth. The mesial and distal surface recordings were far more inconsistent and required close evaluation and discernment. Occlusal surfaces were least likely to present with demineralization and were most often rescored as fluorosis only.

EDI/ICDAS

Figures 6 and 7 represent average numbers of 0s, 1s, 2s, and 3s recorded for all tooth surfaces in all subjects using the EDI and ICDAS indices, respectively. There were no statistically significant changes found across time with either index (Appendix, Tables 4 & 5). For the EDI, 0s were the most commonly assigned score, followed by 1s, then 3s, then 2s. For the ICDAS, tooth surfaces were scored as 0 most frequently, followed by a score of 1, then 2, and very rarely 3.

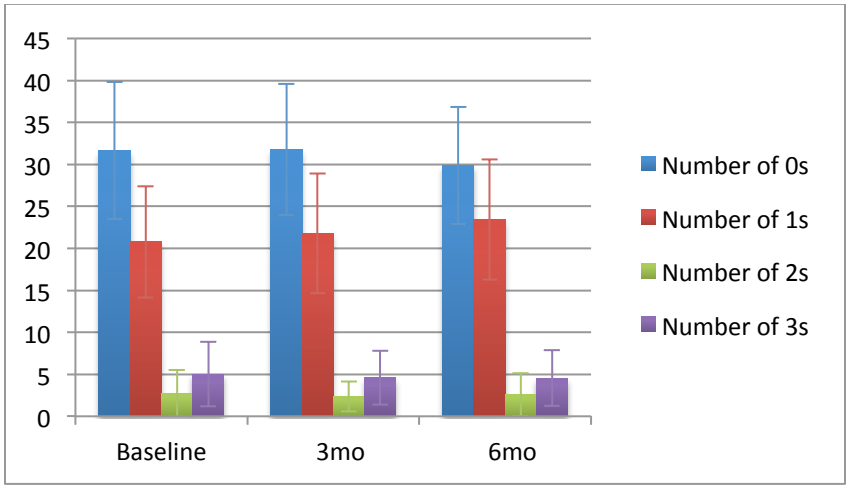


Figure 6: Average EDI score distribution

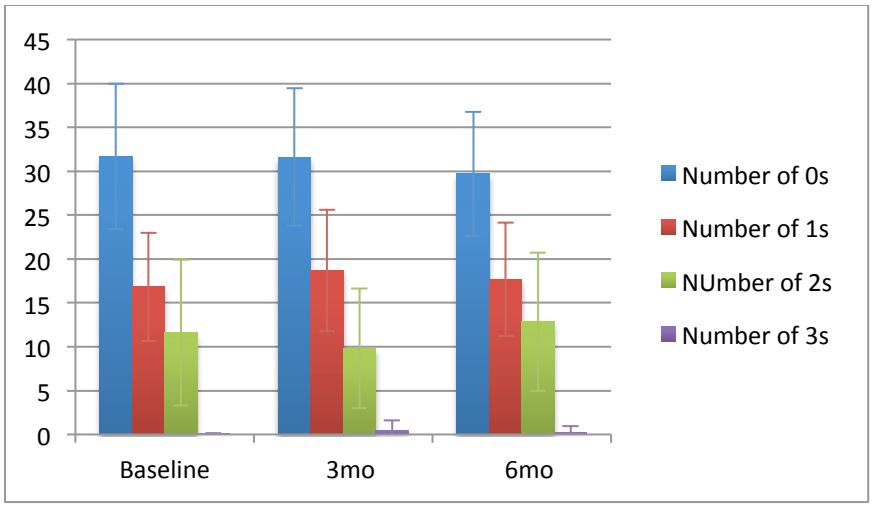
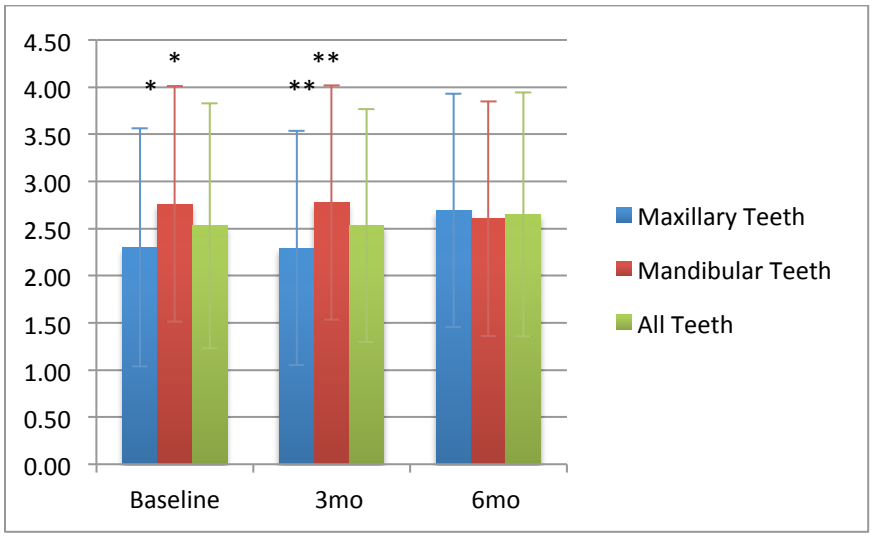


Figure 7: Average ICDAS score distribution

Figure 8 shows the average sum of EDI scores of individual teeth across maxillary, mandibular, and all teeth. There were no statistically significant changes in EDI sums over time; however, there was a statistically significant difference in maxillary and mandibular EDI sums at baseline (2.23 +/- 0.58 versus 2.79 +/- 0.72, respectively, n=37, $p<0.005$) and at 3 months (2.29 +/- 0.59 versus 2.79 +/- 0.71, respectively, n=37, $p<0.05$). There was no difference at 6 months (Appendix, Table 6).



*p<0.005, **p<0.05

Figure 8: Average EDI sum

Figure 9 shows the average highest ICDAS score per tooth for maxillary, mandibular and all teeth. There was no statistically significant change in the highest ICDAS score recorded in subjects' teeth over time, nor was there a significant difference between maxillary and mandibular teeth at any time point (Appendix, Table 7).

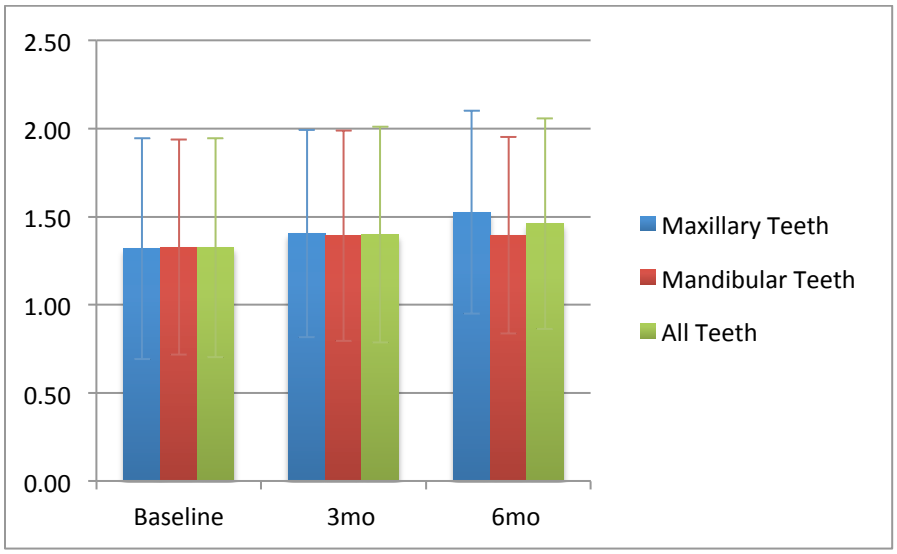


Figure 9: Average highest ICDAS score

In order to examine the effect of surface location on demineralization, Figures 10 and 11 show average EDI and ICDAS scores according to tooth surface across time. Gingival surfaces show higher EDI and ICDAS scores. Mesial and distal surfaces show EDI and ICDAS scores similar to one another, but less than gingival surfaces. Occlusal surfaces often received EDI/ICDAS scores of 0 (Appendix, Tables 8 & 9).

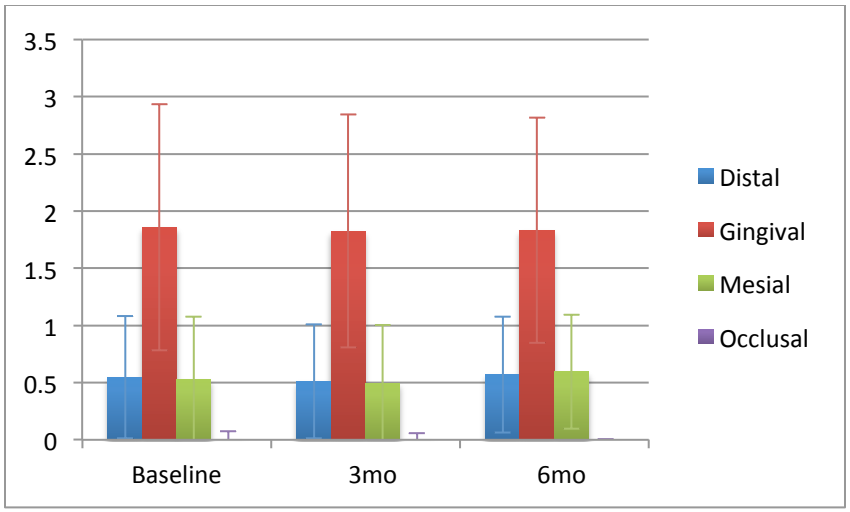


Figure 10: Average EDI score per tooth surface

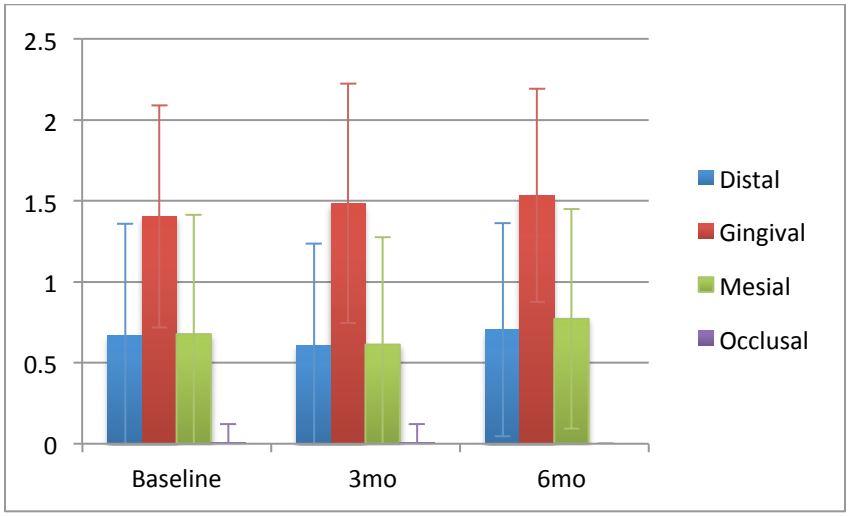


Figure 11: Average ICDAS score per tooth surface

Judgment of caries activity by the Nyvad criteria

In the first 6 months of the study, there were no lesions noted that changed from active to inactive.

Light Fluorescence Imaging

SOPROlife

It became clear when reviewing SOPROlife images of the same teeth over time that accurate assessment of demineralization with this technique was challenging. Angulation of

image exposure greatly affects the visualization of the tooth surface due to glare (Figure 12). Bracket interference and shadows were difficult to avoid and complete surface visualization was nearly impossible (Figure 13). In addition, enamel loss was challenging to differentiate from remineralization in this system. For example, in Figure 14, the subject was known to have experienced significant enamel loss on the gingival surface of various incisors. However, on the SOPROlife images, this appears as remineralization of enamel. The SOPROlife system was also inadequate in distinguishing demineralization from fluorosis, as WSLs and fluorosis appeared identical (Figure 15). And if gingival margins were not completely free of plaque or bonding material, these appeared white like WSLs. Finally, reduced dentin thickness in teeth like the lower incisors and upper laterals gave the false impression of complete demineralization, as entire facial surfaces appeared completely white (Figure 16). For all these reasons, the use of SOPROlife images was limited to secondary referencing (after digital photographs) where inconsistencies in measurements were found.

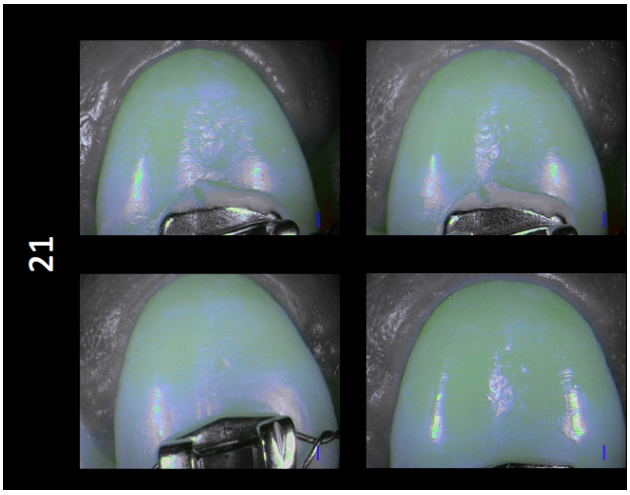


Figure 12: SOPROlife, glare. Upper left central incisor at baseline (top right), 3 months (top left), 6 months (bottom right), and 12 months (bottom left). Note difficulty in accurately assessing changes in white spots due to camera glare and angulation.

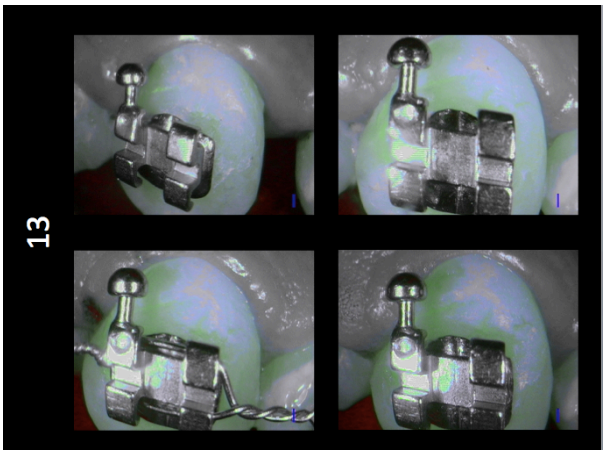


Figure 13: SOPROlife, shadow. Upper right canine at baseline (top right), 3 months (top left), 6 months (bottom right), and 12 months (bottom left). Note bracket hook shadow on the gingival surface, making accurate assessment of demineralization extent impossible.

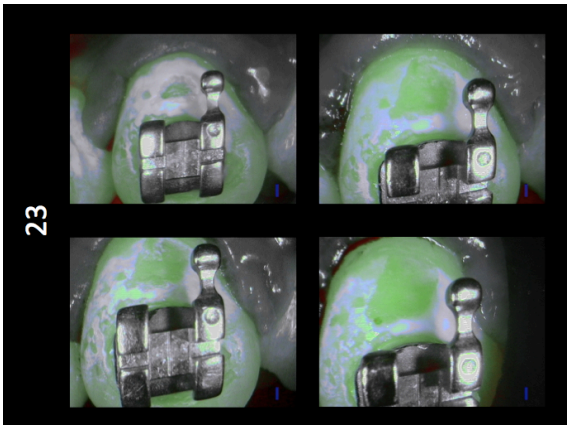


Figure 14: SOPROlife, enamel loss. Upper left canine at baseline (top right), 3 months (top left), 6 months (bottom right), and 12 months (bottom left). Note enamel loss at the central portion of the gingival surface appearing as remineralization.

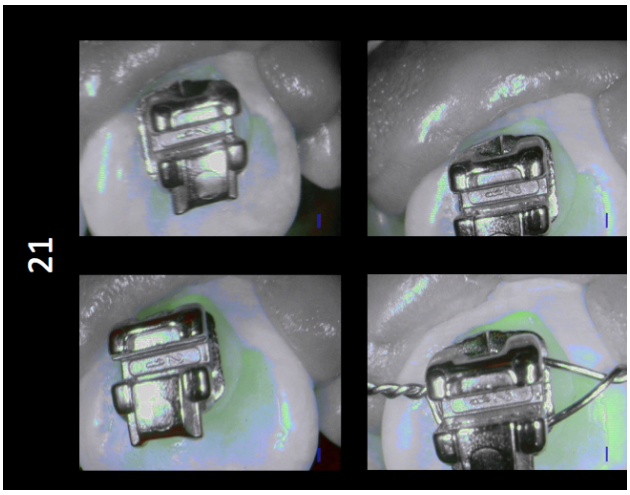


Figure 15: SOPROlife, fluorosis. Upper left central incisor at baseline (top right), 3 months (top left), 6 months (bottom right), and 12 months (bottom left). Note extensive fluorosis on the mesial, distal and occlusal surfaces, which are impossible to differentiate from demineralization.

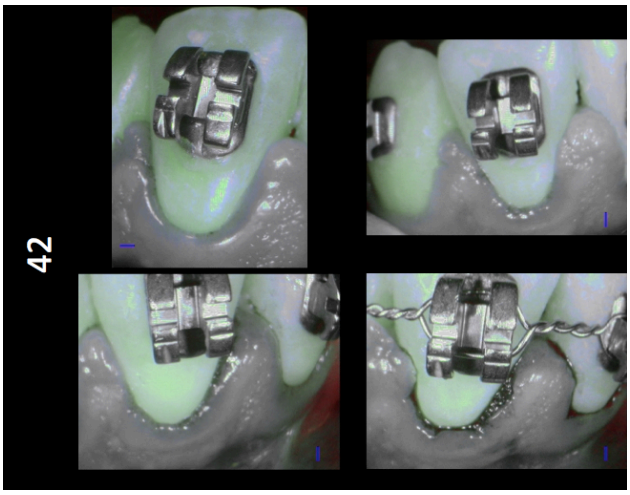


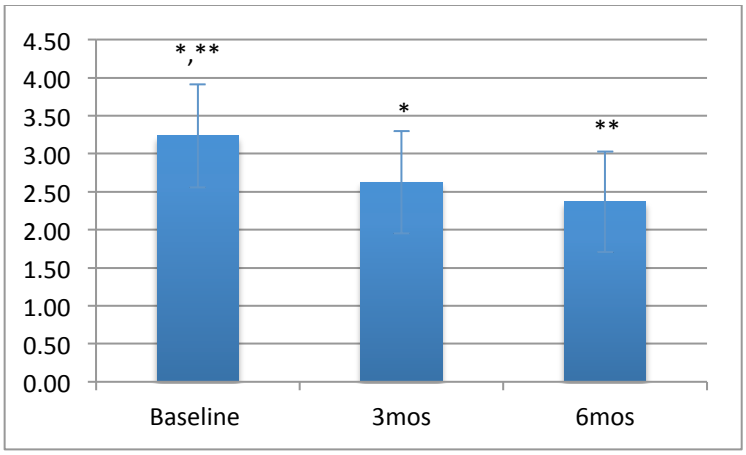
Figure 16: SOPROlife, reduced dentin thickness. Lower right lateral incisor at baseline (top right), 3 months (top left), 6 months (bottom right), and 12 months (bottom left). Note completely white appearance of most surfaces due to reduced dentin thickness.

QLF

Like the SOPROlife system, we were unsuccessful in interpreting QLF images obtained for study teeth at various time points. Although we routinely removed wires to better visualize tooth surfaces, the presence of brackets made it extremely difficult to maneuver the camera head close enough to the tooth surface to obtain accurate images. There may also have been absorption or refection of light by brackets. Most importantly, it was nearly impossible to compare tooth images across time points for the same patient with a nearly identical angulation of the camera head for each tooth,. For these reasons, QLF images were not assessed in the analysis presented here.

Plaque levels

Figure 17 shows average plaque scores over time. There was a statistically significant decrease in plaque scores from baseline to 3 months (3.3 +/- 0.66 to 2.7 +/- 0.67, n=37, $p<0.001$) and from baseline to 6 months (3.3 +/- 0.66 to 2.4 +/- 0.66, n=37, $p<0.001$) (Appendix, Table 10).

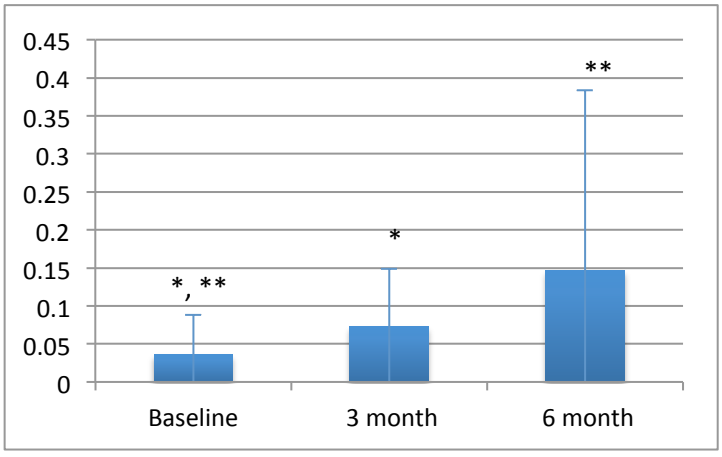


*,** p<0.001

Figure 17: Average plaque score

Salivary fluoride levels

Fluoride values greater than 1.00 were removed from the analysis. Large numbers do not represent errors in laboratory processing (as this technique is very accurate), but rather suggest the presence of foci of concentrated fluoride in the saliva sample, which must be excluded in order to avoid skewing data. Evaluating average salivary fluoride levels over time shows statistically significant increases from baseline to 3 months (0.036 +/- 0.052 to 0.073 +/- 0.076, n=37, p<0.05) and from baseline to 6 months (0.036 +/- 0.052 to 0.15 +/- 0.24, n=37, p<0.01) (Figure 18; Appendix, Table 11).



* p<0.05, ** p<0.01

Figure 18: Average salivary fluoride level

DISCUSSION

EDI/ICDAS

Combining data for all subjects, we expected the analysis presented here to show either improvement (subjects in the experimental group having received the added benefit of supplementary fluoride, calcium and phosphate in bioavailable forms on a daily basis and high doses of fluoride every three months, and subjects in the control group experiencing less WSLs because of improved oral hygiene habits from study participation) or no change at all (negative changes in the control group cancelling out positive changes in the experimental group). Despite the improvement noted in all subject plaque levels over time, and despite increases in all salivary fluoride levels over time, there were no improvements noted in WSL extent or severity.

Our study subjects displayed no or mild signs of demineralization (ICDAS scores of 0 and 1), and most surfaces were less than 50% covered (EDI scores of 0 and 1) in WSLs at all three time points examined. Even where entire surfaces were covered with decalcification (EDI of 3), localized enamel breakdown (ICDAS of 3) was quite rare. Although our study subjects were considered at risk for caries, there was a protective mechanism at play for both groups, preventing progression to loss of enamel. This can most readily be explained by the protective effect of topical fluoride (toothpaste, drinking water, etc.) available to all study subjects.

As the majority of surfaces received scores of 0 and 1 for the EDI, the average EDI sum per tooth was between 2 and 3. Similarly, as the majority of ICDAS surfaces were scored as 0 or 1, the average highest score per tooth was between 1 and 2. While there was no statistically significant change over time in EDI sums, the difference in sums between maxillary and mandibular teeth got smaller over time. That is, the significant difference that existed between average maxillary and mandibular EDI sums at baseline and at 3 months diminished by 6

months, suggesting a slight worsening in extent of WSLs in maxillary teeth and a slight improvement in WSL extent in mandibular teeth. Mandibular teeth have the added benefit of proximity to salivary glands and saliva pooling, providing both greater salivary clearance of plaque and greater access to concentrated levels of calcium, phosphate and fluoride. Maxillary teeth experience less salivary clearance of plaque and less access to remineralizing elements. There was, however, no significant difference in WSL severity between maxillary and mandibular teeth. This might reflect improvement in one group and worsening in the other, or the protective capabilities of fluoride in both groups for all teeth in the oral cavity, as mentioned above.

We found that a larger portion of the gingival surfaces of study teeth was covered with WSLs than any other surface, and WSLs found on the gingival surface showed greater severity of demineralization than any other surface. Naturally, gingival surfaces are prone to poorer plaque clearance than incisal edges; in orthodontic patients, this effect is severely magnified, as brackets make proper hygiene at gingival surfaces extremely challenging. To a lesser extent, the mesial and distal surfaces were prone to WSLs due to difficulty cleaning under orthodontic wires. Orthodontic providers and their teams should focus their oral hygiene instruction efforts on educating patients about areas in the mouth where the caries process is most likely to take hold, progress, and cause esthetic concerns.

Plaque

The reduction in plaque levels from ~3 to ~2 means that subjects on average presented with a band of plaque wider than one mm (covering one third of the tooth surface) at the gingival margin at baseline, which improved on average to a thin band of plaque less than one mm at 6 months. The decrease in plaque levels signifies improvement in oral hygiene across all subjects

participating in the study. The oral hygiene instruction reinforced at each study visit, as well as the effect of study participation and frequent monitoring are likely explanations for this improvement.

The Turesky modification of the Quigley and Hein plaque index utilized in this study is not particularly specialized for orthodontic patients. We may have considered using the Bonded Bracket Plaque Index (after Kilicoglu et al.), as this index is geared towards describing plaque levels around orthodontic brackets.[58] A score of 0 is given when there is no microbial plaque on the bracket or tooth surface, 1 if microbial plaque presents only on the bracket, 2 if microbial plaque is found on the bracket and tooth surface, but does not spread towards the gingiva, 3 if microbial plaque spreads toward the papilla, 4 if microbial plaque covers part of the gingiva, and 5 if the gingiva is totally covered with plaque. Additionally, plaque levels can be evaluated by dividing the tooth surface according to bracket and wire position, as described by the Williams modification of the Silness and Loe index (Figure 19).[58] Ultimately, although our index was not perfectly suited for orthodontic patients, its focus on plaque assessment starting from the gingiva has great utility in the study of WSLs.

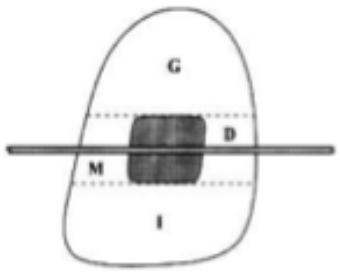


Figure 19: Modification of the Silness and Loe index (as described by Williams). The tooth is divided into mesial (M), distal (D), gingival (G), and incisal (I) regions around the orthodontic bracket and wire to assess plaque levels.

Salivary fluoride

Perhaps the most dramatic change presented here is the increase in salivary fluoride levels seen in all subjects over time. As only half of our study sample was receiving supplementary fluoride, calcium, and phosphate on a daily basis and high doses of fluoride

quarterly, the changes seen in the collective sample can be explained in two possible ways. The first is that the increase in salivary fluoride levels in the experimental group was so significant that it shifted over the entire sample mean. The second is that participation in the study helped patients in the control group improve their compliance with the use of fluoride toothpaste (which was dispensed to them) and over-the-counter fluoride mouth-rinse (which was recommended to them). The answer to this question will be available once group assignments are revealed and the data is re-analyzed by treatment group.

Activity

The lack of change recorded in WSL activity might suggest that 6 months was too short a time span of intervention for significant improvements to be observed. Studies evaluating WSLs *in vivo* during orthodontic treatment are rare. Most studies evaluate product efficacy after orthodontic treatment is complete and for a far shorter time period (*e.g.*, 8 weeks).[33] Because we evaluated subjects in fixed appliances, we are justified in our decision to follow patients for the span of a year, as even 6 months may be too short a time to determine if the products being studied can withstand the plaque burden caused by fixed orthodontic appliances. We will be better able to assess product effect on lesion activity with data available from the 12-month time point.

Digital imaging

Some of the most recent studies published evaluate WSLs using primarily digital photographs.[10, 33] Our experience using digital photography in the reevaluation of teeth for the first 15 subjects made clear how difficult and questionable it can be to compare *in vivo* images at different time points with any level of accuracy. Assuming camera settings are kept

identical, angulation, distance, glare, shadows, and saliva are just some of the factors necessary to duplicate for accurate comparison.

The use of light fluorescence imaging for WSL evaluation of bracketed teeth has not been reported in the literature, nor can this study at this point of presentation support its use for reasons outlined above. A reproducible technique for clinical evaluation should be the gold standard for investigating WSLs.

Limitations

We were not able to blind subjects to the study products with sham products for the control group. Applying fluoride varnish (without CPP-ACP) to control group subjects in the same frequency as MI Varnish application in the experimental group would also have been useful in understanding the effect of high doses of fluoride with and without calcium and phosphate supplementation on WSLs. Unfortunately, our industry sponsor did not support this addition to the study design.

There were short-comings in the EDI index for describing the extent of WSLs in a clinically relevant manner. Any sign of enamel changes on the facial surface of a tooth, as detected with loupes and a 5 second air-dry, necessitated a score of at least 1, even if WSLs were not grossly visible. It was very difficult for teeth to score 0 on the gingival, mesial, or distal surfaces because of the frequency of a thin line of demineralization present at the gingival collar of most teeth. Even if a tooth experienced a clinically significant decrease in WSL appearance, should any decalcification have remained, the EDI score would have remained a 1. Improvements were therefore difficult to represent numerically with this index. Similarly, ICDAS scoring followed EDI scoring (*i.e.*, an EDI surface score of 1 could not be scored lower than a 1 on the ICDAS criteria), suggesting that our study design made the improvement of

ICDAS scores difficult to ascertain as well. In addition, the division of facial tooth surfaces into quadrants around the orthodontic bracket inaccurately portrays the true extent of WSLs at the mesial and distal tooth surfaces. WSLs are most often seen at the gingival halves of these surfaces, where orthodontic wires most commonly pass. However, the other half of these demarcated zones is really the incisal portion of the tooth, where WSLs rarely occur. It is therefore difficult to assign scores of 2s at these surfaces (>50% decalcification); despite the presence of large WSLs, these surfaces often received scores of 1. Thus, the EDI criteria underestimated WSL extent on the mesial and distal surfaces of teeth in severe cases of WSLs more than in mild ones. Perhaps division of the tooth surface around the orthodontic bracket as in the modification of the Silness and Loe index as described by Williams might have been a better technique for judging WSL extent. The challenge for future research in this area is to find more clinically relevant indices to accurately assess WSL changes.

Performing this study in a population of patients with high levels of even mild fluorosis required close clinical calibration. Very good levels of intra-rater reliability were demonstrated after the study investigators learned to distinguished fluorosis from demineralization. While it is recommended for future investigators to screen rigorously and exclude patients with any signs of fluorosis, this may be unrealistic in many communities. Investigators should, therefore, be prepared to perform strict calibration for WSL differentiation in these populations.

CONCLUSION

- Gingival surfaces experience greater surface coverage of WSLs and higher severity of demineralization than mesial, distal and occlusal surfaces.

- Plaque levels improve across subjects in a study with quarterly oral hygiene reinforcement.
- Salivary fluoride levels increase over time across all subjects receiving topical fluoride.
- It is difficult to use digital photographs or digital light fluorescence imaging to accurately evaluate surfaces for changes in WSLs across time points.

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APPENDIX

Table 1: Subject demographics

Females	17
Males	23
Age at Baseline	16y0m+/- 3y9.6m
Average CariScreen value	8600 +/- 2409

Table 2: Average number of days between study visits

	Days	Standard Deviation
Baseline – 3 months	94.84	10.04
3 months – 6 months	91.45	14.08

Table 3: Intra-rater reliability analysis

Subject	Kappa	Standard Error	95% CI	Weighted Kappa	Assessment

96	0.760	0.073	[0.617, 0.903]	0.818	Very good
79	0.754	0.079	[0.600, 0.908]	0.735	Good
80	0.786	0.068	[0.653, 0.919]	0.808	Very good
28	0.811	0.064	[0.686, 0.936]	0.830	Very good

Overall:	0.781	0.035	[0.711, 0.850]	0.806	Very good

Table 4: Average EDI score distribution

	Baseline Average	Weighted Variance	3 month	Weighted Variance	6 month	Weighted Variance
0	32.08	8.32	31.72	7.90	29.86	6.98
1	20.67	6.58	21.72	7.21	23.43	7.14
2	2.51	2.37	2.35	1.79	2.56	2.53
3	5.10	3.70	4.62	3.24	4.54	3.31

Table 5: Average ICDAS score distribution

	Baseline	Weighted Variance	3 month	Weighted Variance	6 month	Weighted Variance
0	32.13	8.42	31.56	7.94	29.70	7.09
1	16.75	5.77	18.67	7.03	17.67	6.45
2	11.40	8.38	9.70	6.90	12.83	7.87
3	0.027	0.16	0.43	1.16	0.18	0.73

Table 6: Average EDI sum

	Baseline	Weighted Variance	3 month	Weighted Variance	6 month	Weighted Variance
Maxillary	2.23*	1.24	2.29**	1.25	2.69	1.24
Mandibular	2.78*	1.24	2.78**	1.24	2.60	1.24
All	2.50	1.29	2.54	1.25	2.65	1.29

*$p<0.005$, **$p<0.05$

Table 7: Average highest ICDAS score

	Baseline	Weighted Variance	3 months	Weighted Variance	6 month	Weighted Variance
Maxillary	1.32	0.62	1.40	0.58	1.53	0.57
Mandibular	1.33	0.61	1.39	0.59	1.39	0.55
All	1.32	0.62	1.40	0.61	1.46	0.59

Table 8: Average EDI score per tooth surface

	Baseline	Weighted Variance	3 month	Weighted Variance	6 month	Weighted Variance
Distal	0.51	0.50	0.50	0.50	0.57	0.50
Gingival	1.91	1.06	1.85	1.01	1.83	0.99
Mesial	0.49	0.50	0.49	0.51	0.59	0.50
Occlusal	0.01	0.07	0.003	0.06	0	0

Table 9: Average ICDAS score per tooth surface

	Baseline	Weighted Variance	3 month	Weighted Variance	6 month	Weighted Variance
Distal	0.62	0.67	0.60	0.63	0.70	0.66
Gingival	1.43	0.68	1.49	0.73	1.53	0.66
Mesial	0.65	0.72	0.61	0.66	0.77	0.68
Occlusal	0.01	0.11	0.01	0.11	0	0

Table 10: Average plaque score

Baseline	Weighted Variance	3 month	Weighted Variance	6 month	Weighted Variance
3.30*,**	0.66	2.65*	0.67	2.37**	0.66

*,** $p<0.001$

Table 11: Average salivary fluoride level

Baseline	Standard Deviation	3 month	Standard Deviation	6 month	Standard Deviation
0.036*,**	0.052	0.073*	0.076	0.15**	0.24

* $p<0.05$, ** $p<0.01$

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