## Title

The Effect of Light-Cured Fluoride Varnish to Remineralize White Spot Lesions and Change the Salivary Microbiome: A Pilot Split-Mouth Randomized Clinical Trial



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# The Effect of Light-Cured Fluoride Varnish to Remineralize White Spot Lesions and Change the Salivary Microbiome: A Pilot Split-Mouth Randomized Clinical Trial

A thesis submitted in partial satisfaction

of the requirements for the degree

Master of Science in Oral Biology

by

Joseph Michael Mullen

2022

ABSTRACT OF THE THESIS

The Effect of Light-Cured Fluoride Varnish to Remineralize
White Spot Lesions and Change the Salivary Microbiome:
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by

Joseph Michael Mullen

Master of Science in Oral Biology

University of California, Los Angeles, 2022

Professor Renate Lux, Chair

Orthodontic treatment impedes the oral hygiene of patients, leading to plaque accumulation and the development of white spot lesions (WSL), which are visible areas of demineralized enamel. Our study aims to investigate the efficacy of different commercially available fluoride varnishes to treat these white spot lesions after the completion of orthodontic treatment and evaluate their effect on the oral microbiome. The present pilot study used a split-mouth design, with each of the eight subjects receiving different treatment on the left and right sides of their mouth. Subjects were assigned to one of three groups using stratified permuted block randomization, with each group receiving two of the following three options: placebo varnish (petroleum jelly), 5% sodium fluoride varnish (Acclean, Henry Schein), and a resin-

modified glass ionomer light-cured fluoride varnish (Vanish XT Extended Contact Varnish, 3M). There are four total visits for this study: T1 (baseline taken within one month of braces removal), T2 (day 30), T3 (day 90), and T4 (day 180). Several series of photographs were taken at each visit to assess their oral health and the appearance of the WSL. Stimulated saliva was collected, and the severity of the WSL was assessed using laser fluorescence (DIAGNOdent pen, Kavo), after which the varnishes were applied according to their group allocation.

Clinical photographs were assessed by a trained and blinded scorer using the Turesky-modified Quigley Hein Plaque Index (TQHPI) and the International Caries Detection and Assessment System (ICDAS). These index measurements, along with the DIAGNOdent scores, were used to assess the efficacy of the different interventions at remineralizing WSLs. Salivary loads of total bacteria, *Candida albicans*, and *Streptococcus mutans* were determined by plating saliva samples on agar plates containing Brain Heart Infusion (BHI), Sabouraud Dextrose (SAB), and Mitis Salivarius-Bacitracin (MSB) respectively.

Although the small sample size of this pilot study made it difficult to achieve statistical significance, several notable trends were identified. Average TQHPI levels stayed relatively stable, with modest decreases seen in the placebo and light-cured fluoride varnish (LCFV) groups. DIAGNOdent scores decreased in all groups, with the 5% varnish group showing a significant decrease from T1-T3 and the LCFV showing the greatest percent change from T1-T4. ICDAS scores increased in the 5% and placebo groups and decreased in the LCFV group. CFUs representing total bacterial load, *S. mutans*, and *C. albicans* all decreased by an average of 60.9 – 99.9 percent, showing decreased oral colonization in the 6 months after completion of orthodontic treatment. These preliminary trends need to be verified in future studies that include more subjects to reach statistical significance.

The thesis of Joseph Michael Mullen is approved.

Nini Chaichanasakul Tran

Bo Yu

Carl Maida

Renate Lux, Committee Chair

University of California, Los Angeles

2022

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## Introduction

The oral microbiome harbors a rich and diverse community that includes bacteria, fungi, and other microbes [1]. When the oral cavity shifts from a healthy to a diseased state, shifts in the makeup of the microbial communities are also observed. These imbalances in the microbiome can cause it to change from being commensal and benign to being inflammatory (as observed in periodontal disease) or acidogenic (as observed in dental caries) [2]. When an acidogenic community is present in dental plaque, the acid generated by the microbes can cause demineralization of the associated tooth surfaces.

Many studies have investigated the effect of orthodontic appliances on the composition of oral microbial communities. Preliminary results from previous research published by our lab support that orthodontic treatment does initiate a shift in the oral microbiome, but the nature of that change may be patient specific [3]. To identify clinically-meaningful changes in the microbiome, species of interest include *Streptococcus mutans*, a primary etiological agent in the development of dental caries, other cariogenic bacteria, and known periodontal pathogens [4]. While some research has not found significant increases in *Streptococcus spp.* in patients undergoing orthodontic treatment [5], a systematic review of the literature published in 2018 found significant increases in counts of *S. mutans*, *Lactobacillus spp.*, and a percentage of potentially pathogenic gram-negative bacteria [6]. Subgingival plaque analysis noted a microbial shift and increase in some periodontal pathogens in the first few months of treatment, but with long-term follow-up the levels of periodontal pathogens returned to pre-treatment levels [7].

Some research has also been performed looking at the synergistic effects of other microbes in enhancing the pathogenicity of acidogenic bacteria, such as *S. mutans*. Analysis of plaque colonies containing *S. mutans* alone compared to those containing both *S. mutans* and

*Candida albicans* found greater biofilm mass and CFU counts in the co-species biofilms [8]. The investigators looked at the structure of these co-species biofilms and found *C. albicans* surrounding the *S. mutans* microcolonies, and their presence increased the production of exopolysaccharides (EPS) that protected the *S. mutans* colonies and increased the mass of biofilm. Koo et al. also reported a synergistic effect between *S. mutans* and *C. albicans* to cause dental caries and increase biofilm adhesion [9].

During orthodontic treatment, acidogenic plaque can more easily accumulate around the brackets and cause smooth-surface demineralization, also referred to as white spot lesions (WSL), due to their white and chalky appearance (see Figure 1). These lesions represent an early form of dental caries and are associated with biofilm containing several pathogenic taxa, including *S. mutans*, *Scardovia wiggsiae*, *Bifidobacterium Cluster 1*, *Granulicatella elegans*, and *Veillonellaceae sp. HOT 155* [10]. The above-mentioned synergistic interaction between *S. mutans* and *C. albicans* may also contribute to the development of acidogenic biofilm that causes orthodontic WSL, since some research has shown a significant increase in *C. albicans* levels after the start of treatment [11].

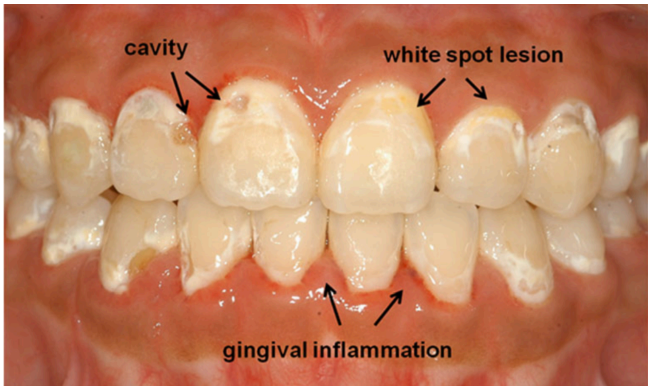



*Figure 1: Appearance of post-orthodontic white spot lesions [12].*

Unfortunately, white spot lesions are a common side effect of orthodontic treatment. Meta-analysis of studies on the incidence and prevalence of WSL revealed an incidence of 45.8

percent (ranging from 23.4-72.9 percent) and a prevalence during treatment of 68.4 percent (ranging from 33.8-97 percent) [13]. White spot lesions can develop very early in orthodontic treatment. A paper published in the Angle Orthodontist found that there is a sharp and significant increase in WSL during the first 6 months of treatment, with a slower continued increase in the number of WSL up to 1 year after having braces cemented [14]. Their distribution within the mouth shows higher prevalence in maxillary compared to mandibular teeth, appearing most commonly on lateral incisors, followed by canines, premolars, and central incisors [15, 16]. These demineralized lesions can cause irreversible damage, requiring dental restorations in 15 percent of orthodontic patients to restore the tooth and improve their smile esthetics [12].

Available literature on the topic has focused on two methods to reduce the prevalence of WSLs: prevention and remineralization. Several commercial products are available and marketed to prevent the development of orthodontic white spot lesions, including fluoride varnish, uniquely formulated orthodontic cements, and smooth surface sealants. A recent systematic review and meta-analysis found three interventions that proved effective: (1) active patient reminders, (2) fluoride varnish, and (3) smooth surface sealants [17]. Smooth surface sealants are most often either composed of glass ionomer (GI) or resin-modified glass ionomer (RMGI), which possess fluoride releasing properties. These sealants, which are also marketed as light-cured fluoride varnishes, are applied around the bracket base of fixed orthodontic appliances at the time of bonding with the aim of preventing demineralization caused by dental plaque.

An *in vitro* study performed on extracted human premolars evaluated the efficacy of RMGI, activated phosphate fluoride gel, fluoride toothpaste with tri-calcium phosphate, and sodium fluoride (NaF) mouthwash to prevent demineralization in an acidic environment. The investigators found that all teeth had some level of demineralization present, but the smallest

increase was found in the teeth treated with the RMGI varnish [18]. Several *in vivo* split-mouth studies have also been performed on premolars planned for extraction which have studied the efficacy of RMGI light-cured fluoride varnish (LCFV). Mehta et al. reported in the American Journal of Orthodontics and Dentofacial Orthopedics that premolars treated with LCFV showed little to no sign of demineralization under polarized light microscopy up to 4 months after beginning treatment, while the control teeth showed significant demineralization that increased in depth from 30 to 120 days [19]. Two other similarly designed studies found that teeth treated with LCFV showed little to no signs of demineralization compared to teeth treated with conventional NaF varnish [20, 21].

Despite these findings of the efficacy of LCFV to prevent WSL, little research has been performed on its ability to remineralize these lesions after removal of orthodontic brackets. Most research on remineralization has focused on other products, including casein phosphopeptide-amorphous calcium phosphate (CPP-ACP, or MI Paste), traditional NaF varnish, and NaF toothpaste. MI Paste is one of the most commonly prescribed therapeutics, and while some research has showed that CPP-ACP can reduce the depth of demineralized lesions [22], most studies have found that it does not remineralize WSLs more than regular home care [23-25]. A systematic review on the subject reported that the only therapy that showed significantly improved remineralization was multiple applications of NaF varnish [24, 26, 27]. However, very few studies have investigated whether LCFV will remineralize WSLs. One *in vitro* experiment published in 2012 found a significant loss in fluorescence (indicating improved mineral content) after the application of GI-containing materials, with a lower surface roughness also observed in the LCFV group [28]. Another randomized controlled trial compared LCFV to resin infiltration to improve WSLs, finding that the LCFV resulted in significantly improved restoration of color

and lightness [29]. The relative lack of research on this promising material to remineralize WSLs represents a knowledge gap in the literature that warrants further investigation.

**Objectives and Specific Aims**

The goal of this study is to research the efficacy of different fluoride products to remineralize post-orthodontic white spot lesions. We hypothesized that teeth with WSL treated with LCFV will remineralize significantly more than teeth treated with traditional sodium fluoride (NaF) varnish or placebo varnish. We propose the following specific aims:

1. Investigate the efficacy of LCFV, 5% NaF varnish, and placebo varnish to treat WSL as measured by a visual index (ICDAS) and DIAGNOdent scores.
2. To determine the effect of the different varnishes on the levels of biofilm accumulation, as measured by the TQHPI [30].
3. Evaluate changes in the salivary load of microorganisms, specifically *S. mutans* and *C. albicans*, in orthodontic patients over the first six months after completion of treatment.

**Design and Methodology**

To best determine the efficacy of LCFV to remineralize post-orthodontic WSL, we conducted a split-mouth randomized clinical trial. The study criteria and methodology were approved by the UCLA Institutional Review Board under IRB#20-001077 (Appendix A). Subjects nearing the end of their orthodontic treatment were identified and recruited with the following inclusion criteria:

1. Males and females, 12-19 years of age, inclusive, at the time the Assent and Informed Consent Form was signed

2. Systemically healthy, as determined by the investigators
3. Subject is approaching the end of their orthodontic treatment using metal fixed oral appliances (brackets) on at least the maxillary arch
4. Subject has at least two visible white spot lesions in separate oral quadrants at the time of recruitment on teeth treated with fixed oral appliances
5. Subject is willing to use only oral care products that fall within the standard of care (manual toothbrush and NaF toothpaste) for the duration of the study
6. Subject is willing to postpone dental cleanings between the baseline and final visit (since subjects will receive cleanings 6 months apart at both the baseline and final visit, the standard of care will be maintained)
7. Subject is willing and able to comply with oral hygiene and diet instructions

The exclusion criteria for our subjects included the presence of advanced periodontal disease, a medical condition for which antibiotics are recommended prior to dental visits/procedures, pathologic oral lesions, or any other condition/illness that would compromise normal immune function. Subjects who used systemic or topical antibiotics within 30 days prior to the screening were also excluded.

The investigators determined eligibility for inclusion through during the screening process in the UCLA orthodontic clinic. Once the subject was determined eligible, informed consent was obtained and they were assigned a unique study identification number. All teeth with WSL present were identified and recorded, and the subject was randomly assigned to one of the following three groups via stratified permuted block randomization:

**Group 1:** LCFV on upper left (UL) and lower right (LR) quadrants and 5% NaF varnish on upper right (UR) and lower left (LL) quadrants

**Group 2:** Placebo varnish on UL and LR quadrants and LCFV on UR and LL quadrants

**Group 3:** 5% NaF varnish on UL and LR quadrants and placebo varnish on UR and LL quadrants

Four strata were identified for the study to help balance the age and gender distribution of the groups: (1) 12-15 year old males, (2) 12-15 year old females, (3) 16-19 year old males, and (4) 16-19 year old females. Permuted block sizes of 1-3 and a final allocation sequence was determined using a random number and sequence generator [31]. A printed group assignment was placed in a lined, sealed envelope labeled with the strata and sequence number. Once a patient was recruited, the investigators took a video of themselves writing the subjects name or ID number on the sealed envelope before opening it to assign them to a treatment group.

Previous literature studying traditional 5% NaF varnish found a mean change in DIAGNOdent score of 7.56 (17.66 ± 5.36 reduced to 10.10) versus the mean change with placebo of 3.09 (16.19 ± 5.70 reduced to 13.10) [26]. Based on these differences, a conservative estimate of sample size would be $n=26$ per treatment group, or $n=78$ total, to achieve 80% power and an $\alpha=0.05$. However, this does not take into account the split-mouth design of the study; since each subject will provide data for two of the three varnishes (placebo, traditional, and experimental), achieving 26 subjects per group would only require 39 subjects total (13 with placebo and experimental, 13 with placebo and traditional, and 13 with traditional and experimental). However, in order to accurately calculate the required sample size, we would need to know the correlation between observations on the two sides of the mouth, which is not available in the literature. Therefore, considering an $n=39$ to be the low end of the spectrum and $n=78$ to be high end of the spectrum, we plan to ultimately enroll $n=60$ subjects total (20 in each

split-mouth treatment group) to achieve the desired power. The present pilot study includes data from eight subjects who have already been recruited.

There are four total visits for this study: T1 (baseline, day 0, collected within one month of braces removal), T2 (one month), T3 (three months), and T4 (6 months). All samples were collected in the morning hours. To avoid disrupting the oral microbiome and plaque, patients were instructed not to brush their teeth the morning of each study visit and to fast from food and drink (except water, which could be drunk up to 1 hour prior to the visit). At the beginning of each appointment, a questionnaire regarding the use of fluoride products in the previous 6 months and other relevant questions was completed (Appendix B). Stimulated saliva was collected by having the patient chew on a piece of parafilm wax and spit into a sterile saliva collection tube labeled as follows: [subject ID][S][visit]. The tube was placed on ice and taken up to the lab, where seven serial dilutions were performed on each sample. Dilutions 2-7 were each plated on three different agar plates: Brain Heart Infusion (BHI) to assess total bacterial load, Sabouraud Dextrose (SAB) to approximate the load of *C. albicans*, and Mitis Salivarius-Bacitracin (MSB) to approximate the load of *S. mutans*.

After the saliva collection was completed, several drops of Trace plaque-disclosing liquid were placed under the tongue and the subject swished for 30 seconds, coloring the biofilm a bright pink color for easy scoring (see Figure 2). Cheek retractors were used to take photos of the teeth, with at least all buccal surfaces visible back to the first molars. The three photos (one frontal and two buccal shots) were taken with the teeth slightly apart, as shown in Figure 2. Photos were captured using the same Canon Rebel T6i camera, equipped with a 60 mm macro lens and Canon ring flash with the following settings: ISO 800, f=18, shutter speed 1/200.

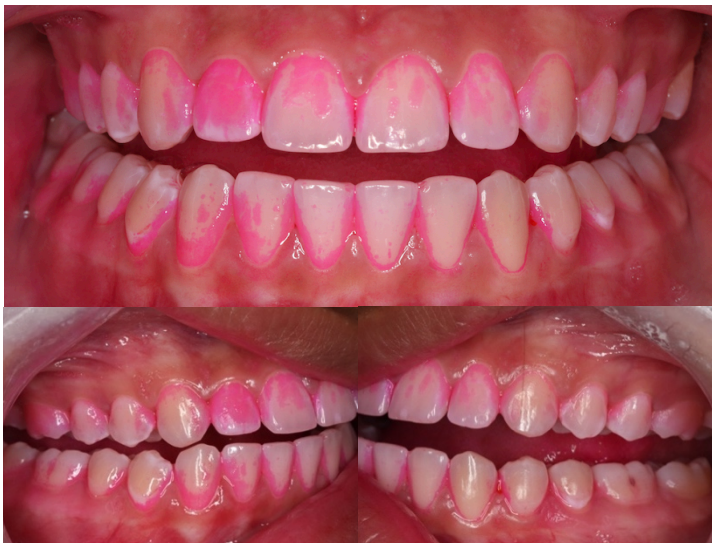


*Figure 2: Frontal (upper panel) and buccal (lower panel) photos of subject 5 with plaque disclosed*

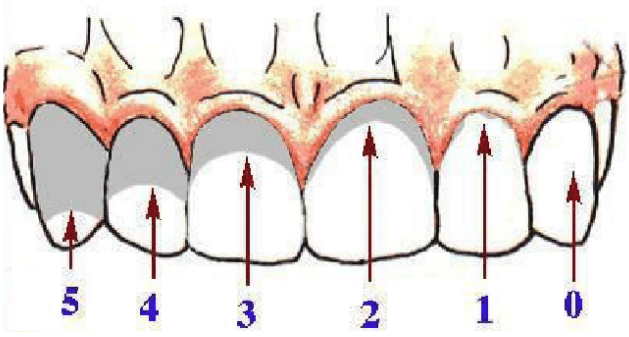
After the TQHPI photos were taken, a dental prophylaxis was performed with a prophy cup and pumice and hand scalers. Following the prophylaxis, two additional sets of the three photos detailed in the previous paragraph were taken: one with the teeth moist, and one after drying the teeth for 15 seconds with a dental air syringe. The teeth with visible WSLs identified at the start of the study were then scored using the DIAGNOdent pen. After calibrating the DIAGNOdent using the manufacturer's calibration disc and a non-affected area of a maxillary incisor, each WSL was scored three times and recorded on the Laser Fluorescence Data Sheet (Appendix C).

Varnishes were then applied to the buccal surfaces of all teeth in each quadrant of mouth and each tooth, regardless of the varnish used, was light cured to maintain patient blinding to the treatments they received. Before leaving, patients were given an Oral Hygiene Study Instructions information sheet (Appendix D) and supplied with a new soft-bristled toothbrush and tube of over-the-counter NaF toothpaste. At each recall appointment the same protocols were followed, including reapplying the varnishes. Although varnish reapplication may not be clinically

necessary for the LCFV, is a logistical necessity because the LCFV would obscure the DIAGNOdent scores. Thus, the LCFV must be removed and reapplied at each timepoint to obtain accurate measurements. At T3, subjects were given another new toothbrush (consistent with the ADA recommendation of changing your toothbrush every 3 months), and at T4 no varnishes were reapplied since the study was completed.

Subjects were compensated for their participation by receiving $14 cash at each visit to cover gas and parking. Upon completion of all four study visits, they also received an additional $50 cash and an extra set of clear thermoformed plastic retainers.

The TQHPI and ICDAS scoring was completed by a trained dental student who did not perform any clinical data collection, allowing her to be blinded to the treatments received. The photos were de-identified and randomized using a random number generator, ensuring the blinded scorer would not know which patient nor which timepoint they were scoring [31]. The TQHPI was scored from the buccal surfaces of teeth #3-14 and #19-30 using the three plaque-disclosed photographs, excluding the first premolars since the biofilm on those teeth was disrupted with the collection of dental plaque being used in a different study (see Figure 3) [30].




**Scoring Criteria**
**Score 0:** No plaque
**Score 1:** Flecks of plaque on gingival margin
**Score 2:** Continuous band of plaque on cervical margin up to 1mm thick
**Score 3:** Band of plaque >1mm but less than 1/3 tooth surface
**Score 4:** Plaque covers between 1/3 and 2/3 tooth surface
**Score 5:** Plaque covers >2/3 tooth surface

*Figure 3: Turesky-modified Quigley Hein Plaque Index scoring from 0-5*

The visual appearance of the WSLs was assessed using the ICDAS classification, which is shown below in Table 1 [32].

*Table 1: Scoring criteria for ICDAS*

| ICDAS | |
|---|---|
| 0 | Sound |
| 1 | First visual change in enamel |
| 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 |

The DIAGNOdent, TQHPI, and ICDAS data were assessed for normality using the Shapiro-Wilk test and assessed for significance using the Mann-Whitney U test with $\alpha=0.05$. The number of colonies on the plated saliva samples were counted at the various dilutions and CFUs were calculated by averaging the results of non-outlier dilutions. The averaged CFU counts were compared for significance using a t-test with $\alpha=0.05$.

## Results

*Patient Demographics/Compliance*

Eleven subjects were recruited to participate in the study. Two of the subjects did not come to the first collection and were removed from the study, one subject missed their T2 appointment due to a medical issue, and one subject was excluded from the pilot study due to the presence of several large cavitated lesions that made her data significant outliers. The demographic data and number of collections at each timepoint are summarized below in Table 2.

*Table 2: Subject demographic and timepoint data*

| **Patient Demographic Data** | |
|---|---|
| Number of Subjects | 8 |
| *- Group 1 (LCFV, 5%)* | *4* |
| *- Group 2 (Placebo, LCFV)* | *3* |
| *- Group 3 (5%, Placebo)* | *1* |
| Average Age (at T1) | 16y11m |
| *- Standard Deviation* | *+/– 1y10m* |
| # Males | 5 |
| # Females | 3 |
| Total # Sample Collections | 27 |
| *- T1* | *8* |
| *- T2* | *7* |
| *- T3* | *7* |
| *- T4* | *5* |

Subjects were largely compliant with study instructions, as summarized below in Table 3. They brushed an average of two times per day and flossed less than one time per day (average 0.7). Subjects complied with the instructions to not brush their teeth the morning of the collection and come fasting in 24 of the 27 total timepoints. The only data that appeared to be potentially affected by lack of compliance were the BHI CFU count for subject 5 at T3 and the SAB and MSB CFU counts for subject 7 at T2.

*Table 3: Subject compliance data*

| | # Visits (out of 27) | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Subject brushed teeth in morning | 2 / 27 | 0 | 1 | 1 | 0 |
| Subject didn't come fasting | 3 / 27 | 0 | 2 | 1 | 0 |
| Subject took antibiotics in past month | 0 / 27 | 0 | 0 | 0 | 0 |
| Subject used additional fluoride products | 3 / 27 | 0 | 0 | 1 | 2 |
| Subject used adjuct oral hygiene products | 5 / 27 | 4 | 0 | 1 | 0 |
| Subject used non-study electric toothbrush | 1 / 27 | 1 | 0 | 0 | 0 |
| Subject received cleaning from outside DDS | 1 / 27 | 0 | 0 | 0 | 1 |
| Average # times subjects brushed/day | 2.00 | 2.19 | 1.93 | 1.86 | 2.00 |
| Average # times subjects flossed/day | 0.70 | 0.48 | 1.07 | 0.57 | 0.70 |

There were 54 total teeth with visible WSLs present that were included in the analysis, most of them incisors and premolars, with even distribution of maxillary and mandibular teeth.

The number of teeth with WSLs treated with 5% varnish was lower than the number treated with the placebo and LCFV. A summary of the teeth included in the analysis for the DIAGNOdent and ICDAS data is shown below in Table 4.

*Table 4: Characteristics of teeth included in data analysis*

| | # Teeth | Maxillary | Mandibular | Incisors | Canines | Premolars | Molars |
|---|---|---|---|---|---|---|---|
| Placebo | 20 | 8 | 12 | 6 | 1 | 8 | 5 |
| 5% NaF | 13 | 8 | 5 | 4 | 4 | 2 | 3 |
| LCFV | 21 | 10 | 11 | 6 | 4 | 7 | 4 |
| TOTAL | 54 | 26 | 28 | 16 | 9 | 17 | 12 |

*ICDAS/Visual Appearance Results*

Three of the eight subjects subjectively reported improvement in the appearance of their WSLs during the study, one from each of the three study groups. The ICDAS scoring data was assessed for normality using the Shapiro-Wilk test, which returned a W of 0.847. This score indicates the data is not normally distributed, so the Mann Whitney U-test was used to assess significance instead of a regular t-test. As explained in the previous section, a higher ICDAS score indicates a more visible/severe carious lesion.

There were no statistically significant changes noted in any of the varnish groups, although notable trends were visible, as shown in Figure 4 below. The lack of significance is likely due to (1) the small sample size of this pilot study, and (2) the non-parametric nature of the data, which prevented use of the more powerful t-test to assess significance. The placebo and 5% varnishes showed a similar trend of decreasing slightly at T3, then increasing at T4. The LCFV showed a notable downward trend across all four timepoints. From T1 to T4, teeth treated with both the placebo and 5% varnishes increased in their score by 8.77 and 25.57 percent respectively, while teeth treated with LCFV decreased in their score b 14.74%. The data and p-values for all the analyses used are included below in Tables 5 and 6.

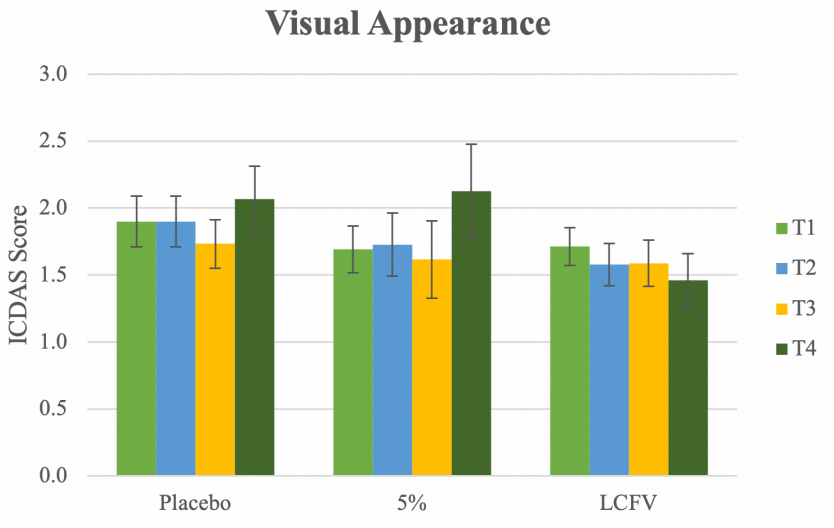



*Figure 4: ICDAS scores in teeth treated with different varnishes*

*Table 5: Average ICDAS scores of various varnishes and percentage change from baseline*

| | **Average ICDAS Score** | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | *T1-T4 % Change* |
| Placebo | 1.90 | 1.90 | 1.73 | 2.067 | 8.77% |
| 5% | 1.69 | 1.73 | 1.62 | 2.125 | 25.57% |
| LCFV | 1.71 | 1.58 | 1.59 | 1.462 | -14.74% |

*Table 6: p-values comparing changes in ICDAS scores from T1-T4*

| | ***p-values (calculated from Mann Whitney U-Test)*** | | | | | |
|---|---|---|---|---|---|---|
| | T1-T2 | T1-T3 | T1-T4 | T2-T3 | T2-T4 | T3-T4 |
| Placebo | 0.906 | 0.495 | 0.431 | 0.474 | 0.526 | 0.227 |
| 5% | 0.743 | 0.891 | 0.133 | 0.781 | 0.212 | 0.256 |
| LCFV | 0.465 | 0.521 | 0.391 | 0.972 | 0.846 | 0.814 |

*DIAGNOdent Results*

The Shapiro-Wilk test returned a W of 0.864, indicating the DIAGNOdent scoring data was not normally distributed, so significance was calculated with the Mann Whitney U-test. The T1 data for the 5% varnish was significantly higher than both the placebo (p=0.00789) and

LCFV (0.00662) groups. Although there was some variability in the data, the DIAGNOdent scores in all three groups decreased during the study. As mentioned in the design and methodology section, there is some inherent variability in the DIAGNOdent scoring, despite using the aforementioned calibration protocols; however, this was mitigated by taking three separate scores for each tooth at each timepoint and averaging them. The greatest change was visualized in the 5% varnish group from T1-T3, which showed a statistically significant 34 percent drop, before jumping back upward at T4. The outcomes of the data analysis can be visualized in Figure 5 and Table 7, with the p-values reported in Table 8.

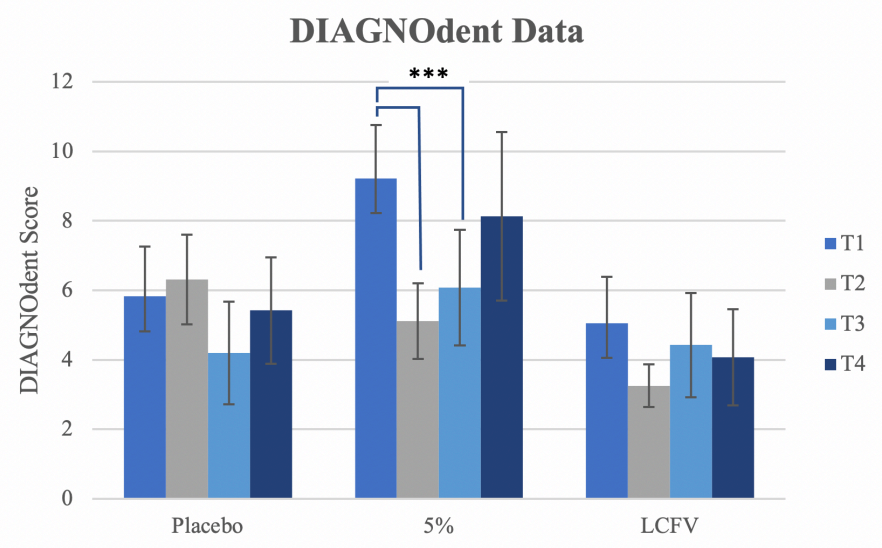



*Figure 5: DIAGNOdent scores of teeth with WSL treated with different varnishes*

*Table 7: Average DIAGNOdent scores of various varnishes and percentage change from baseline*

| | **Average DIAGNOdent Score** | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | *T1-T4 % Change* |
| Placebo | 5.83 | 6.32 | 4.19 | 5.42 | -6.95% |
| 5% | 9.22 | 5.12 | 6.08 | 8.13 | -11.91% |
| LCFV | 5.06 | 3.25 | 4.42 | 4.08 | -19.38% |

*Table 8: p-values comparing changes in DIAGNOdent scores from T1-T4*

| | ***p-values (calculated from Mann Whitney U-Test)*** | | | | | |
|---|---|---|---|---|---|---|
| | T1-T2 | T1-T3 | T1-T4 | T2-T3 | T2-T4 | T3-T4 |
| Placebo | 0.882 | 0.263 | 0.907 | 0.171 | 0.828 | 0.350 |
| 5% | 0.045 *** | 0.040 *** | 0.384 | 0.839 | 0.591 | 0.384 |
| LCFV | 0.606 | 0.377 | 0.403 | 0.645 | 0.590 | 0.983 |

Since only five of the eight included subjects completed T4, the DIAGNOdent analysis was repeated with only the five subjects who had finished all four visits (see Figure 6 and Table 9 below). Similar trends were noted, but the magnitude of the increase in score at T4 was less severe in the 5% NaF group, and the drop from T1-T3 in the 5% NaF group no longer achieved significance at α=0.05. However, a few of the jumps did achieve borderline significance, as noted in Figure 6. The LCFV data in this group also showed an even more notable downward trend.

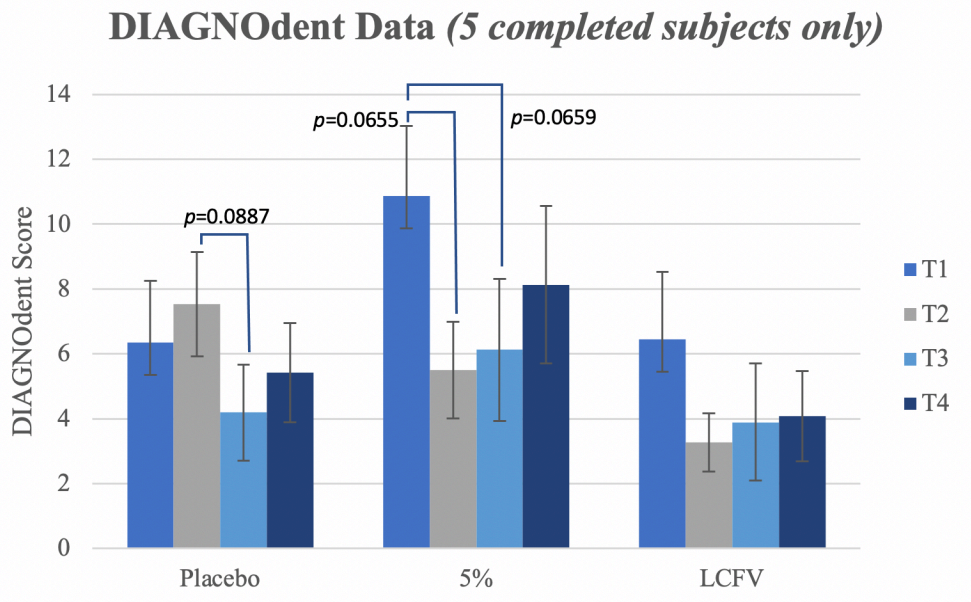



*Figure 6: DIAGNOdent scores of teeth from the 5 subjects with WSL treated with different varnishes who completed all study time points*

*Table 9: Average DIAGNOdent scores from first 5 completed subjects and percentage change from baseline*

| | **Average DIAGNOdent Score *(5 completed subjects only)*** | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | *T1-T4 % Change* |
| Placebo | 6.35 | 7.53 | 4.19 | 5.42 | -14.60% |
| 5% | 10.88 | 5.50 | 6.13 | 8.13 | -25.29% |
| LCFV | 6.45 | 3.27 | 3.89 | 4.08 | -36.83% |

*TQHPI Results*

The Shapiro-Wilk test returned a W of 0.9355, indicating the DIAGNOdent scoring data was not normally distributed, so significance was calculated with the Mann Whitney U-test. For most of the subjects the average TQHPI stayed notably stable over the 6 months (see Figure 7 and Table 10).

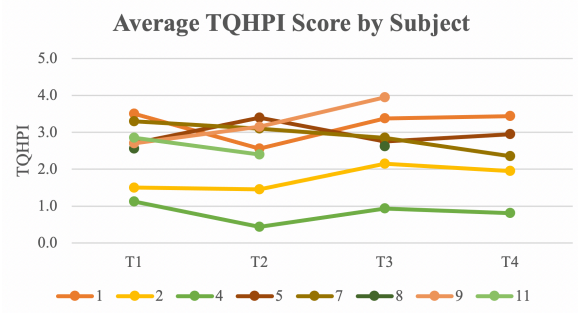



*Figure 7: Trends in average TQHPI score*

*Table 10: Average TQHPI score by subject*

| | **Average TQHPI by subject** | | | |
|---|---|---|---|---|
| Subject | T1 | T2 | T3 | T4 |
| 1 | 3.50 | 2.56 | 3.38 | 3.44 |
| 2 | 1.50 | 1.45 | 2.15 | 1.95 |
| 4 | 1.13 | 0.44 | 0.94 | 0.81 |
| 5 | 2.70 | 3.40 | 2.75 | 2.95 |
| 7 | 3.30 | 3.10 | 2.85 | 2.35 |
| 8 | 2.56 | | 2.63 | |
| 9 | 2.70 | 3.15 | 3.95 | |
| 11 | 2.85 | 2.40 | | |
| Average | 2.53 | 2.36 | 2.66 | 2.30 |

When the data were analyzed on the level of the tooth (irrespective of the patient it came from), a small downward trend was noted in the placebo and LCFV groups. However, such minor changes in the TQHPI score do not represent a statistically nor clinically significant change, rather indicating relatively stable oral hygiene in the subjects during the study period. The TQHPI data averaged by varnish treatment are summarized below in Figure 8 and Tables 11-12.

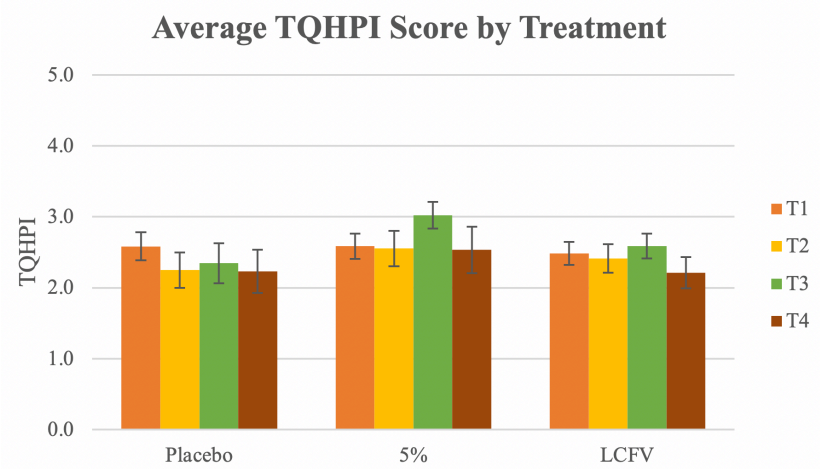



*Figure 8: TQHPI scores of teeth treated with different varnishes*

*Table 11: Average TQHPI scores of various varnishes and percentage change from baseline*

| | **Average TQHPI Score** | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | *T1-T4 % Change* |
| Placebo | 2.58 | 2.25 | 2.35 | 2.23 | -13.65% |
| 5% | 2.59 | 2.55 | 3.02 | 2.54 | -1.98% |
| LCFV | 2.48 | 2.41 | 2.59 | 2.21 | -11.04% |

*Table 12: p-values comparing changes in TQHPI scores from T1-T4*

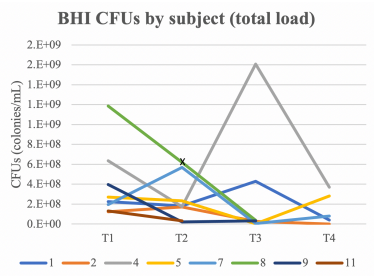
| | ***p-values (calculated from Mann Whitney U-Test)*** | | | | | |
|---|---|---|---|---|---|---|
| | T1-T2 | T1-T3 | T1-T4 | T2-T3 | T2-T4 | T3-T4 |
| Placebo | 0.400 | 0.520 | 0.331 | 0.624 | 0.908 | 0.827 |
| 5% | 0.689 | 0.128 | 0.649 | 0.111 | 0.857 | 0.155 |
| LC | 0.717 | 0.645 | 0.356 | 0.458 | 0.598 | 0.205 |

*Salivary CFU Results*

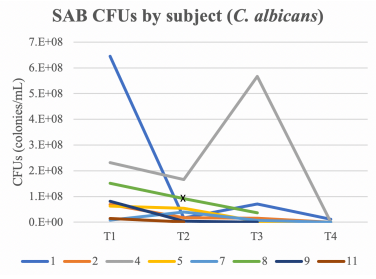
Colony counts on all three plating media (BHI, SAB, and MSB) showed significant decreases from baseline to T4, despite not reaching statistical significance when using the t-test. This lack of statistical significance is again likely due to the small sample size of this pilot study. A few of the timepoints, such as Subject 4/T3, appear to be possible outliers, which could be due

to plating error or contamination. Overall downward trends were noted among most subjects, indicating that in the 6 months after completion of orthodontic treatment the salivary loads of *S. mutans* and *C. albicans* decrease. The patient-level trends in the data are noted below in Figures 9A-C and Tables 13A-C.

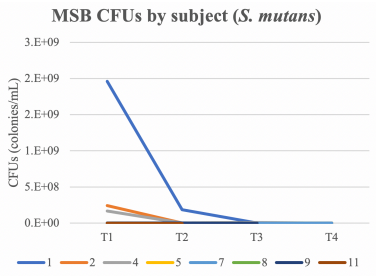
A.




B.




C.




*Figures 9A–C (left to right): Salivary CFU counts by subject, indicating trends in salivary total bacterial load (BHI), C. albicans (SAB), and S. mutans (MSB) respectively*

*Tables 13A–C (left to right): Salivary CFU counts by subject, showing trends in salivary total bacterial load (BHI), C. albicans (SAB), and S. mutans (MSB) respectively*

A.

| BHI Scores (by subject) | | | | |
|---|---|---|---|---|
| Subject | T1 | T2 | T3 | T4 |
| 1 | 2.25E+08 | 1.82E+08 | 4.31E+08 | 3.89E+07 |
| 2 | 1.24E+08 | 1.73E+08 | 2.75E+07 | 1.21E+06 |
| 4 | 6.35E+08 | 1.75E+08 | 1.61E+09 | 3.71E+08 |
| 5 | 2.71E+08 | 2.32E+08 | 3.44E+06 | 2.81E+08 |
| 7 | 1.94E+08 | 5.68E+08 | 6.05E+06 | 8.15E+07 |
| 8 | 1.19E+09 | | 3.68E+07 | |
| 9 | 3.97E+08 | 2.11E+07 | 3.30E+07 | |
| 11 | 1.31E+08 | 3.19E+07 | | |
| Average | 3.96E+08 | 1.98E+08 | 3.06E+08 | 1.55E+08 |

B.

| SAB Scores (by subject) | | | | |
|---|---|---|---|---|
| Subject | T1 | T2 | T3 | T4 |
| 1 | 6.45E+08 | 1.66E+07 | 7.05E+07 | 1.18E+07 |
| 2 | 6.85E+07 | 1.77E+07 | 1.53E+07 | 4.50E+05 |
| 4 | 2.32E+08 | 1.65E+08 | 5.67E+08 | 8.65E+04 |
| 5 | 6.23E+07 | 5.46E+07 | 2.55E+06 | 2.00E+04 |
| 7 | 8.10E+06 | 3.99E+07 | 7.53E+06 | 1.80E+04 |
| 8 | 1.51E+08 | | 3.62E+07 | |
| 9 | 8.10E+07 | 3.85E+06 | 9.50E+03 | |
| 11 | 1.47E+07 | 9.76E+05 | | |
| Average | 1.58E+08 | 4.27E+07 | 9.99E+07 | 2.46E+06 |

C.

| MSB Scores (by subject) | | | | |
|---|---|---|---|---|
| Subject | T1 | T2 | T3 | T4 |
| 1 | 1.96E+09 | 1.86E+08 | 2.22E+06 | 1.61E+05 |
| 2 | 2.41E+08 | 5.90E+05 | 2.25E+06 | 5.79E+05 |
| 4 | 1.68E+08 | 2.80E+06 | 8.72E+06 | 9.95E+04 |
| 5 | 1.52E+06 | 2.47E+06 | 3.94E+05 | 4.36E+05 |
| 7 | 1.40E+04 | 0.00E+00 | 1.17E+05 | 1.55E+03 |
| 8 | 4.60E+05 | | 1.29E+05 | |
| 9 | 4.54E+05 | 5.15E+05 | 3.13E+04 | |
| 11 | 3.53E+05 | 2.00E+05 | | |
| Average | 2.96E+08 | 2.75E+07 | 1.98E+06 | 2.56E+05 |

Since saliva composition represents the entirety of the oral microbiome, it was not possible to separate the data in this pilot study to analyze possible varnish-specific effects. However, when studied in aggregate, there seems to be a notable decrease in salivary levels of both fungi (approximating *C. albicans* levels) in the SAB agar and *Streptococcus spp.*

(approximating *S. mutans* levels) in the MSB agar. This is suggestive that the oral salivary microbiome becomes less pathogenic in the first 6 months after completion of orthodontic treatment. The combined data for all included subjects can be visualized in Figure 10 and Table 14, with Table 15 showing the p-values for each timepoint comparison.

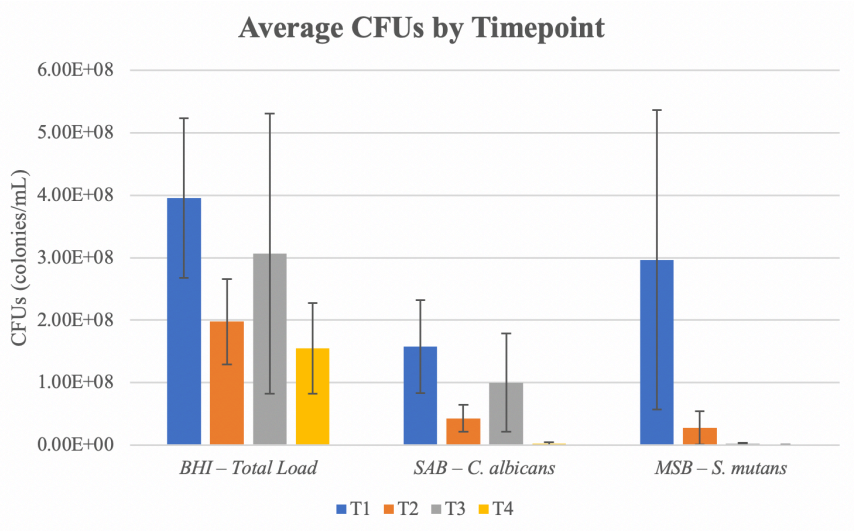



*Figure 10: Average CFU counts of all subjects in three agar media (BHI, SAB, MSB)*

*Table 14: Average CFU counts of all subjects and percentage change from baseline*

| | **Average CFUs** | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | *T1-T4 % Change* |
| BHI | 3.96E+08 | 1.98E+08 | 3.06E+08 | 1.55E+08 | -60.93% |
| SAB | 1.58E+08 | 4.27E+07 | 9.99E+07 | 2.46E+06 | -98.44% |
| MSB | 2.96E+08 | 2.75E+07 | 1.98E+06 | 2.56E+05 | -99.91% |

*Table 15: p-values comparing changes in CFU counts from T1-T4*

| | ***p-values (calculated from t-test)*** | | | | | |
|---|---|---|---|---|---|---|
| | T1-T2 | T1-T3 | T1-T4 | T2-T3 | T2-T4 | T3-T4 |
| BHI | 0.213 | 0.726 | 0.192 | 0.651 | 0.682 | 0.594 |
| SAB | 0.185 | 0.602 | 0.132 | 0.496 | 0.154 | 0.325 |
| MSB | 0.318 | 0.274 | 0.358 | 0.353 | 0.410 | 0.253 |

**Discussion**

Although LCFV and other fluoride-containing products have been well-established as an effective tool to prevent the development of WSLs, the present study is the first in vivo split-mouth RCT evaluating the ability of LCFV to remineralize WSLs compared to 5% NaF varnish and a placebo [18-21]. Regarding the ICDAS results, it is notable that the LCFV was the only intervention that showed a consistent downward trend over the six months of the study. Most of the WSLs included in this study had not reached the level of localized enamel breakdown, resulting in a majority of the scores falling between 0-2. At T4 the LCFV group had on average scores of approximately 0.6 points lower than the other two groups, which could signify a slightly clinically significant improvement in their appearance. However, the improvement is still modest, so the results of this pilot study would suggest that the LCFV should not be marketed or suggested as a therapy to predictably make WSLs visually disappear.

The DIAGNOdent data revealed an interesting trend in the placebo and 5% groups, with a distinct jump back up at T4. This appears to be in part because fewer T4 samples had been collected for the present study, so the T4 data is averaged from a smaller number of subjects. When the data was analyzed to only include the five subjects who had completed all four study visits, the jump upward at T4 was still present, but less pronounced. This upward trend at T4 could potentially be due to either subject burnout with oral hygiene instructions, although subjects reported the lowest levels of brushing/flossing at T3, not T4, which makes this explanation less likely. Another possibility is that the jump represents a relapse in the remineralization effect of the 5% NaF varnish, since the time period between T3-T4 was the longest in the study (3 month). It is possible that a T4 jump was not observed in the LCFV group because the relapse was mitigated by the longevity of the LCFV's contact with the tooth. The

smaller 5-subject group also revealed a more pronounced drop in score in the LCFV group – but whether this will remain the case with a larger sample size is still uncertain. The drop from T1-T3 in the 5% varnish group was one of the only changes that achieved statistical significance despite the small sample size, which could in part be due to the larger average DIAGNOdent score at baseline. If the decreasing trends noted in this pilot study are similar to what would be seen in a larger sample, the drop in DIAGNOdent score could achieve statistical significance. A possible future direction to expedite the increase the sample size could be to increase the age range of the study to capture young adults who may have started their treatment as adolescents.

The TQHPI data stayed relatively constant over the study period, which makes intuitive sense especially for the placebo and 5% varnishes. Since those varnishes would be removed when the patient brushed their teeth that night, if not sooner, they would be unlikely to significantly increase or decrease the amount of plaque that would be seen accumulated on the tooth 1-3 months later. If a subject's plaque score were considered an approximation of their oral hygiene habits, the consistency of the TQHPI data could indicate excellent stability of the subjects' hygiene over the course of the study, mitigating its effect as a confounding factor. The LCFV would have the potential to alter the plaque accumulation if it stayed intact on the surface of the teeth for the entire 1-3 months – however, personal experience with the varnish demonstrated that it would have likely chipped or broken off before the patient returned for the next follow-up visit. The chipping/removal of the LCFV could also have been expedited with the repeated insertion and removal of clear thermoformed plastic retainers, which a majority of the subjects used in their prescribed retention protocol. An interesting follow-up study could be to monitor the longevity of the LCFV by evaluating how many days/weeks/months it stays on the tooth in an average patient.

*Limitations of the Study*

An important limitation of this study, as mentioned before, is the small number of subjects included. As additional subjects continue to be recruited, this limitation will be mitigated and more significant results may be identified from the data.

Additionally, although SAB is the agar medium of choice to select for and enumerate *C. albicans*, it is important to note that it is not specific for this organism and other fungal microorganisms can also be found, especially other *Candida spp.* such as *C. glabrata* or *C. parapsilosis* [33]. Similarly, MSB is not selective for only *S. mutans*, and other bacteria have been identified on MSB agar, including *Staphylococcus epidermidis* and *Enterobacter kobei* [34]. *S. mutans* colonies were found to be relatively small compared to some of the other microorganisms found on MSB, so large colonies were excluded from the MSB counts in calculating CFUs [34]. However, despite these limitations, these media are widely accepted in the microbiological field as the best choices to reasonably approximate the load of *C. albicans* and *S. mutans* in a microbial community.

Another limitation of this study is that the analyses on the tooth level treated each tooth as a separate entity, but the overlap of subjects from which each tooth was evaluated could represent a confounding variable that the present study did not account for. The data analysis also did not account for the intra-subject effects that the varnishes could have at sites distant to their point of application – for example, whether LCFV applied on the UR quadrant could affect remineralization in the LR quadrant. Previous research has shown that RMGI products can affect remineralization up to 7mm away from the location of the material, but the most pronounced effects are seen only within a 1mm perimeter of the product [35]. Furthermore, if the subjects

were using thermoformed plastic retainers (as a majority of subjects did), the retainers would aid in keeping the effects of the material more localized by covering the clinical crowns of the teeth.

*Clinical Implications*

The CFU data showed distinct downward trends over the 6-month period, which would seem to indicate that the oral microbiome becomes less pathogenic after the removal of braces. This also demonstrates face-value validity, since clinical experience has shown that many patients with braces, particularly adolescents, have difficulty maintaining optimal oral hygiene with the physical barriers of the brackets and wires impeding ideal brushing and flossing. The removal of those appliances would subsequently allow improved hygiene practices, resulting in a less pathologic environment. Despite this fact, the magnitude of the decrease in all three media (BHI, SAB, and MSB) was surprisingly large.

An important factor that could have contributed to this drastic improvement is that all subjects received a professional dental prophylaxis at each timepoint, totaling four cleanings in six months. Most individuals only receive one prophylaxis every six to twelve months, so it is reasonable to assume that the higher frequency of dental cleanings could have contributed to the improvement in the CFU data, and even possibly some of the improvements noted in the DIAGNOdent and ICDAS scores.

## Conclusions

This study was unable to achieve statistical significance in most of its analysis due to the small sample size, which represents a significant limitation of this pilot analysis. However, there are still some distinct trends visible in the data that, if held consistent with a larger sample, would lead us to make the following tentative conclusions:

- Multiple applications of LCFV to teeth with post-orthodontic WSLs may result in a clinically significant improvement in their appearance compared to teeth treated with 5% NaF varnish and a placebo varnish, as measured by the ICDAS index.
- All three varnishes resulted in mild remineralization as scored by the DIAGNOdent pen, with a statistically significant drop in the 5% varnish group from T1-T3 and the greatest percent change from baseline visualized in the LCFV group.
- None of the varnishes appear to have a significant impact on the level of plaque accumulation on the teeth, as measured by the TQHPI.
- There appears to be a sizable decrease in total bacterial load and the representative levels of *C. albicans* and *S. mutans* over the first 6-7 months after completion of orthodontic treatment with braces, as measured by CFU counts of saliva samples cultured in various agar media.

An increased sample size is needed to achieve the significance and power needed to draw definitive conclusions on the efficacy of the LCFV compared to 5% NaF varnish and the placebo varnish.

**Disclosures**

This study was funded by a grant from 3M Investigator Sponsored Research, #2020-ISR-000199. The authors are grateful to 3M for sponsoring this research project, and a financial breakdown of the funds/products supplied can be found in Appendix E.

# Appendix A

Below the approval notice for the study by the UCLA Institutional Review Board.

University of California Los Angeles
10889 Wilshire Blvd, Suite 830
Los Angeles, CA 90095-1406

http://ora.research.ucla.edu/ohrpp
General Campus IRB: (310) 825-7122
Medical IRB: (310) 825-5344

**APPROVAL NOTICE**

| | |
|---|---|
| **DATE:** | 3/16/2022 |
| **TO:** | JOSEPH MULLEN, DDS<br>DENTISTRY |
| **FROM:** | JAMES MC GOUGH, MD<br>Chair, MIRB3 |
| **RE:** | IRB#20-001077-CR-00002<br>2022 Review for IRB#20-001077<br>Investigating the ability of light-cured fluoride varnish to remineralize post-orthodontic white spot lesions and alter the oral microbiome |

The UCLA Institutional Review Board (UCLA IRB) has approved the submission listed below. UCLA's Federalwide Assurance (FWA) with Department of Health and Human Services is FWA00004642.

**Submission and Review Information**

| | |
|---|---|
| Type of Submission | Continuing Review |
| Type of Review | Expedited Review |
| Approval Date for this Submission | 3/16/2022 |
| Expiration Date of the Study | 3/15/2023 |
| Funding Source(s) | 1) 3M CORPORATION (INCL 3M HEALTH CARE & 3 M PHARMACEUTICALS<br>*Grant PI*: JOSEPH MULLEN<br>*Grant Title*: 3M Investigator Sponsored Research<br>*Grant Number*: 2020-ISR-000199 |
| Initial IRB Approval Type & Date for this Study | IRB Review: Expedited 6/23/2020 |

**Regulatory Determinations**

-- **Children as Subjects** - The UCLA IRB determined that the research meets the requirements of 45 CFR 46.404 and 21 CFR 50.51 for research involving children as subjects. [One parent's permission is sufficient.]

-- **Expedited Review Category(ies)** - The UCLA IRB determined that the research meets the requirements for expedited review per 45 CFR 46.110 and 21 CFR 56.110, categories 1b, 3, 4, 5, 6, and 7.

-- **HIPAA General Waiver** - The UCLA IRB waived the requirement for HIPAA Research Authorization for medical record review to identify potential participants.

**Documents Reviewed included, but were not limited to:**

| Document Name | Document Version # |
|---|---|
| 20-001077- Adult Consent, Parent Permission, Youth Assent Form_CLEAN_1-12-22 (1).pdf.pdf | 0.01 |
| 20-001077- Screening Script CLEAN_1-12-22 (1).pdf.pdf | 0.01 |
| 20-001077- Study Advertisement_CLEAN_11-30-21.pdf.pdf | 0.01 |

# Appendix B

University of California, Los Angeles

**Oral Health Questionnaire**

**Investigating the ability of light-cured fluoride varnish to remineralize post-orthodontic white spot lesions and alter the oral microbiome**

Name: Visit #: 1 2 3 4

Have you been treated with any **systemic antibiotics** (e.g. pills) or **topical antibiotics** (e.g. mouthrinse/ ointment) in the past 30 days (or since your last visit)?

YES (please describe _____) NO

Have you used any **fluoride-containing products** BESIDES over-the-counter sodium fluoride toothpaste in the past 30 days (or since your last visit)?

YES (please describe _____) NO

Have you used any other **adjunctive oral hygiene therapies** (e.g. mouthrinse, hydrogen peroxide) in the past 30 days (or since your last visit)?

YES (please describe _____) NO

In the past 30 days (or since your last visit), have you been using a **manual or electric toothbrush**?

MANUAL ELECTRIC

In the past 30 days (or since your last visit), how many times a day have you **brushed your teeth** on average?

_____

In the past 30 days (or since your last visit), how many times a day have you **flossed your teeth** on average?

_____

In the past 30 days (or since your last visit), which of the following interdental cleaning products have you used? (circle all that apply)

REGULAR FLOSS FLOSS PICKS WATERPIK INTERDENTAL BRUSHES

Since your last visit, have you noticed any change in the white spots on your teeth? (describe where in mouth)

IMPROVED_____ STAYED THE SAME_____ GOTTEN WORSE_____

When was your last professional dental cleaning?

_____

What kind of retainer have you been wearing since the last study visit (circle one for each arch)?

*Upper retainer:* HAWLEY ESSIX *Lower retainer:* HAWLEY ESSIX

# Appendix C
## Data Collection Sheet

**Orthodontic White Spot Lesion Varnish Study**
Data Collection Sheet

*Note: Do NOT include any patient identifying information*

Subject ID#: _____ Operator Name: _____

Date: _____ Study Visit _____
*(1, 2, 3, 4)*

Did they brush their teeth? Yes No

Did they fast (food/drink)? Both Food only Drink only No

| Tooth number | DiagnoDent Score | | |
|---|---|---|---|
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 9 | | | |
| 10 | | | |
| 11 | | | |
| 12 | | | |
| 13 | | | |
| 14 | | | |
| 15 | | | |

| Tooth number | DiagnoDent Score | | |
|---|---|---|---|
| 18 | | | |
| 19 | | | |
| 20 | | | |
| 21 | | | |
| 22 | | | |
| 23 | | | |
| 24 | | | |
| 25 | | | |
| 26 | | | |
| 27 | | | |
| 28 | | | |
| 29 | | | |
| 30 | | | |
| 31 | | | |

Time plaque collected: _____ Time plaque processed: _____

Time saliva collected: _____ Time saliva processed: _____

Label collection tubes:
[subject ID][X=maxillary, D=mandibular][visit][R=right, L=left]
Saliva: [subject ID][S][visit]

**SALIVA MEASURES** *(circle one for each)*

1) **Resting Flow Rate**: dry lower lip, time how long until droplets form

**LOW** (>60sec) **Normal** (30-60sec) **High** (<30sec)

2) **Salivary Consistency**: assess resting saliva in mouth

**High Viscosity** (Sticky/Frothy) **Increased Viscosity** (Frothy/Bubbly) **Normal Viscosity** (Watery/clear)

3) **pH (Resting Saliva)**: Spit pooled saliva into small cup, insert one end of pH strip for 10sec, save other end

**RECORD pH**: _____

**Highly Acidic** (5.0-5.8) **Moderately Acidic** (6.0-6.6) **Healthy Saliva** (6.8-7.8)

4) **Quantity of Stimulated Saliva**: chew wax 30sec, spit into STERILE TUBE, continue spitting until either >5mL collected or 5min pass, then spit small amount extra in second small cup

**mL collected (if <5mL)**: _____

**Very Low** (<3.5mL) **Low** (3.5-5.0mL) **Normal** (>5.0mL)

5) **pH (Stimulated Saliva)**: insert other end pH strip 10sec into cup

**RECORD pH**: _____

**Highly Acidic** **Moderately Acidic** **Healthy Saliva**

6) **Buffering Capacity**: use pipette to place 3 drops stimulated saliva onto strip, turn strip on side to drain excess onto gauze, wait 2 minutes then score

**RECORDED SCORE**: _____

**Very Low** (0-5) **Low** (6-9) **Normal** (10-12)


Green = 4 points
Green/Blue = 3 points
Blue = 2 points
Blue/Red = 1 point
Red = 0 points

## Appendix D

University of California, Los Angeles

**ORAL HYGIENE STUDY INSTRUCTIONS**

**Investigating the ability of light-cured fluoride varnish to remineralize post-orthodontic white spot lesions and alter the oral microbiome**

**INTRODUCTION**

You have enrolled in a study being conducted by Joseph Mullen, DDS, Renate Lux, PhD, Nini Tran, DDS, PhD, and associates from the School of Dentistry at the University of California, Los Angeles. As a part of your participation in this study, we ask that you comply with the following oral hygiene instructions, which are consistent with the standard of care for oral hygiene and for patients receiving treatment with fluoride varnish:

Instructions for today:

- Do not brush or floss your teeth in the next 4 hours
- Eat only soft food for the next 4 hours
- Do not consume hot drink or alcohol (including mouth rinses) for at least 4 hours
- Brush your teeth and floss before going to bed this evening

Instructions for the duration of the study:

- Brush your teeth twice each day (morning and night) using a soft-bristled manual toothbrush and a pea-sized amount of over-the-counter sodium fluoride toothpaste.
- Floss your teeth twice each day (morning and night).
- Refrain from using supplemental fluoride or antibiotic therapies (e.g. fluoride mouthrinse, antibiotic mouthrinse) for the duration of your involvement in the study, unless they are prescribed by your dentist or physician. If you are prescribed any antibiotic therapy (systemic or topical) by your dentist or physician, follow their instructions and inform Dr. Joseph Mullen by calling him at (916) 770-9035.
- At today's visit you received a professional dental cleaning. Please refrain from receiving additional professional cleanings for the next six months, since you will receive another professional cleaning at your final study visit. This is consistent with the standard of care, which is to receive a cleaning every six months.

If you have any questions, comments or concerns about the research, you can talk to the one of the researchers. Please contact Joseph Mullen, DDS, at (916) 770-9035, or the faculty sponsor, Renate Lux, PhD, at (310) 206-5660.

**UCLA Office of the Human Research Protection Program (OHRPP):**

If you have questions about your rights as a research subject, or you have concerns or suggestions and you want to talk to someone other than the researchers, you may contact the UCLA OHRPP by phone: (310) 206-2040; by email: participants@research.ucla.edu or by mail: Box 951406, Los Angeles, CA 90095-1406.

## APPENDIX E

## Budget and Justification

| Category | Amount | Description/Justification |
|---|---|---|
| Lab Test Fees | $9,600 | Microbiome Analysis - 16S sequencing for 1 sample per patient per timepoint (60 subjects * 4 visits * 1 sample/visit * $40/sample = $9600) |
| Patient Costs | $6,120 | Study participant reimbursement for 60 subjects (parking for 4 study visits at $13 each + $50 for study completion = $102 * 60 subjects = $6120) |
| Product Costs | $2,500 | 10 tubes of 3M™ Vanish™ XT Extended Contact Varnish * $250/tube = $2,500<br><br>0.7ml per application * 40 subjects assigned to XT varnish * 3 applications per subject (baseline, 1m, 3m) = 84ml (8.5 tubes), plus 1.5 tubes to account for attrition of subjects who withdraw from study early. |
| Bio statistical/ Analytical Assistance | $1,960 | Biostatistician fees ($98/hour for UCLA-affiliated investigators * 20 hours = $1960) |
| Journal Fees | $1,000 | Journal Fees for publication in Journal of Dental Research |
| Study Supplies | $400 | 240 prophy angles w/ paste (to perform four prophylaxis treatments on each of the 60 subjects) |
| Study Supplies | $400 | 2 toothbrushes and 2 tubes of toothpaste for each of the 60 subjects |
| Lab Equipment | $300 | Lab Supplies (1 case of 1.5mL screw-top plaque collection tubes and 1 case of plates) |
| Product Costs | $100 | Traditional 5% NaF Varnish (to be applied in 40 subjects, Groups 1 and 3) |
| **TOTAL PROPOSED COST** | **$22,380** | |

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