PROTOCOL NUMBER: CHLA-18-00389/UP-19-00405

TITLE: Treatment of Interproximal Carious Lesions on Primary Molar Teeth with SDF and Super Floss Application versus SDF without Super Floss versus Fluoride varnish alone: a pilot Phase 3 Randomized Controlled Trial

STUDY PHASE: III

STUDY ARMS:
1) 38% Silver Diamine Fluoride + Superfloss + Fluoride Varnish
2) 38% Silver Diamine Fluoride + Fluoride Varnish
3) Fluoride Varnish

IND OR IDE #: N/A

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AMENDMENTS/REVISIONS: N/A

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1.0 BACKGROUND AND HYPOTHESES

1.1 The vast majority of caries observed in children ages 5 to 10 are on interproximal surfaces[1,2]. Carious lesions on these surfaces are challenging to control and arrest due to difficulty reaching the contact area (in between teeth), limited salivary access, and poor flossing compliance in children and adolescents when flossing themselves[3]. Currently, for initial non-cavitated, demineralized lesions in these high caries risk patients, the standard of care has been to: reinforce oral hygiene instruction, advise in reducing the frequency of fermentable carbohydrate consumption, observe the incipient lesion at recall visits via bitewing radiographs every 6 months, and promote remineralization of the carious lesion with in-office application of topical fluoride (i.e. 5% sodium fluoride varnish) over all teeth[4,5]. Depending on the patient and the circumstances of their oral health condition, the practitioner may consider treating these lesions with restorations but a conservative, preventive approach is usually the initial goal of treatment.

Silver diamine fluoride (SDF) has been used for many years internationally, but in August 2014, SDF was cleared by the FDA as a medical device to treat dental hypersensitivity.[6] More recently, the FDA granted SDF "Breakthrough Therapy Designation" on October 30, 2016 for caries arrest in children and adults in the United States under the product: Advantage Arrest Silver Diamine Fluoride 38% by Elevate Oral Care.[6] The current formulation of 38% SDF is 25% silver for its antimicrobial effects, 5% fluoride for remineralization, and 8% ammonia to keep the silver and fluoride in solution.[6,7] It has been touted as a very safe[6,7] and possible noninvasive alternative and/or adjunct to topical fluoride treatment or restorative treatment.[6] SDF has been shown to remineralize demineralized enamel or dentin, inhibit collagenases to protect dentin collagen from destruction, and have bactericidal properties to cariogenic bacteria including streptococcus mutans.[7,8] Additionally, after the silver ions have killed the bacteria, they may be re-activated to do so again against subsequent or neighboring bacteria in a "zombie effect" to prevent incidence of new caries as explained by Horst et al.[7] The silver and fluoride ions can penetrate up to 25 microns into enamel[9] and 50-200 microns in dentin[10], and promote remineralization while decreasing the depth of lesion.[11] Repeat applications, with a biannual maintenance regimen, have shown to arrest caries over time[6].

Most of the existing protocols for SDF application include direct microbrush topical application to the exposed carious lesion. However, with interproximal caries, microbrush application is difficult due to the unexposed broad contact area. The decision to treat initial interproximal lesions in children can be difficult when weighing patient behavior, extent of treatment and prognosis considerations. While there are other methods to treat interproximal caries such as resin infiltration, Mattos-Silveira et al. reports that there was a significant difference in children's discomfort when comparing resin infiltration to flossing or SDF application to initial interproximal caries lesions.[12] Currently, there is another study by Mattos-Silveira et al. in progress in Brazil that looks at the application of SDF in arresting interproximal carious lesions.[13] This study uses a lower concentration (30%) than the current product used in the United States. Based on another study (Mei, ML et.al.) the 38% concentration of SDF appears to have a greater inhibition of metalloproteinases when compared to the 30% SDF and potentially this could lead to a greater arresting effect[14].

Elevate Oral Care[15] and others[16] have suggested applying SDF to interproximal caries with the use of a woven or spongy floss, such as Oral-B Super Floss®. Like typical floss, Super Floss is made of nylon, but the non-waxed fibers have an interlocking network which gives it its spongy texture. Its sponginess is presumed to be more absorbent, allowing for easier delivery and application of SDF to interproximal carious lesions due to limited access with a microbrush. In 1981, Tsutsumi showed a significant reduction in the progress of existing caries and significant reduction in incidence of new caries in human primary molars when comparing flossing with plain unwaxed floss to SDF applied with unwaxed floss[17]. Therefore, Super Floss should be considered as a method for SDF delivery to interproximal lesions, but there is currently no information as to the efficacy of this treatment modality. **The purpose of this study is to investigate whether 1) SDF application using Super Floss can halt the progression/arrest and/or reverse/remineralize the initial interproximal caries and 2) whether SDF applied with Super Floss is more effective in arresting or remineralizing incipient lesion in comparison to:**

a) **SDF applied without Super Floss or**
b) **Fluoride varnish applied alone**

1.2 We hypothesize that:

1.21 SDF applied with Super Floss can reliably arrest and/or remineralize initial interproximal lesions.

1.22 SDF applied with Super Floss will be more effective than SDF applied without Super Floss or fluoride varnish applied alone in arresting initial interproximal caries.

2.0 OBJECTIVES AND PURPOSE

2.1 The primary aim of this prospective study is to test if 38% SDF application using Super Floss can reliably arrest caries in superficial interproximal lesions as determined by comparing time lapse bitewing radiographs.

2.2 The secondary aim is to test if SDF applied with Super Floss will be more effective than SDF applied without Super Floss or fluoride varnish applied alone in arresting initial interproximal caries also as determined by comparing time lapse bitewing radiographs.

3.0 STUDY DESIGN

3.1 Caries criteria is defined into 5 groups based on the zone of radiolucency using the International Caries Classification and Management System (ICCMS)[18]. This classification will be used by the present study to describe whether carious lesions have progressed based on comparing radiographs taken over time[19] and allow for statistical analysis. The positioning of these bitewing radiographs must be consistent in showing the contact area and preventing overlap in the proximal teeth in question. ICCMS Classification is described as follows (Appendix I):

i. 0: No radiolucency/no caries – not included in study

ii. RA: Initial Stages – These are the target interproximal lesions for the present study

- 1: Radiolucency in the outer half of enamel,
- 2-Dentin-enamel Junction (DEJ): lesions extending within the inner half of enamel but not to DEJ,
- 2+DEJ: lesions extending within the inner half of enamel including the DEJ,
- 3: lesions extending to the outer one-third of dentin past the DEJ, and

iii. RB: Moderate Stages and beyond – These lesions will not included in present study because it is currently the standard of care to provide appropriate dental restorative treatment to repair the caries.

3.2 Each child with interproximal lesions on deciduous molar teeth meeting eligibility criteria RA Category 1, 2, or 3 will be invited to participate. Those who agree to participate will be randomly allocated to one of three treatments: Fluoride varnish (Control) application alone versus SDF application without Super Floss (Control) versus SDF application with Super Floss (Intervention). Daily flossing and oral hygiene instruction will be taught to patients of all study groups. Inclusion, Exclusion, and Withdrawal criteria are below in Section 5.

3.3 Follow-Up: Recall examinations and treatment reapplication will be done at 3 months, 6 months, and 12 months after the initial visit in accordance with current American Academy of Pediatric Dentistry (AAPD) guidelines for high caries risks patients[5]. Bitewing radiographs will be taken at 6 months and 12 months. This is in accordance with the "Prescribing Dental Radiographs…" guidelines as approved by the AAPD[20], which state that bitewing radiographs in high risk children with dental caries are recommended every 6-12 months.

3.4 Providers: Residents and Attendings at the Ostrow School of Dentistry at USC, Pediatric Residency Program. All residents and attendings participating in this study will be trained and standardized on how to identify lesions of interest on radiographs, obtain consent, and apply the medications.

3.5 Tracking of study participants: We will count the number of participants who:

1. Those who are asked to participate
2. Those who consent
3. Those who drop out before randomization/after randomization
4. Those who withdraw by their own choice for various reasons
5. Those who are lost to follow up

Note: We will only collect and store clinical data for those participants who continue throughout the duration of the project. If a participant decides to drop out of the study at any time, then we will no longer collect data from the medical record during their ongoing clinical care. We will also discard any previous data we have collected on the participants that drop out.

4.0 DRUG/DEVICE INFORMATION

4.1 Drug/Device Name

4.1.1 Advantage Arrest 38% Silver Diamine Fluoride

4.1.2 Voco ProFluorid Varnish

4.1.3 Oral-B Super Floss

4.2 Manufacturer:

4.2.1 Elevate Oral Care (346 Pike Rd Suite 5, West Palm Beach, FL 33411)

4.2.2 VOCO GmbH (Anton-Flettner-Str. 1-3 D-27472 Cuxhaven, Germany)

4.2.3 The Procter & Gamble Company (8700 Mason-Montgomery Road Mason, OH 45040)

4.3 Chemical makeup, based on manufacturer's MSDS:

4.3.1 SDF: Ag(NH3)2F. The silver is antibacterial, the fluoride aids in remineralization of the tooth, and the ammonia suspends the two in solution. One drop contains 25 μL and 9.5mg of SDF.

CAS #	Ingredient	Percentage (%)
7440-22-4	Silver (Ag)	24 - 27
1336-21-6	Ammonia (NH3)	7.5 - 11
16984-48-8	Fluoride (F)	5 - 6
3844-45-9	FD&C Blue #1	<1
7732-18-5	Deionized Water	≤62.5

4.3.2 Fluoride varnish: 5% NaF

CAS #	Ingredient	Percentage (%)
64-17-5	Ethanol	10 - 25
7681-49-4	Sodium Fluoride	2.5 - 5

4.3.3 Super Floss: No hazardous ingredients as defined by OSHA and/or WHMIS.

4.4 Dose/Formulation:

4.4.1 SDF: One drop contains 25 μL and 9.5mg of SDF, which can be applied to ~5 lesions.

4.4.2 Fluoride Varnish: 1 mL suspension contains 50 mg NaF, equivalent to 22.6 mg fluoride ion. 1 unit package = 0.4 mL, which is 20 mg NaF. Approximately 0.25mL is used for patients with primary dentition, and 0.40mL is used for adolescent patients or permanent dentition.

4.4.3 Super Floss: N/A

4.5 Toxicity or adverse effects,:

4.5.1 SDF: SDF is corrosive to metal and glass, and can be an eye, skin, mucosal, respiratory, and gastrointestinal irritant. Repeated overexposures may cause mottled teeth or fluorosis on teeth/bones (not in the dosage used in this study).

In the UCSF Protocol by Horst et al. 2016, they found SDF to be extremely safe, citing not one adverse event in over 80 years of use in Japan and mild temporary side effects found in 3 of 1,493 subjects in 9 clinical trials. The lethal dose (LD50) of SDF, determined by female and male mouse and rat studies, was found to be: 520 mg/kg by oral administration, and 380 mg/kg by subcutaneous administration. Considering a small child weighing 10kg with caries, the dose would be 0.95mg/kg (One drop of SDF contains 9.5mg and one drop is usually adequate to treat one patient). For that 10kg child, the relative safety margin of using an entire drop would be: 380 mg/kg LD50 / 0.95 mg/kg dose = 400-fold safety margin.

4.5.2 Fluoride varnish:

- Sodium Fluoride: Oral rat LD50: 52 mg/kg
- Ethanol: Oral rat LD50: 7060 mg/kg

Fluoride varnish can be an irritant with direct eye contact and prolonged skin contact. If ingested, it may cause salivation, nausea, vomiting, abdominal pain, headaches, or dizziness. Repeated overexposures may cause fluorosis.

For a similar calculation above for a 10kg child, the relative safety margin of using an entire 0.4 mL unit (one package) of fluoride varnish would be: 52 mg/kg LD50 / 2.0 mg/kg = 26-fold safety margin.

4.5.3 Super Floss: There are no toxic or adverse effects listed, and there are no hazards when used as directed.

4.6 Non-medical Side Effects:

4.6.1 SDF: SDF stains carious lesions. It also can have a bitter or metallic taste. It can also stain or darken skin with a ‘temporary tattoo’ which resolves in 2-14 days after normal skin exfoliation. SDF can also stain hard surfaces like table tops as well as clothing.[7]

4.6.2 Fluoride Varnish: N/A

4.6.3 Super Floss: N/A

4.7 Risk:

4.7.1 SDF: Minimal

4.7.2 Fluoride Varnish: Minimal

4.7.3 Super Floss: N/A

4.8 Storage:

4.8.1 SDF: Store in a cool dry place in provided closed container.

4.8.2 Fluoride Varnish: Store in a cool, dry place in original sealed package away from direct sunlight.

4.8.3 Super Floss: N/A

4.9 Stability, based on manufacturer's MSDS:

4.9.1 SDF is a light sensitive liquid. Prolonged exposure to light or evaporation will cause precipitation.

4.9.2 Fluoride varnish is a stable product.

4.9.3 Super Floss: N/A

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria:

5.1.1 ASA I and ASA II children, aged 3-12, with interproximal decay on deciduous molars identified by radiographs. These teeth will not have previous restorations.

5.1.2 Radiographic decay within enamel or extending to the dentin-enamel junction based on International Caries Classification and Management System (ICCMS)[18] Categories 1 through 3 – see above. Categories 1, 2 and 3 signify the extent of the "Initial Stages" of an interproximal carious lesion with radiolucency in the outer 1/2 of enamel, inner 1/2 of enamel, and outer 1/3 of dentin respectively.

5.1.3 Interproximal lesions present without existing restorations, recurrent decay, or adjacent teeth with existing restorations.

5.1.4 Behavior of the children should be within a Frankl 3 or 4 category[21], indicating a "Positive" and "Definitely Positive" behavior rating, which would allow for safe and controlled execution of the proposed protocol.

5.2 Exclusion Criteria:

5.2.1 Children who are not ASA I or ASA II.

5.2.2 Children who are allergic to or intolerant of SDF

5.2.3 Children who have known sensitivity to silver or heavy metal-ions, or have abnormal skin sensitization.

5.2.4 Children who have ulcerative gingivitis or stomatitis.

5.2.5 Carious interproximal lesions on primary molars in ICCMS Category 4, 5, or 6[18], which signify the extent of the carious lesion radiographically reaching the middle 1/3 of dentin, inner 1/3 of dentin, and into the pulp respectively.

5.2.6 Behavior of a child within the Frankl 1 or 2 category[21], indicating a "Definitely Negative" and "Negative" behavior, which may compromise safe application of SDF.

5.3 Withdrawal Criteria

5.3.1 Patients can withdrawal voluntarily at any time.

5.3.2 Any tooth which has progression of caries beyond ICCMS Category 3 will be withdrawn from the study. These teeth will be treated with appropriate restorative dental treatment according to the standard of care.

5.3.3 Any qualifying tooth that subsequently requires a restoration or extraction due to trauma or exfoliates prematurely will be withdrawn from the study.

5.3.4 Any study tooth whose adjacent tooth exfoliates before the end of this study and exposes the interproximal contact will be withdrawn from the study.

6.0 STRATIFICATION/DESCRIPTIVE FACTORS/RANDOMIZATION SCHEME

6.1 We have not defined any *a priori* stratification factors.

6.2 Descriptive factors: Age, Gender, Race,, Date of Initial exam, Date of Recall exam/Follow-up, , Number of posterior caries on primary teeth that qualify for this present study, DMFS, Which interproximal surface (mesial or distal), Which primary molar, and Radiographic depth of Lesion based on ICCMS Categories (see Table 1 in Appendix).

6.3 Once participants have met eligibility criteria and have undergone the informed consent process (parental permission and assent, if applicable), they will be randomized to one of the three treatments 1:1:1 using a cluster-randomized design. That is, participants, not teeth, will be randomized, though multiple teeth per participant may be treated. Using the uniform distribution, the statistician will generate a random number sequence. We will use blocked randomization (blocks of six) to better ensure an even distribution across groups. Once group

assignments have been made, the statistician will prepare numbered, opaque envelopes containing the random study arm assignments, which the investigators will use sequentially, as study participants accrue (i.e., the study identification number will match the number on the envelope).

7.0 STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN

7.1 Treatment protocol after patients are randomized into one of three treatment groups:

7.1.1 Fluoride varnish application *(Control - Group 1)*: Bulk saliva removed with saliva ejector. All teeth wiped dry with cotton gauze. One unit dose (0.4 ml) of fluoride varnish will be applied to all dentition then interproximal surfaces of all teeth will be flossed.

i. Professionally applied fluoride varnish has been shown to inhibit demineralization and enhance remineralization in early enamel caries.[22] Current Caries Risk Assessment (CRA) protocol indicates fluoride varnish applications at 3-6 months intervals for high caries risk patients.[4,5]

7.1.2 SDF application with Super Floss *(Intervention - Group 2)*: Petroleum jelly will be applied to lips and face to protect the skin from staining. Teeth will then be dried with an air-water syringe or wiped with gauze. Super Floss will be flossed through contact and left in place. The practitioner will apply SDF to the superfloss on the buccal or lingual aspect and then over the occlusal embrasure. Provider can visualize SDF being absorbed to the other end of the superfloss due to the blue tint of the SDF. Super floss will remain between the teeth, allowing the SDF to absorb, for one minute. Super floss is then removed and excess SDF removed with gauze or cotton roll. Fluoride varnish is applied over all teeth afterwards per high risk CRA protocol and current standard of care[4,5].

7.1.3 SDF application without Super Floss (*Intervention Control - Group 3*): Petroleum jelly will be applied to lips and face to protect the skin from staining. Teeth will then be dried with an air-water syringe or wiped with gauze, and teeth with interproximal caries isolated. SDF will be applied with microbrush over the occlusal embrasure of the interproximal surfaces of the target teeth. Excess SDF removed with gauze or

cotton roll. Fluoride varnish is applied over all teeth afterwards per high risk CRA protocol and current standard of care[4,5].

i. This control (secondary aim) is to determine if indirect/nearby SDF application can also be absorbed by the lesion and arrest caries.

7.2 Drug studies:

AGENT	DOSE	ROUTE	DAY	ReRx INTERVAL	NOTES
38% SDF	9.5mg/drop 1 drop = 25 µL	Topical	1	3 months, 6 months, 12 months	
5% Sodium fluoride varnish (2.26% [22,600ppm] fluoride)	22.6mg/mL 1 unit = 0.4mL	Topical	1	3 months, 6 months, 12 months	Fluoride varnish will be applied to each group.

7.3 Criteria for removal from treatment

7.3.1 If a carious lesion is arrested or remineralizes in any treatment group, this is considered a stable lesion. The patient will continue on with that treatment modality (ie. reapplication at 3 months, 6 months, and 12 months) until the tooth exfoliates or other comprehensive dental treatment is required. That lesion will continue to be monitored for any change in progress.

7.3.2 Treatment will be discontinued if a patient experiences any persistent eye, skin, oral mucosa, respiratory, and gastrointestinal irritation that are considered Grade 2 and above adverse events.

7.3.3 Treatment will be discontinued if a patient experiences any allergic reactions or sensitivities to SDF.

7.3.4 Patients will discontinue treatment if or when a carious lesion progresses to ICCMS category 4 or beyond. The patient will be notified and scheduled for routine operative dental procedures (ie. restorations) to treat the carious lesion.

7.3.5 Treatment will be discontinued if patient becomes intolerant to SDF or their behavior does not allow for safe and proper application of protocol.

7.3.6 A patient may always be removed from treatment whenever he/she wishes.

8.0 ASSESSMENT OF EFFICACY AND SAFETY

8.1 Assessment of Efficacy will be based on size/depth of carious lesion comparisons between initial (Day 1) dental radiographs and subsequent time lapse radiographs taken at 6 and 12 month visits. This frequency (every 6 months) of radiographs is the current standard of care for patients with high caries risk[4,5], including those with lesions being monitored.

8.2 Side effects/Toxicities to be monitored.

8.2.1 Side effects/toxicities that the patient is to be asked about or examined at each evaluation while on treatment include: Eye, skin, mucosa, respiratory, and gastrointestinal irritation.

8.2.2 No long-term toxicities to be monitored after completion of therapy.

8.3 No dosage change based on Grade 1 Adverse Events (AE)/toxicity. If the patient develops temporary Grade 1 AEs as listed above and parents would like to continue treatment, the same regimen will resume. Serious adverse events have not been documented in previous studies and are not expected in our study as well.

8.4 Adverse Event Reporting:

8.4.1 We do not foresee any morbidity or mortality associated with any of the proposed treatment modalities. Participants will be advised that if any adverse events do occur such as irritation to the eyes, skin, mucosa, respiration, and gastrointestinal tract, then they can call the CHLA Pediatric Dental Clinic during business hours or there will be a pediatric dental resident on-call after hours. We will also report the event to the IRB within 48 hours.

8.4.2 Places for submitting reports: Adverse Event Reports will be submitted only to the IRB as there are no sponsors of the proposed research.

9.0 CLINICAL EVALUATIONS AND STUDY CALENDAR

Parameter	Treatment Day 1	3 Month Follow-Up	**Recall Visit (6 months and 12 months)**
Comprehensive Medical and Dental History	X		
Updated Medical and Dental History		X	X
Oral Examination	X	X	X
Dental Radiographs	X		X
Assessment and Treatment Planning	X		X
Informed Consent/Assent	X		

Dental Prophy (cleaning)		X		X
SDF application (with or without use of Super Floss)		X[1]	X[1]	X[1]
Fluoride varnish application*		X	X	X
Parameter				
Comprehensive Medical and Dental History	X			
Updated Medical and Dental History			X	X
Oral Examination	X		X	X
Dental Radiographs	X			X
Assessment and Treatment Planning	X			X
Informed Consent/Assent	X			
Dental Prophy (cleaning)	X			X
SDF application (with or without use of Super Floss)	X[1]		X[1]	X[1]
Fluoride varnish application*	X		X	X

1 SDF application with or without the use of Super Floss is determined by which treatment group the patient is in.

* All treatment groups will get fluoride varnish application.

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

The primary outcome is arrest of interproximal caries on a primary molar, as measured by lack of change in ICCMS classification indicating caries progression on radiographic examination, (higher score indicates progression). The outcome will be evaluated at three time points: baseline, 6 months and 12 months. As the treatments cannot be made to appear similar to one another, neither the treating dentist or the patient/parent(s) will be blinded to treatment status. However, evaluation of the outcome will made by comparing time lapse (ie. comparing intial xrays vs. at 6 months vs. at 1 year) bitewing radiographs and visualizing if the carious lesion has remineralized, arrested, or progressed

11.0 SPECIAL INSTRUCTIONS:

11.1 Not applicable

12.0 DATA COLLECTION AND MONITORING

As the study is small and preliminary in nature, there are is no formal monitoring committee. However, the statistician will monitor the progress of the study to assess accrual rate and data quality throughout the progress of the study. Once collected, the data will be cleaned,

beginning with range checks, evaluation of outliers, crosstabs between variables of interest and visual examinations.

13.0 STATISTICAL CONSIDERATIONS

13.1 The proposed pilot Phase III randomized controlled clinical trial aims to determine which of the following treatments is superior in arresting dental caries among patients presenting with interproximal caries scoring RA1 – RA3 on the International Caries Classification and Management System (ICCMS): (1) Fluoride varnish (FV, current standard of care, control), (2) Super floss + SDF + FV, (3) SDF + FV. We hypothesize that group 2 will have the best outcomes, as measured by no change or reversal in ICCMS classification indicating no caries progression or remineralization on radiographic examination, (higher score indicates progression) followed by group 3. As we could find no data on ICCMS-defined progression or remineralization rates for either Super Floss or fluoride, we were unable to calculate power. Instead, we provide estimates of recruitment based on feasibility. We expect to accrue approximately 20 participants, with 2 qualifying teeth per participant, in each group.

10% loss to follow up has increased dramatically to ~48%. Given this unexpected setback, we now need to continue recruiting participants to reach sufficient power. We originally planned to enroll 39 subjects in the study, but would now like to add 21 new participants for a total of 60 participants.

Descriptive statistics will be calculated for all demographic and clinical characteristics to assess the degree to which randomization was successful using means and standard deviations or medians and interquartile ranges for continuous variables and counts and percentages for categorical variables.

The primary analysis will be by intent-to-treat. The primary outcome is the change in ICCMS category, as assessed by radiographic evidence, from baseline to 12 months, with a planned interim analysis to evaluate the endpoint at 6 months for early indications of efficacy. We will calculate the median difference at each time point and use an individual-level model with an adjustment to the standard error to account for the clustering. First we will evaluate the association between treatment groups and the change in ICCMS score from baseline at 6 and 12 months using a cluster-adjusted chi-squared test. We will further assess

the relationship between these two variables using generalized estimating equations, specifying a Poisson (or negative binomial, if overdispersed) family and a log link. We will also specify an exchangeable correlation matrix and a robust standard error.

Since the study is randomized, we do not anticipate needing to adjust for any confounders. However, should we find any differences in demographic or clinical variables at baseline, we will investigate them as potential confounders, which will be defined as those variables which alter the odds ratio by >15% when included in the model.

For those with missing data on outcomes or other covariates to be included in the model, we will consider multiple imputation methods if the amount of missing data is <10%. To assess the impact of missing data, we will compare those with missing data to those not missing data with respect to demographic and clinical variables to assess comparability. Should imputation be utilized, a sensitivity analysis will be conducted excluding those for whom data was imputed to assess the impact of imputation.

Model fit will be evaluated by inspection of residuals and influential points. As we are conducting an interim analysis, we will use a two-sided Bonferroni-corrected p-value of a=.025 for statistical significance. Finally, to assess the degree to which the two raters agree with respect to classifying the outcome of the x-rays, we will calculate and report Kappa. All statistical analyses will be done using Stata 15.0 (College Station, TX).

Power Analysis

We calculated power using a cluster-randomized design. We were unable to locate any previous studies examining the impact of these three treatments (or any two) using the same classification system as our outcome. However, we were able to obtain an estimate of 0.0 to 0.25 for the interclass correlation coefficient (ICC) (Honkala et al., 2011). Assuming the mean ICC, and assuming a clinically significant mean difference of 0.2, a standard deviation of 0.2, a covariance of cluster sizes of 0.65, two caries on average per child, and a two-sided adjusted Type I error rate of 0.025 to account for the three time points at which the data will be analyzed, we will have 80% power with 11-12 participants per group, for a total of 33-36 participants (66-72 caries). Since we anticipate significant loss-to-follow-up over the course

of the study, we will inflate the sample size by ~48% and recruit a total of 54-60 participants (108-120 caries). Sample size was calculated using PASS Software v.14.0 (Kayesville, UT).

This study is powered to detect an effect in a population of children who are predominantly non-white. Therefore, the sample size as calculated is likely to result in a sample size with reasonable power to detect an effect near the size outlined above, if perhaps slightly larger.

14.0 REGISTRATION GUIDELINE

14.1 Participants will be randomized as described in Section 6.3. Randomization envelopes will be available to the investigators, who need only select the next one in the series to accomplish randomization. No stratification parameters have been specified.

14.2 The forms and records needed for registration: Informed Consent; Assentfor Younger Subjects ; and Tracking Form.

Note: At the time of registration, two copies of a signed and dated patient Informed Consent form with Bill of Rights must be available (a copy for patient's dental chart; a copy for the subject/parent; and the original for the PI's file).

15.0 BIOHAZARD CONTAINMENT

15.1 Not applicable

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice.

17.0 REFERENCES

1. Skeie MS, Raadal M, Strand GV, and Espelid I. Caries in primary teeth at 5 and 10 years of age: a longitudinal study. European Journal of Paediatric Dentistry 4:194-202. 2004.

2. Ferro R, Besostri A, and Olivieri A. Caries prevalence and tooth surface distribution in a group of 5-year-old Italian children. Eur Arch Paediatr Dent. 10(1):33-7. 2009.

3. Hujoel PP, Cunha-Cruz J, Banting DW, and Loesche WJ. Dental flossing and interproximal caries: a systematic review. J Dent Res 85(4):298-305. 2006.

4. Ramos-Gomez FJ, Crystal YO, Ng MW, Crall JJ, and Featherstone JD. Pediatric dental care: prevention and management protocols based on caries risk assessment. J Calif Dent Assoc. 2010 Oct;38(10):746-61.

5. Caries-risk Assessment and Management for Infants, Children, and Adolescents. Pediatric Dentistry, 2017:39(6), 197-204.

6. Horst JA. Silver Fluoride as a Treatment for Dental Caries. Adv Dent Res. 2018 Feb: 29(1):135-40.

7. Horst JA, Ellenikiotis H, UCSF Silver Caries Arrest Committee, and Milgrom PM. UCSF Protocol for Caries Arrest Using Silver Diamine Fluoride: Rationale, Indications, and Consent. J Calif Dent Assoc 2016:44(1):16-28.

8. Zhao IS, Gao SS, Hiraishi N, Burrow, MF, Duangthip D, Mei ML, Lo ECM, and Chu CH. Mechanisms of silver diamine fluoride on arresting caries: a literature review. Int Dent J. 2017.

9. Suzuki T, Nishida M, Sobue S, Moriwaki Y. Effects of diammine silver fluoride on tooth enamel. J Osaka Univ Dent Sch. 1974 Sep; 14():61-72.

10. Chu, C., & Lo, E. Microhardness of dentine in primary teeth after topical fluoride applications. Journal of Dentistry, 2008:36(6), 387-391.

11. Conteras V, Toro M, Elías-Boneta A, and Encarnación-Burgos A. Effectiveness of silver diamine fluoride in caries prevention and arrest: a systematic literature review. Gen Dent. 65(3): 22-29. 2017.

12. Mattos-Silveira J, Floriano I, Ferreira FR, Vigano MEF, Mendes FM, and Braga MM. Children's discomfort may vary among different treatments for initial approximal caries lesions: preliminary findings of a randomized controlled clinical trial. International Journal of Pediatric Dentistry 25(4): 300-304. July 2015.

13. Mattos-Silveira J, Floriano I, Ferreira FR, Vigano MEF, Frizzo MA, Reyes A, Novaes TF, Moriyama CM, Raggio DP, Imparato JCP, Mendes FM, and Braga MM. New proposal of silver diamine fluoride use in arresting approximal caries: study protocol for a randomized controlled trial. Department of Pediatric Dentistry, School of Dentistry, University of Sao Paulo (Brazil) Trials: 15:488. 2014.

14. Mei ML, Li QL, Chu CH, Yiu CK, and Lo EC. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. Dent Mater 28(8): 903-908. 2012.

15. "Advantage Arrest Silver Diamine Fluoride 38% Information - Dr. Jeanette MacLean Superfloss SDF Technique interproximal application" (Youtube video). Elevate Oral Care. Web. 18 July 2018.

16. Bendit J, and Young DA. Silver diamine fluoride: The newest tool in your caries management toolkit. Dent Acad of Continuing Educ. 2017 July: 1-7.

17. Tsutsumi, N. 1981. Studies on topical application of Ag (NH3)2F for the control of interproximal caries in human primary molars: 3. Clinical trial of Ag(NH3)2F on interproximal caries in human primary molars. Jpn J Pediatr Dent. 19(3):537–545.

18. Ismail A., Pitts N., Tellez M., Banerjee A., Deery C., and Authors of the ICCMS. The International Caries Classification and Management System (ICCMS™) An Example of a Caries Management Pathway. BMC Oral Health, 2015, Vol 15,Suppl 1, S9.

19. Feldens CA, Tovo MF, Kramer PF, Feldens EG, Ferreira SH, and Finkler M. An in vitro study of the correlation between clinical and radiographic examinations of proximal carious lesions in primary molars. J Clin Pediatr Dent. 27(2):143-7. 2003.

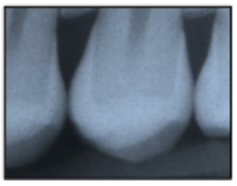
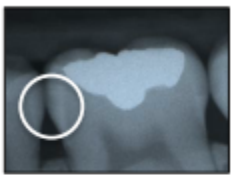
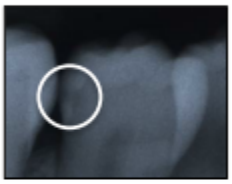
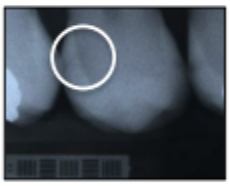
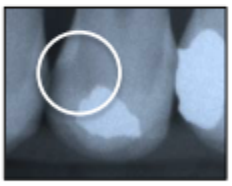
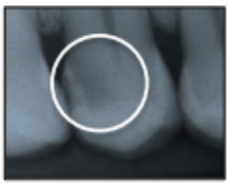
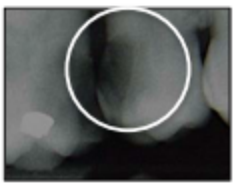
20. American Academy of Pediatric Dentistry. Prescribing Dental Radiographs for Infants, Children, Adolescents, and Individuals with Special Health Care Needs. Pediatr Dent 2017;39:205-7.

21. American Academy of Pediatric Dentistry. Guideline on Behavior Guidance for the Pediatric Dental Patient. Pediatr Dent 2016;37:180-93.

22. Gao S, Zhang S, Mei M, Lo E, and Chu C. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment - a systematic review. BMC Oral Health, 2016(16):12.

APPENDICES

Appendix I: Table of the ICCMS Radiographic Scoring System of Carious Lesions

ICCMS™ Caries Categories	ICDAS Radiographic scoring system			
	0	**No radiolucency**		No radiolucency
	RA: Initial stages	**RA 1**		Radiolucency in the outer ½ of the enamel
		RA 2		Radiolucency in the inner ½ of the enamel ± EDJ (enamel-dentin junction)
		RA 3		Radiolucency limited to the outer 1/3 of dentin
	RB: Moderate stages	**RB 4**		Radiolucency reaching the middle 1/3 of dentin
	RC: Extensive stages	**RC 5**		Radiolucency reaching the inner 1/3 of dentin, clinically cavitated
		RC 6		Radiolucency into the pulp, clinically cavitated

Red box encloses Categories 1-3, which are the target lesions for this study.