# Evaluation of Silver Diamine Fluoride in Reduction of Plaque and Salivary Oral Bacteria in Children with Early Childhood Caries (ECC)

BY

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CAPSTONE

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| | |
|---|---|
| AAPD | American Academy of Pediatric Dentistry |
| ASA | American Society of Anesthesiologists |
| ATP | Adenosine Tri-Phosphate |
| CAT | Caries risk assessment tool |
| DMFT | Decayed Missing and Filled Teeth |
| ECC | Early Childhood Caries |
| FDA | Food and Drug Administration |
| IRB | Institutional Review Board |
| MS | Mutans Streptococci |
| NHANES | National Health and Nutrition Examination Survey |
| OPRS | Office for the Protection of Research Subjects |
| PHI | Protected Health Information |
| PI | Principal Investigator |
| RLU | Relative Light Unit |
| SDF | Silver Diamine Fluoride |
| UIC | University of Illinois at Chicago |

## 1. INTRODUCTION

### 1.1 Background Information

Despite increasing use of dental care services and available preventative products, caries continues to be a significant challenge facing youth in the United States and is the leading chronic disease of childhood.[1] "Early childhood caries (ECC) is defined as the presence of one or more decayed, missing (due to caries), or filled tooth surfaces in the primary teeth of a child of 71 months of age or under."[2] This disease has become even more prevalent for minority youth such as Hispanic and non-Hispanic black populations.[3,4] Data collected by Crall et al in 2005 from the 2004 NHANEs study documented approximately 60 percent of children overall will experience caries in their primary teeth by age five.[5] A study in 2016 showed improvement in prevalence of dental caries in a certain age group; documenting only 18% of children 2-5 years old experiencing dental caries (Figure 1). In terms of demographics of children most affected, Figure 2 shows prevalence across different ethnicities ages 2-19 years: Hispanic 52.0%, Non-Hispanic Asian 42.6%, Non-Hispanic Black 44.3%, Non-Hispanic White 39.0%.[6]

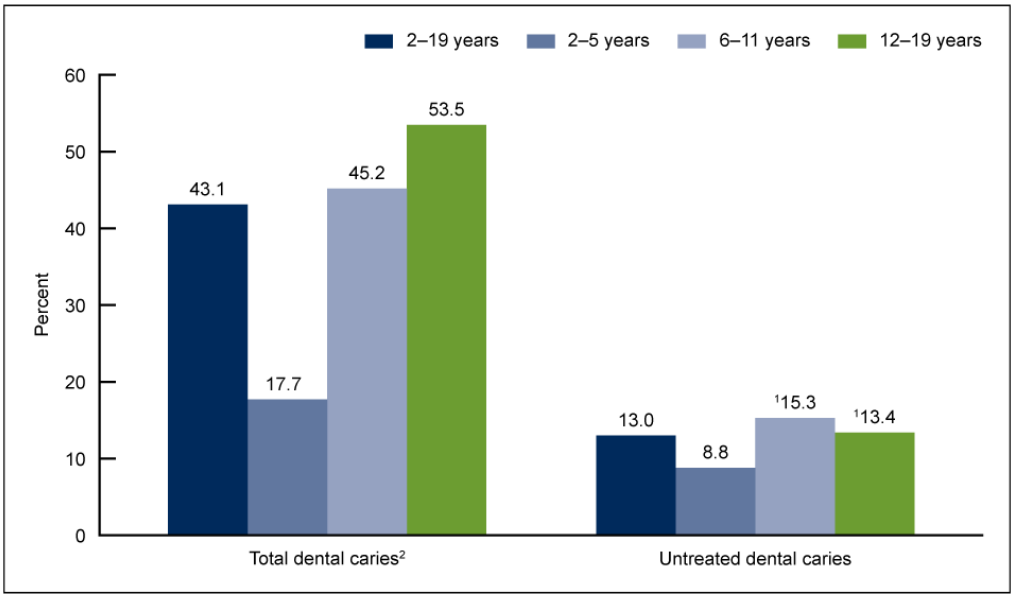


Figure 1. Prevalence of total dental caries and untreated dental caries in primary or permanent teeth among youth aged 2-19 years, by age: United States, 2015-2016. Source: NCHS, National Health and Nutrition Examination Survey, 2015-2016

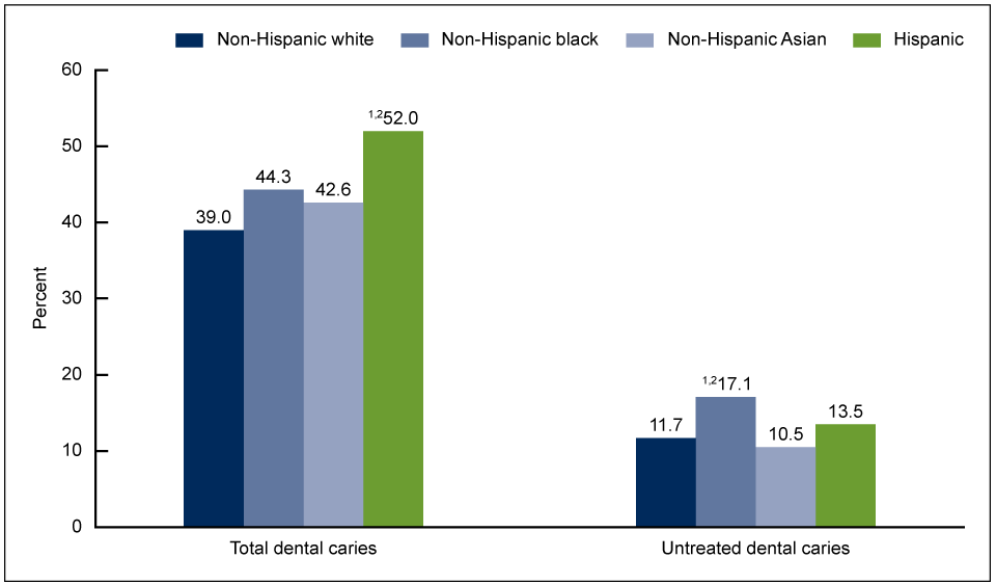


Figure 2. Prevalence of total dental caries and untreated dental caries in primary or permanent teeth among youth aged 2-19 years, by race and Hispanic origin: United States, 2015-2016. Source: NCHS, National Health and Nutrition Examination Survey, 2015-2016

The detrimental effects for young children as a result of this disease process include: compromised learning, communication, nutrition, and other activities essential for proper growth and development.[7,8] The disease process is complex and multifactorial but is mediated by protective factors (fluoride, salivary buffering capacity, and host immunity) and risk factors such as frequent carbohydrate exposure, poor oral hygiene, and biofilm formation.[9]

Traditionally, the treatment of dental caries has been focused on surgical management of repairing lesions and less centered on the disease process itself.[1,10-12] Recently, personalized healthcare and medical management of caries has been suggested to be a more effective prevention and treatment rather than treatment of the disease consequences(cavities).[1] Some strategies for employing this patient centered approach are: use of antimicrobials, re-mineralizing agents, salivary stimulation, and most importantly behavior modification.[11] SDF has also been recently suggested by the Illinois Department of Public Health to be used as an interim management of caries during the acute stages of COVID outbreak.[13]

## 1.2 **Microbiology of ECC**

The etiology of this disease is often simply defined by four major components: cariogenic bacteria, fermentable carbohydrates, susceptible teeth and host, as well as time for the process to develop.[1] Dental caries is a disease caused by accumulation of microbial biofilm on teeth surfaces but can have interactions within the oral cavity with

saliva fluids. Previously dental caries was determined to be explained by the Specific Plaque Hypothesis while the paradigm has shifted in recent years to an Ecological Plaque Hypothesis. The difference between these two mechanisms is primarily from being caused by a specific pathogen or select few to a view that there is a large microbiome with complex interactions that mediates the caries process. However, a great deal of research points to mutans streptococci (MS) species and Lactobacilli as having large contributions to the etiology of caries in the Ecological Plaque Hypothesis. Due to the complex interactions within the oral microbiome, it is extremely difficult to make conclusions and associations that hold true across all populations as to how bacteria within the oral cavity contribute to caries.[14,15] A common conclusion is that, when this microbiota shifts to high levels of specific pathogens, it will create dysbiosis and disease will manifest, in this case dental caries. The bacterial species most often implicated as major contributors to the caries process are primarily MS and lactobacillus species. These bacteria form colonies within plaque present on tooth surfaces, and will metabolize dietary nutrients to produce acidic byproducts that demineralize and damage the underlying tooth structure.[16] High levels of these bacterial species are associated with increased caries risk.[17,18] Research from Caufield *et al*. has also proposed further implications from these oral bacteria within the GI microbiota which indicates interactions from the tooth surface to the carrier, saliva.[19] For these reasons, this study was focused on the interaction with silver diamine fluoride (SDF) and these two bacterial species.

## 1.3 Silver Diamine Fluoride (SDF)

### 1.3.1 SDF Overview

Silver Diamine Fluoride has emerged as a new product that has become available for dentists in the United States after being approved by the Food and Drug Administration (FDA) as of April 2015 for desensitization. However, it has a frequent well-accepted use for caries management as an alternative therapy to prevent caries progression. It is composed of silver ions which act as an antimicrobial, fluoride for re-mineralization, and ammonia as a stabilizing agent.[12] Use of SDF is a simple, non-invasive method for treatment that has been shown to be effective in arresting active caries in primary teeth.[20] There are a variety of clinical uses for SDF, one of which being treatment for children at extreme caries risk such as those with early childhood caries (ECC) and severe early childhood caries (S-ECC) to prevent caries progression.[12]

### 1.3.2 SDF Effectiveness

Most studies have evaluated effectiveness of SDF by recording tooth staining and hardness following SDF treatment. In a recent systematic review including 8 studies using 38% SDF, the reported average proportion of arrested dentinal carious lesions has been found to be 81%.[21] *In vitro* and *In vivo* studies investigating effects of SDF on bacterial species within plaque and saliva samples have shown varying results. A recent study by Mitwalli et. al found no significant differences in microbial plaque samples following single time SDF application.[22] This finding is also consistent with results from studies completed by Milgrom and Horst where no significant differences were noted in plaque samples following SDF application.[11,23] Some evidence has revealed decreases in

bacterial concentrations within plaque and saliva samples while others have found no changes within composition of these samples.[11,24-26]

### 1.3.3 **SDF Mechanism of Action**

Silver ions within the SDF solution contribute to the antimicrobial effect by breaking membranes of bacteria, denaturing proteins, and inhibiting DNA replication. Ionic silver has also been shown to deactivate nearly all macromolecule within its environment which can contribute to the bactericidal role. The SDF solution can delay caries progression by forming a thin layer of silver-protein conjugate on the decayed tooth surface which increases resistance to acid dissolution and host enzymatic digestion. In addition, fluoride within the solution aids in this process by formation of hydroxyapatite, fluorapatite, silver chloride, and metallic silver. Fluoridated tooth surface has been extensively shown to be more resistant to acid degradation than normal tooth structure.[23] The treated lesions will increase in mineral density and hardness which will contribute to decrease in the lesion depth. SDF will also interact with the host physiology by inhibiting proteins that break down exposed dentin organic components such as matrix metalloproteinases, cathepsins, and bacterial collagenases. In-vitro studies have found that lesions treated with SDF are more resistant to subsequent biofilm formation and progression, presumed to be related to remnant ionic silver. When bacteria killed by silver ions are added to live bacteria, silver ions are re-activated and the dead bacteria will then have a transference effect where they will then contribute to the killing of the live bacteria. This phenomenon explains how silver deposited on lesions can have sustained antimicrobial effects.[12]

### 1.3.4 **SDF Ideal Application Frequency**

Previous studies exploring the topic of the ideal application frequency are limited and have shown mixed results. The 2017 guideline published by the AAPD following a systematic review found that one-time SDF application arrest rates ranged from 47-90 percent. However, the effectiveness of lesion arrest decreases over time, which indicates successive application is necessary. Half of treated lesions had reverted back to active lesions at 24 months if no further intervention occurred.[27] Studies have shown unanimously that annual application of SDF is more effective at caries arrest when compared to 5% sodium fluoride varnish.[21] Bi-annual SDF application has been shown to increase the caries arrest rate compared with annual application. Additionally, three time per year application showed even higher arrest rates.[21,28,29] Individuals with higher plaque indices and lesions covered in plaque displayed lower rates of arrest, therefore addressing other risk factors such as plaque presence may increase the rate of successful treatment outcomes.[28] The current standard recommended by the AAPD is 6 month application frequency but this is based on limited quantity and quality of evidence. Most studies have determined application frequency based on clinical caries arrest but not evaluating more objective measures such as microbiologic effects. Further research has been proposed to determine the ideal application frequency when considering all factors involved.

## 1.4 **CariScreen**

The current AAPD caries risk assessment tool advocates use of microbiological testing to be used for pediatric dental patients. Studies have shown that patients with active

caries have much higher concentrations of MS and lactobacilli in their saliva and plaque than do disease free individuals. Longitudinal studies have shown there are increases in MS and lactobacilli over time with the progression of caries.[18] Traditionally, laboratory culturing methods have been used for quantification of the bacterial counts in plaque or saliva samples; however, these methods often tend to be laboratory intensive and expensive. Recently, a commercially available chair side meter known as CariScreen (Version 1.4, Albany, Oregon) has become available to quantify oral bacteria samples and assess patients' caries risk. The diagnostic tool uses adenosine triphosphate (ATP) bioluminescence to measure visible light release from dental plaque after mixture with a luciferase enzyme reaction within a swab. Luciferin and luciferase produce light when activated by ATP, a metabolic by-product of metabolizing bacteria.[30] ATP is then quantified using a bioluminometer which can measure the light output in relative light units (RLUs). This allows the identification of non-specific oral bacterial load and biofilm activity levels. A similar method has been used previously in the food production industry to rule out bacterial contamination prior to human consumption. Previous studies have used the CariScreen meter in pediatric dental patients to determine plaque bacteria levels and caries risk assessment.[18,31]

## 1.5 **Oral Health Care for Children**

The most important component to the management of dental caries in children is prevention of the disease altogether. One aspect of this prevention has been the American Academy of Pediatric Dentistry (AAPD) recommendation to establish a dental home by the eruption of the first tooth or 12 months of age, whichever comes first. A

typical initial examination allows pediatric dentists to provide recommendations for diet, oral hygiene, dental prophylaxis, clinical examination, acquiring dental radiographs if indicated, and topical application of fluoride. Caries risk assessment is an important measure to determine an individual's risk of developing caries based on demographic information, habits, preventative care, genetic factors, and many others. The amount of mutans streptococci present within a child's saliva has been shown to be a good predictive factor of that child's caries risk in the near future. This evaluation has been shown by Edelstein in 2016 to be a more predictive measure for caries risk alone than the entire AAPD Caries Risk Assessment Tool cumulatively.[32] Therefore studies evaluating effects on oral bacteria and their correlation with caries risk are warranted.

## 1.6 **Purpose**

This is a prospective, longitudinal pre- and post- interventional study that aims to evaluate the effects of application of 38% SDF on microbial load within plaque and saliva samples at 3 months and 6 months intervals.

The objectives of the study are:

- Determine the oral bacterial load in dental plaque of ECC children using the chairside CariScreen unit at baseline, 3, and 6 months after silver diamine fluoride (SDF) application
- Determine the oral bacterial load in saliva of ECC children using bacterial culture method at baseline, 3, and 6 months after silver diamine fluoride (SDF) application.

- The differences in bacterial levels at different sampling time points will be compared to reveal the efficacy of SDF in reducing oral bacterial load.

### 1.7 **Hypotheses**

The Null Hypothesis of the study is:

- $H_0$: There is no statistical difference between the plaque bacterial level when measured by ATP score in patients receiving a single versus two time application of SDF over a 6 month period.
- $H_0$: There is no statistical difference between the oral bacterial level in saliva when measured by traditional bacterial culture in patients receiving a single versus two time application of SDF over a 6 month period.
- $H_0$: There is no statistical difference in presence of visible plaque in patients when receiving a single versus two time application of SDF over a 6 month period.

## 2. **MATERIALS AND METHODS**

### 2.1 **Overview**

University of Illinois-Chicago (UIC) IRB approval was attained for this study: protocol # 2017-0342. Pediatric patients were recruited from the University of Illinois Chicago College of Dentistry Department of Pediatric Dentistry. Patients were screened for inclusion within the study and if determined to be eligible, the purpose, risks, and benefits of the study were reviewed with parents. If parents were interested in being included in the study, informed consent was obtained and signed. This study was a

prospective, longitudinal pre- and post- intervention study with randomization into two treatment groups. A total of 40 participants (20 in each group) were recruited for the study. The amounts of fluoride or silver used during treatment did not approach the 5 mg/kg probable toxic dose for fluoride or the 380 mg/kg lethal dose for silver respectively[29]. One clinical operator conducted the study, and provided all treatment to patients including application of SDF, collection of saliva, and CariScreen plaque swabs. This operator reviewed manufacturer protocols for the CariScreen meter and underwent training and calibration using the device. Baseline data and outcome measures were recorded electronically on a data collection form within Microsoft® Excel 2016 and transferred to SPSS for statistical analysis.

## 2.2 Study Site, Participants and Enrollment Process

### *2.2.1 Study Site*

This study was carried out at the University of Illinois-Chicago College of Dentistry, Department of Pediatric Dentistry. The clinic provides care for an extensive number of patients requiring dental treatment under general anesthesia which provided a sufficient number of patients for inclusion within this study.

### *2.2.2 Operator*

One designated and trained operator, a pediatric dental resident, administered all oral swabs, saliva collection, as well as SDF applications for the purposes of this study. These procedures were performed according to manufacturer recommendations and instructions.

### *2.2.3 Study Participants*

Study subjects were recruited from the UIC Post-graduate Pediatric Dental Clinic. Eligible patients were identified by the clinical operator (capstone candidate, Dr. Austin LaMay) through the axiUm® electronic health record system. This individual then evaluated patients during a comprehensive oral examination, if patients were diagnosed with early childhood caries requiring treatment under general anesthesia, the parents were informed their children were eligible for recruitment in the study. Inclusion and exclusion criteria were specified for the purposes of this study. Forty patients were expected for recruitment in the study based on a power analysis completed using data from previous studies conducted within the department.

*2.2.4 Inclusion Criteria*

The inclusion criteria for the participants are summarized in Table 1 and were as follows:

1) Children between the ages of 2 and 6 years old. This group represents children with possible diagnosis of ECC and make up the majority of children requiring treatment under general anesthesia for complete oral rehabilitation in a hospital setting.
2) Participants were required to be healthy and classified according to the American Society of Anesthesiology (ASA) class 1.
3) Participants were required to have extensive caries requiring treatment under general anesthesia. The study aims to determine the effect of SDF on bacteria within saliva and plaque samples.
4) Participants needed to be cooperative enough to allow an intra-oral cotton swab and collection of saliva.

5) Participants needed to be cooperative enough for SDF application.
6) Participants were not taking or did not have any antibiotic medication within 10 days of data collection.
7) Participants did not have fixed orthodontic or any other oral appliances.
8) English or Spanish speaking literacy of the parent/guardian was required. The study documentation, and informed consent form were translated into Spanish for inclusion of that population as well.

2.2.5 *Exclusion Criteria:*

The list of the exclusion criteria for this study included:

1) Children younger than 2 years of age and older than 6 years of age were excluded from the study.
2) Children with medical status categorized as ASA II to VI. Patients with significant medical history were excluded from study because of possible confounding effects of their health status.
3) Children with orthodontic or other oral appliances.
4) Children too uncooperative to obtain cotton swab or saliva sample.
5) Children taking or have had an antibiotic medication in the past 10 days.
6) Children allergic to silver.
7) Non-English or Non-Spanish speaking parents/guardians and patients, due to concerns obtaining informed consent.

***Table I: Summary of Inclusion and Exclusion Criteria***

| | Inclusion Criteria | Exclusion Criteria |
|---|---|---|
| **Patient** | • Two to six years of age<br>• Healthy (ASA I)<br>• Treatment planned to receive comprehensive dental care under general anesthesia<br>• English or Spanish speaking Parent/patient<br>• Pediatric patients who have sufficient cooperation to allow obtaining swab sample as part of the ATP meter test as well as collecting saliva sample<br>• Is not taking or did not have any antibiotic medication within 10 days of data collection<br>• Does not have fixed orthodontic or any other oral appliances. | • Medically Compromised (ASA*II to VI)<br>• Patients older than 6 years of age or younger than 2 years of age<br>• Patients presenting with an adult other than the parent or legal guardian<br>• Has orthodontic or other oral appliances.<br>• Too uncooperative to obtain swab sample as part of the ATP meter test.<br>• Is taking or has had an antibiotic medication in the past 10 days.<br>• Patients/Parent who do not speak English or Spanish.<br>• Patients allergic to silver<br>• Patients with history ulcerative gingivitis or stomatitis |

***American Society of Anesthesiologists (ASA)**

## 2.3 Subject Enrollment

Study participants were selected from the pool of patients visiting the clinic at the Pediatric Dentistry Department of the COD at UIC. The study aimed to enroll 40 subjects. The clinical operator reviewed the daily electronic health record (EHR) (axiUm) schedule of the Post-Graduate Pediatric Dental Clinic. Parents of children being referred to UIC COD for dental treatment under general anesthesia were also contacted for their child's

possible inclusion within the study. Following these procedures, a group of 40 participants were obtained to be followed throughout the study period.

Patients presenting to the UIC Department of Pediatric Dentistry had the following performed at their initial visit: the pediatric dental resident (Dr. Austin LaMay)(AL) would perform a comprehensive dental exam, complete medical and dental history, caries risk assessment, extra-oral and intra-oral exam, dental prophylaxis, and fluoride application. He would then determine each child's caries risk based on the caries-risk assessment tool (CAT) which has been developed by the AAPD.[33] This tool takes into account: demographics, oral hygiene practices, dietary habits, and protective factors. If any negative factors were identified with their child's routine practices the pediatric dental resident (AL) would provide recommendations and home care instructions to prevent caries initiation or progression. If possible and when indicated, the pediatric dental resident would take radiographs as outlined by AAPD guidelines for radiographic exposure.[34] Following this procedure, a dental prophylaxis was performed. Based on all findings of the chief complaint, radiographic examination, and clinical examination, a treatment plan would be developed by the clinical operator. In addition to optimal treatment to be performed, the mechanism of which treatment would be carried out would also be discussed with the parent including pharmacologic (nitrous, conscious oral sedation, general anesthesia) and non-pharmacologic behavior management techniques (tell-show-do, distraction, imagery, etc).

Due to the extensive need within the public aid system in Illinois and limited number of dental providers offering access to general anesthesia services for children on

public aid dental insurance, the current wait time for dental treatment under general anesthesia at UIC is approximately 1 year. SDF has been used in the past as an interim therapeutic treatment regimen to delay the progression of caries in patients awaiting general anesthesia at the UIC postgraduate pediatric dental clinic. The AAPD recommends recall status be determined based on a child's caries risk.[33] Children with high caries risk are recommended for 3 month recall dental appointments, while low risk patients only require appointments every 6 months. Children with special healthcare needs are more commonly determined to be at higher risk based on their needs and functional abilities.[33]

Based on caries burden and behavioral indicators it will be determined whether the child would best be treated under general anesthesia, the subject was evaluated for involvement in this study. The clinical operator would review purposes of the study through a patient information leaflet (PIL), potential risks and benefits involved were discussed with parents to determine their enrollment in the study. Adverse outcomes were explained to the parents specifically as it pertains to, expected black stains to carious tooth structure once SDF is applied. If parents agreed to participate in the study, the study consent was completed and signed by the parent and clinical operator (parental permission, Appendix B. Patients and parents were also informed they would be compensated $10 for each follow-up visit required during participating with the study. Once patient enrollment was confirmed, baseline measurements were obtained at the initial appointment and SDF was applied thereafter. Most patients' dental insurances covered SDF treatment, however if the procedure was not covered it was not billed to the patient or insurance company. Participants were assigned a subject number and a master

list of participants linked to the patient's EHR which was kept safely by the clinical operator. The enrolled participants were then randomized to treatment groups in a paired manner by coin flip to determine the intervention group. Participants that were to have SDF applied twice over the 6 month period were designated as Group 2, while participants receiving only a single SDF application were designated at Group 1. Participants that did not meet the inclusion criteria were not enrolled in the study. Treatment recommendations based on their individual needs were determined on a case by case basis and an optimal treatment plan was reviewed with the parents for their care.

## 2.4 Armamentarium

Delivery of SDF required SDF, microbrush, and 2x2 cotton gauze followed by application of 5% fluoride varnish. Silver diamine fluoride 38% sold by Advantage Arrest® (West Palm Beach, FL) was used for this study. Plaque swab and bioluminescent evaluation was completed using the chairside Cariscreen® meter. Sterile 10mL collection vials were used for collection of saliva via the drool method for microbiological culture of bacterial species.

### *2.4.1 SDF*

Elevate® is the manufacturer of Advantage Arrest® which is 38% SDF. SDF can safely be used in children without a silver allergy.

### *2.4.2 ATP Bioluminometer*

The CariScreen protocol involves carefully swabbing the mid-lingual surface of the lower anterior teeth. One firm swipe is required, without contact from the gingiva or any soft tissue. The swab is placed back in its tube, and the snap valve is broken by

bending the bulb forward and backward. The bulb must then be squeezed to expel all liquid down the swab shaft. The tube is gently agitated for 5-10 seconds prior to inserting the swab into the CariScreen Meter, which will provide the result in RLUs from 1-9999.

## 2.5 Procedure

Prior to completing the dental prophylaxis and SDF application, the investigator carried out the ATP bioluminescence swab test as outlined by the CariScreen collection protocol. ATP scores were determined immediately after that swab was collected and documented in the data Excel sheet. Following the bioluminescent swab, unstimulated saliva was collected over 5 minutes via the drool method. This method involved participants placing the sterile collection vial up to their lips and allowed saliva to "drool" into the vial over the course of 5 minutes. This protocol pertained to the initial visit as well as any follow up appointments required based on the group designation of the participant. Thereafter, the Pediatric Dentistry Resident completed the dental prophylaxis and applied the SDF on the dental carious lesions. Participants and parents were provided with oral hygiene recommendations consistent with AAPD guidelines.[9] Standardized oral hygiene instructions included: twice daily parental brushing of child's teeth with fluoridated toothpaste, and daily flossing. Dietary advice included: drinking tap water and milk during the day, no milk or sugar sweetened beverages at night, and limiting juice to a daily intake of 4-6 ounces.

## 2.6 Data Collection

Information was recorded into an Excel file at the initial exam, three month, and six month follow up if applicable:

1. Subject ID number
2. SDF application date
3. Group randomization designation
4. ATP bioluminescence score of the patient
5. Salivary culture data
    a. Brain heart infusion (BHI) agar – Total bacteria level
    b. Mitis salivarius bacitracin agar- Streptococci species
    c. Rogosa agar- Lactobacillus species
6. Visible plaque recording (Y/N)

| Dates/Experimental group designation | Subject ID# | Visible plaque present (Y/N) | ATP bioluminescence score | Salivary Culture data |
|---|---|---|---|---|
| @ Initial | **1** | | | |
| @ 3-months | | | | |
| @ 6-months | | | | |
| @ Initial | **2** | | | |
| @ 3-months | | | | |
| @ 6-months | | | | |
| @ Initial | **3** | | | |
| @ 3-months | | | | |
| @ 6-months | | | | |

Figure 3: Data Colllection Spreadsheet

Enrolled parents/children consisted of two groups who received the following:

1. Group 1: Exam, caries risk assessment, anticipatory guidance including standardized oral hygiene instructions, ATP swab testing, saliva sample, dental prophylaxis, dental radiographs if possible and when indicated, SDF application, and six month recall dental appointment.

2. Group 2: Exam, caries risk assessment, anticipatory guidance including standardized oral hygiene instructions, ATP swab testing, saliva sample, dental prophylaxis, dental radiographs if possible and when indicated, SDF application, and three and six month recall dental appointment.

If the child became eligible for general anesthesia treatment prior to research completion (six months), the child would be excluded from the remaining research procedures in order to receive their definitive treatment. This procedure occurred for one of the study participants and they were subsequently excluded from the study.

## 2.7 Chairside steps of SDF application

1. Visible plaque removed prior to SDF application by dental prophylaxis
2. Teeth dried with air syringe or cotton gauze, whichever was feasible
3. Microbrush dipped into SDF drop until saturated

4. Microbrush applied to carious lesions and allowed to be absorbed
5. 5% Fluoride varnish applied to dentition

### 2.8 Statistical Analysis

Data gathered through all study forms were transferred into Microsoft Excel Spreadsheet (*Microsoft Inc., Redmond, WA, USA*). The data file was stored on a password-protected computer. The Excel data file was then transferred to the IBM SPSS statistical (version 25, IBM corporation, Armunk, NY) software program for statistical analysis. All data were assigned a numerical value in order to complete statistical analysis.

## 3. RESULTS

### 3.1 Number of Participants

Participant enrollment took place over the course of approximately 6 months. The follow up period for both groups took place over the following 6 months, therefore the study period took place over a total of 12 months. Forty participants were recruited for the study, 32 participants completed the necessary follow up appointments entirely. Eight participants (3 Group-1, 5 Group-2) were lost to follow up for a few primary reasons: loss of contact, unexpected comprehensive treatment prior to study completion, and study interruption related to the COVID-19 pandemic. There were no adverse events identified related to the study treatment regimen noted for either group.

### 3.2 Demographics and DMFT Scores

*Table II* shows the demographic distribution for study participants and their group designations. The table represents data for participants that completed study follow up and were used for complete data analysis N=32. Average ages of participants were 3.85 (SD=.9) overall, 3.55 (SD=.6) for Group-1 and 4.15 (SD=1.2) for Group-2 with a range of ages 2-6 years old. Gender distribution for the study was overall 62% male (n=20), 37.5% female (n=12). A similar gender distribution was seen for Group-1: 63.7% male, 35.3% female and Group-2: 60% male, 40% female. Average dmft scores were also calculated for each group independently which were 11.5 (SD=3.17) for Group-1, 12.05 (SD=3.22) for group 2 for an overall average of 11.77 (SD=3.17).

*Table II: Participant Demographics*

| | **Group 1 n=17** | **Group 2 n= 15** | **Total N=32** |
|---|---|---|---|
| **Age (mean in years)** | 3.55 (SD=.6) | 4.15 (SD=1.2) | 3.85 (SD=.9) |
| **Gender (count and percentage)** | M= 11 (63.7%)<br>F= 6 (35.3%) | M= 9 (60%)<br>F= 6 (40%) | M= 20 (62.5%)<br>F= 12 (37.5%) |
| **Average DMFT score** | 11.5 (SD=3.17) | 12.05 (SD=3.22) | 11.775 (SD=3.17) |

### 3.3 ATP Bioluminescence Scores

The baseline mean initial ATP bioluminescence score was 7777 RLU for Group-1 (SD= 1570). Using a related samples t-test a statistically significant decrease for Group-1

was observed and at the end of the study period the mean ATP score had been reduced to 5558 RLU ($p=0.001$, $t=3.35$, 32df, SD = 2237).

When evaluating the mean baseline ATP bioluminescence for Group-2 the score was 6349 (SD=2386). At the 3-month follow up time point particular to Group-2, the mean bioluminescence score had decreased to 5678 RLU (SD=1940). At the end of 6months, the average decreased to 5664 (SD=2391) for Group-2. There was a numerical reduction in RLU but no statistically significant change in ATP bioluminescence score at the end of the study period for Group-2. When comparing the groups, there was an observed decrease of 2,219 RLU for group 1 and 685 RLU for Group-2.

Figure 4 below represents data from the Cariscreen bioluminescent meter during the study period for Groups 1 and 2. Both groups showed a similar average value for the bioluminescence scores at 6 months following SDF application at 5585, and 5664 respectively.

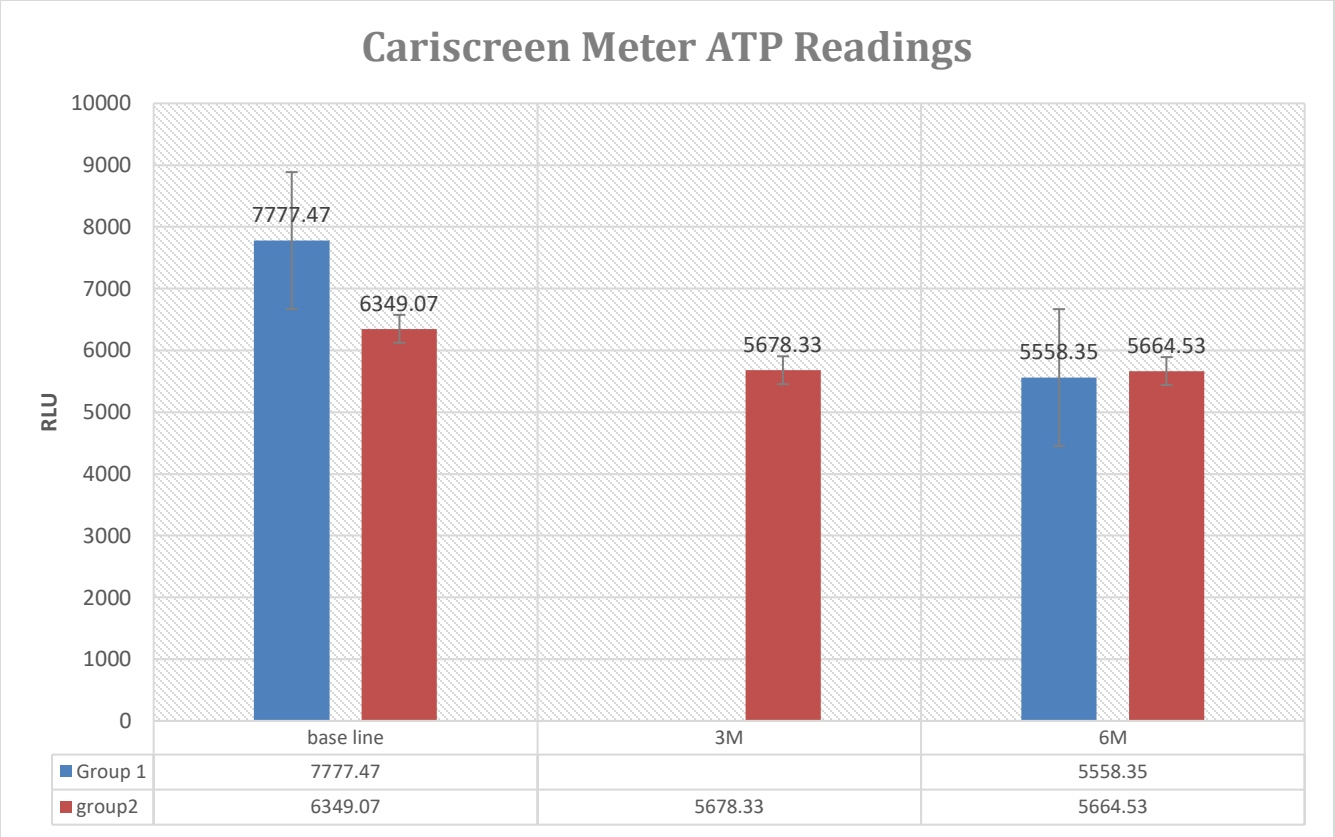


Figure 4: Bioluminescent meter readings following SDF application to measure relative bacteria in plaque on tooth surface

### 3.4 Visible Plaque Presence

At baseline measurement, in Group-1 there were two participants with no plaque present and 18 participants with plaque present. For Group-2 there was one subject with no plaque and 19 participants with plaque present. After interventions throughout the study respective to each study group, using McNemar's Chi-Square for repeated measurements, there were significant decreases in the presence of plaque at the end of the study period for both groups when compared with baseline measurement (p=.000 Group-1, p=.016 Group-2). When comparing the effect of study

interventions with Pearson Chi-Square analysis between Group-1 and Group-2 on presence of plaque before and after SDF application, there was a numerical difference that approached statistical significance ($x^2$=3.46, p=.063). All participants were utilized when determining baseline measurements (n=40) however only 32 participants returned for complete follow up and were used for complete data analysis.

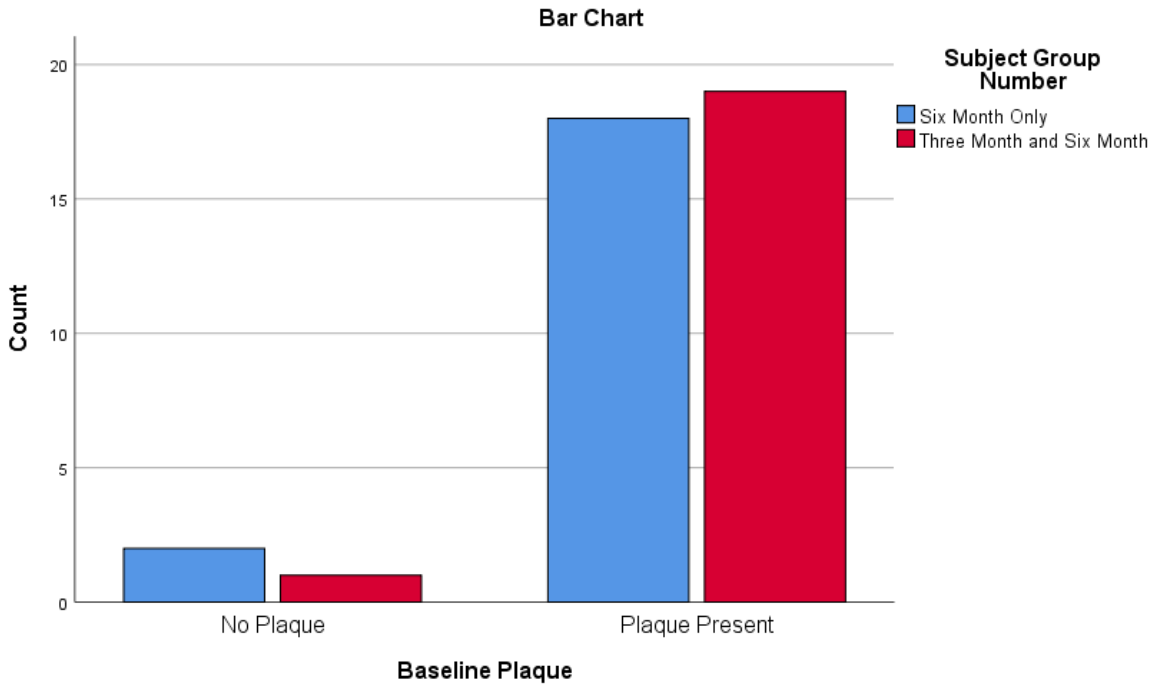


Figure 5: Visible plaque presence at baseline

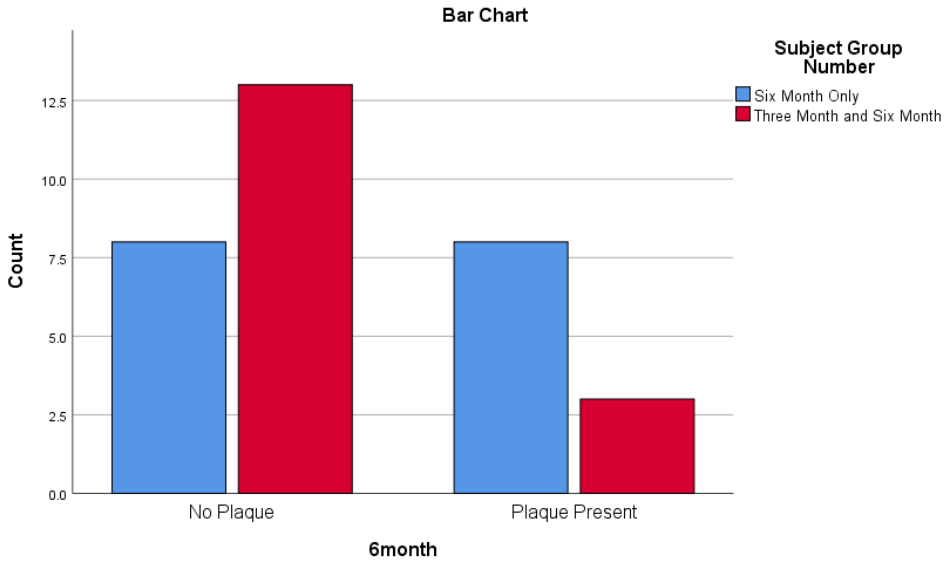


Figure 6: Visible plaque presence at 6 months

*Table III: Visible plaque presence at 6 months following SDF application compared to baseline*

| | Value | df | Significance |
|---|---|---|---|
| **Pearson Chi-Square** | **3.463** | **1** | **.063** |

**McNemar's Chi Square:**

| | Baseline Plaque vs. 6month (pooled) |
|---|---|
| **N** | **32** |
| **Significance** | **.000** |

**Group-1 & 2**

**6 months**

| Baseline Plaque | No Plaque | Plaque Present |
|---|---|---|
| **No Plaque** | **2** | **1** |
| **Plaque Present** | **19** | **10** |

**Group-2**

| | 3 months | |
|---|---|---|
| **Baseline Plaque** | **No Plaque** | **Plaque Present** |
| **No Plaque** | **1** | **0** |
| **Plaque Present** | **7** | **9** |

| | **Baseline Plaque & 6-months (both groups)** | **Baseline Plaque & 3months (Group-2)** |
|---|---|---|
| **N** | **32** | **17** |
| **Significance** | **.000** | **.016** |

### 3.5 Cariogenic Bacterial Culture Data

Table IV depicts data for total bacteria for baseline, 3-month follow up where applicable, and 6-month time points.

*Table IV: Total bacteria in saliva measured by laboratory culture*

| | Total bacteria baseline 0-months | Total bacteria 3-months | Total bacteria 6-months |
|---|---|---|---|
| Group 1 n=17 | $3.5x10^7$ CFU/ml | | $2.4x10^7$ CFU/ml |
| Group 2 n=15 | $2.09x10^7$ CFU/ml | $2.9x10^7$ CFU/ml | $4.7x10^7$ CFU/ml |

Table V depicts data for Lactobacilli species for baseline, 3 month follow up where applicable, and 6 month time points.

*Table V: Lactobacilli measured in saliva by laboratory culture*

| | Lactobacilli baseline 0-months | Lactobacilli 3-months | Lactobacilli 6-months |
|---|---|---|---|
| Group 1 N=17 | $1.10x10^5$ CFU/ml | | $1.03x10^5$ CFU/ml |
| Group 2 N=15 | $1.22x10^5$ CFU/ml | $2.2x10^4$ CFU/ml | $3.18x10^4$ CFU/ml |

Table VI depicts data for Streptococci species for baseline, 3 month follow up where applicable, and 6 month time points.

*Table VI: Streptococci measured in saliva by laboratory culture*

| | Streptococci Baseline 0-months | Streptococci 3-months | Streptococci 6-months |
|---|---|---|---|
| Group 1 N=17 | $4.79x10^5$ CFU/ml | | $5.79x10^5$ CFU/ml |
| Group 2 N=15 | $1.01x10^5$ CFU/ml | $3.4x10^5$ CFU/ml | $1.79x10^5$ CFU/ml |

Figure 7 illustrates the compilation of data for both groups and all bacterial species cultured from saliva over the study time points. Overall there is a trend that bacterial concentrations did not change significantly over time for any of the species cultured: total bacteria, lactobacilli, nor streptococci.

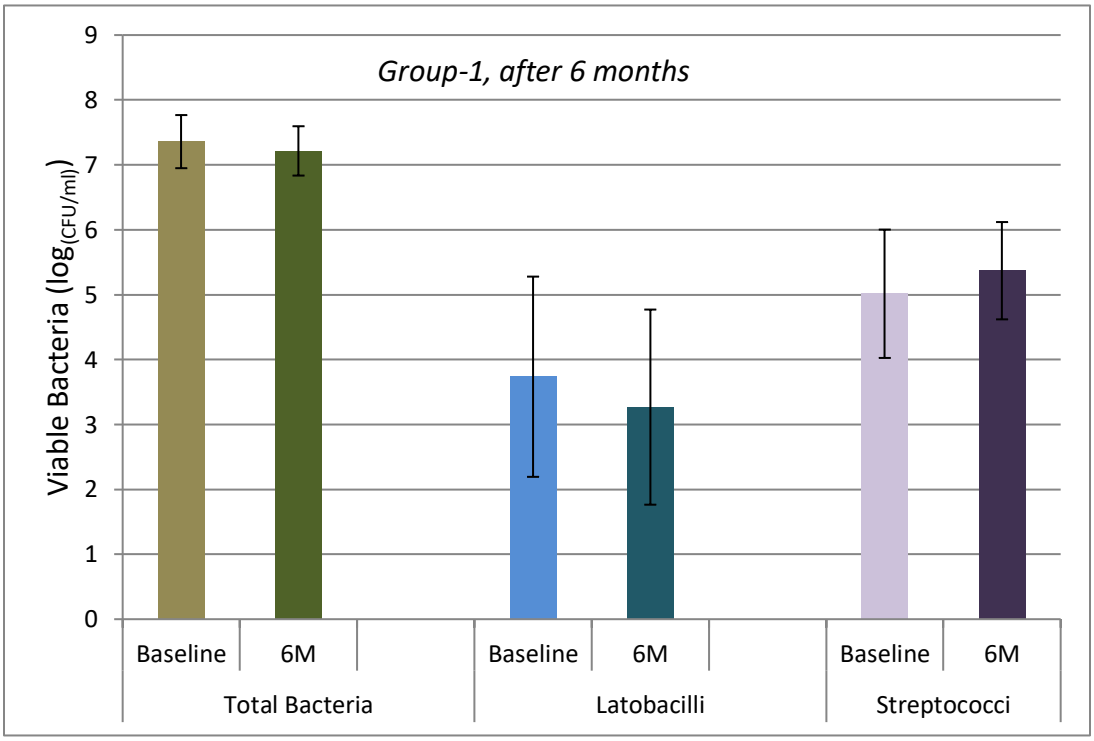


Figure 7: Viable bacteria measured in saliva by laboratory culture following SDF application for Group-1 participants

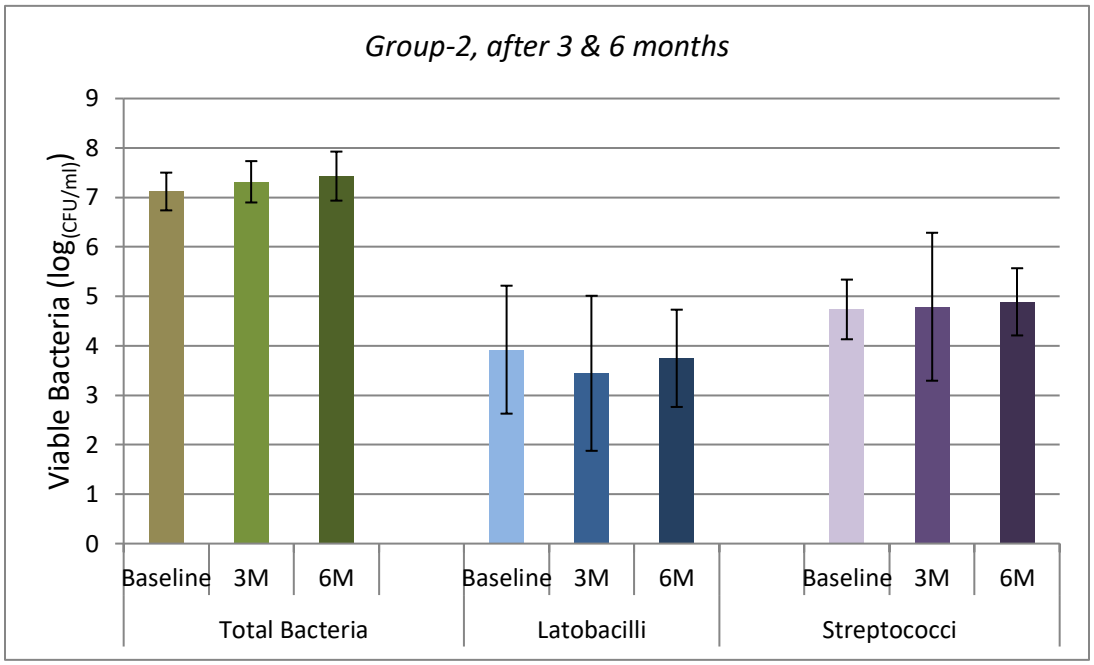


Figure 8: Viable bacteria measured in saliva by laboratory culture following SDF application for Group-2 participants

Statistical analysis to compare the means before and after interventions at each time point was completed using a paired sample t-test. There was no statistically significant differences found when comparing time points between groups or within groups. However, when comparing the proportion of lactobacilli that contributed to the total bacteria, we observed that this proportion decreased over time for both groups after SDF application. The decrease for proportion of lactobacillus species within the total bacteria was more notable for Group-2 compared to that of Group-1. Figure 12 illustrates this change in proportion as a percentage for the study groups.

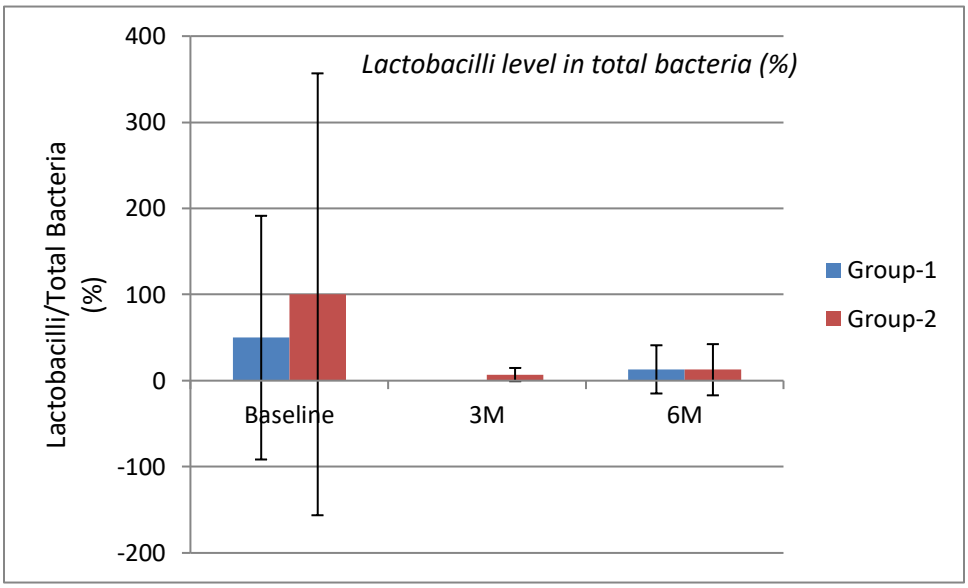

Figure 9: Lactobacilli level in total bacteria (%) measured in saliva following application of SDF

## 4. DISCUSSION

### 4.1 Bioluminometer readings to determine 3 month vs 6 month SDF application frequency

The null hypothesis is that there is no difference in CariScreen meter readings, which detects relative amounts of bacteria within plaque swabs, when SDF is applied on a 3-month frequency vs 6-month basis. We reject the null hypothesis, since there is a statistically significant difference for Group-1. When evaluating the data, we see a trend of a decrease in bioluminescent readings for both groups, with the larger decrease coming from Group-1. This may be due to the higher average reading at baseline which allowed for a greater numerical decrease overall. At the 6-month time point the average bioluminescence scores are very similar for Groups-1 and -2 which shows a trend towards a value ~5600 at that time. If the mean bioluminescence scores would have been more similar for both groups at baseline, it is possible there may have been significance for the

decrease in Group-2 as well. The other possibility for the results we obtained could be due to error in protocol while obtaining plaque swabs for the bioluminescent meter that created error in the results we obtained. It is difficult to control for the environment despite a provider's best attempts to do so in a young child during a clinical scenario. Similar studies evaluating the reliability of the CariScreen meter have had conflicting conclusions. Hallet *et al* suggested in 2013 that the test was not a reliable caries risk assessment tool when compared with other more validated measures. The readings they obtained were three times higher than those suggested by the manufacturer as well as notably inconsistent.[31] Fazilat *et al* determined in 2010 that the meter was reliable to determine oral bacteria in plaque based on data from their study correlating ATP readings with oral streptococci concentrations with high correlation.[18] There is widely accepted evidence that patients involved in research may be particularly diligent with their behavior because they are aware they are being monitored as participants within study. This may contribute as a confounding variable to changes we notice in our outcome measures.[35]

### 4.2 Microbial culture of cariogenic salivary bacteria to determine 3 month vs 6 month SDF application frequency

Evaluating results from bacterial culture of salivary bacteria at baseline and each time point within the study, we did not observe any significant changes. There was a large diversity and variance of oral bacteria between different subjects and varying amounts of bacteria over time even within the same subject. This could be attributed to differing behaviors in diet, oral hygiene, or other activities of daily living. When the data

from all subjects was pooled, there was no significant trend indicating SDF played any role to attribute for differences in the salivary bacteria overall. However, when looking closely at the data, there was an interesting trend that indicated the proportion of lactobacilli compared to total bacteria decreased in both treatment groups following SDF application. This may indicate that SDF may have specific effects on lactobacillus species within saliva. This trend was not observed for the data obtained from streptococcal species.

### 4.3 Plaque Presence

When evaluating results for presence of plaque over the course of the study, we observed a significant change in frequency of plaque present in subjects throughout the study period. Most subjects presented with plaque present at enrollment for the study, however during subsequent recalls we observed a significant decrease in the frequency of plaque present in the study subjects. This shows our data is consistent with evidence that counseling on proper oral hygiene and instruction by dental professionals can be effective for behavior modification in these patients. It also provides insight that there could be possible changes in the microbiota related to the decrease of food debris and plaque accumulation on tooth surfaces. We might expect to see a decrease in species of cariogenic bacteria within plaque or saliva that might be attributed to this decrease in plaque. Past research has shown a decrease in biofilm formation on the surfaces of teeth treated with SDF.[36] This is consistent with our results which may have been attributed to this mechanism or a combination of changes in oral hygiene in addition to this proposed mechanism. It is important that future studies evaluating this topic should control for differences in hygiene that could affect results of oral bacteria measurements.

## 4.4 Comparison to Past Studies

There have been other studies published recently evaluating the effects of silver diamine fluoride on cariogenic bacteria. Many of these studies were laboratory studies to validate the antibacterial activity of SDF on various media or simulated tooth structure. *In vitro* studies by Savas *et al* and Lou *et al* have established that SDF possesses strong inhibitory and bactericidal properties against a wide range of bacterial species.[25,26] However, a few published *in-vivo* studies on the antibacterial effects of SDF have shown conflicting results. One study by Mitwalli *et al*. investigated the effects of SDF on root/cervical carious lesions in adults. At baseline and following application of SDF, plaque samples were obtained from the surfaces of the lesions and DNA sequencing was completed to obtain profiles of bacteria within the plaque samples. There were no significant differences within the bacterial profiles identified pre- and post-intervention. However, there were some changes in the relative abundances of some acidogenic species but none of these were identified as S. mutans or Lactobacillus species.[22]

The second recent publication is from Garrastazu *et al*. evaluating effects on salivary levels of S. mutans following application of SDF compared with those of chlorhexidine in children. Children were randomized to have SDF or chlorhexidine applied to their teeth and followed up at 1, 30, and 90 days post intervention for saliva samples. Saliva samples were cultured on differential media for evaluation of S. mutans levels. The study found that 30% SDF had similar antimicrobial effects as 1% chlorhexidine in creating

a statistically significant reduction of salivary levels of S. mutans at all time points. One of the main controversies with this study is that the authors did not specify if any of these children had caries or any mention of the methods in which they applied these antimicrobials to the children's teeth.

A well-designed study by Milgrom *et al*. published in 2018 measured levels of bacteria on the surfaces of carious lesions and unaffected tooth structure in 66 children before and 2-3 weeks after SDF application. They utilized RNA sequencing methods to determine changes in the abundances of microbes sampled from the tooth surfaces. Contrary to their hypothesis, there was very little difference in the abundances of most bacterial species. Specifically, there were no significant changes in cariogenic bacteria including S. mutans and Lactobacilli which were nearly universally found in all samples pre- and post-intervention despite SDF treatment. Surprisingly, most changes were modest increases as opposed to decreases as their hypothesis predicted. The authors also used genetic sequence evaluation to confirm that there were also no significant changes in expression of antibiotic resistance or metal resistance genes within the samples obtained during the study. These data suggest that SDF is safe and does not promote the development of resistance to its bactericidal mechanisms.

Despite conflicting results on the bactericidal effects of SDF in clinical settings, some studies suggested that it has minimal effect on bacteria within saliva or plaque. Our data supports the concept: SDF has minimal effect on bacterial concentrations within plaque and saliva. The exception to this theory is that there may be some reduction of bacterial species within plaque when measured with the bio luminometer, however this

was only found in 1 treatment group. It is unlikely that these results are reliable because it is very counter-intuitive that a single application of SDF would result in less bacteria in plaque than 2 applications considering many studies have confirmed the bactericidal nature of the compound.

## 4.5 Study Strengths

The study was completed with a single operator and protocol for SDF placement, examination, oral hygiene instruction, and diet counseling. This provided uniformity across participants and treatment groups to ensure the same protocols as much as possible between participants and avoidance the need for inter-operator calibration. The participants were also randomized to the corresponding treatment group which serves as a strength by limiting bias within the study.

The participants were all selected from the same subject group within the pediatric dental clinic. Although a convenient sample and single center study, UIC pediatric patients are considered diverse and they represented a population that was consistent between treatment groups in terms of age, extent of decay, and gender.

The study also utilized several independent outcome variables to measure differences in bacteria throughout the study period. This variation in measurement methods help to establish more consistent conclusions overall because we would expect uniformity across the data across the different methods of measurement.

## 4.6 Study Limitations

One of the study limitations was the relatively small sample size compared to some similar

studies. There were 40 participants initially recruited for the study, of which 32 completed the study. A power analysis was completed prior to the study based on previous comparable research protocols completed at the department utilizing the bioluminometer in detecting differences in bacteria after SDF application. The previous study found a 2382RLU decrease in CariScreen reading following SDF application when evaluated at 3months. Anticipating sample sizes of 20 in each group achieved >80% power when considering mean difference of 2382RLU using the CariScreen ATP device. The previous research found a standard deviation of 861 at baseline and 2237 at the second time point. Our results were not consistent with those from the previous study and ultimately resulted in a deficiency of subjects to determine conclusive results. This is supporting evidence from other studies that the CariScreen meter may not be a reliable measurement tool.

The second limitation of the study was the related to the study period of 6 months. Since the study group was those of a remarkably high disease burden waiting for many months for treatment under general anesthesia, a subset of the study population had teeth with carious lesions that developed abscesses throughout the study period despite study interventions. It is possible that the latter affected the bacterial levels due to the abscesses. This had occurred in a relatively small percentage of the study subjects, however nearly all of these subjects had no differences in total bacteria measured from their saliva despite the presence of the chronic abscess.

Despite the study being conducted in a randomized fashion where participants were placed into groups serially in pairs following enrollment, there were differences in

baseline values between the groups. The conditions of the study did not allow the operator to be blinded as to which treatment group each patient was placed into. This may have introduced some amount of artificial bias related to how the study was conducted or what results were expected.

There was intention to provide a control group that did not receive SDF throughout the study, however due to ethical concerns this was ultimately not possible. Only one parent had declined SDF throughout the enrollment period which was not enough to provide an effective control group. The ethical concern would be if SDF were not offered as a treatment option to a parent and child that would benefit from it's use otherwise. For that reason, there are limitations when interpreting the results of the study without a control group to compare.

## 4.7 Future Studies

To date, studies evaluating the microbiologic effects of SDF in children have been limited. In addition, available data has been conflicting, thus making the conclusive determination as to whether SDF affects microflora of plaque and saliva of upmost importance. Numerous studies report the successful outcomes when SDF was used as a treatment agent against ECC. However, the exact mechanism of SDF leading to the positive clinical benefits remains unclear. Once the understanding of the mechanisms of SDF are understood, this should enable clinicians to more accurately prescribe its use and promote effective treatment regimens. Future studies investigating the antimicrobial effects of SDF could be focused on differences within bacterial profiles within the carious

lesions themselves using traditional culture methods or genetic sequencing of bacteria. Most past research has focused on bacteria on the surfaces of the carious lesions or saliva, but the mechanism of arrest may be restricted to the carious lesions themselves with little effects on the plaque found on surfaces of the lesion or surrounding saliva.

## 5. Study Conclusions

The following conclusions can be made based on the results of this study:

- There is no benefit to applying SDF more frequently than the current 6 month standard when measuring differences in bacteria within plaque using a chairside bioluminometer.
- There is no benefit to applying SDF more frequently than the current 6month standard when measuring differences in salivary bacteria using traditional culture methods.
- There is a significant reduction in frequency of visible plaque for when SDF is applied at both 3 and 6 month time intervals in conjunction with recall appointments.

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## APPENDIX A

OFFICE OF THE VICE CHANCELLOR FOR RESEARCH

UIC

**Continuing Review of Research**

Version: 3.6; Date: 04/12/2018

**Office for the Protection of Research Subjects (OPRS)**
**Institutional Review Board**
FWA# 00000083

201 AOB (MC 672)
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Phone: 312 996-1711
Fax: 312 413-2929
http://research.uic.edu/compliance/irb

| *To Be Completed By the Investigator* | |
|---|---|
| UIC Protocol #: 2017-0342 | Date Application Completed: **08/17/17** |
| Institutional Proposal (IP) # : | Application Document Version #: **1** |

**I. Research Title:** **Evaluation of silver diamine fluoride in reduction of plaque and salivary oral bacteria in children with early childhood caries (ECC)**

**II. Personnel**

**A. Principal Investigator**

| Name (Last, First) | Degree(s) | Net ID |
|---|---|---|
| **Alrayyes, Sahar** | **DDS, MS** | **salray1** |
| Department<br>**Pediatric Dentistry** | College<br>**Dentistry** | University Status<br>☐ Student/Fellow/Resident<br>☒ Faculty/Staff |
| Phone Number<br>**312-996-6414** | UIC E-mail Address<br>**salray1@uic.edu** | |

**B. Faculty Sponsor** – Complete only when PI is a student, fellow, or resident

| Name (Last, First) | Degree(s) | Net ID |
|---|---|---|
| Department | College | |
| Phone Number | UIC E-mail Address | |

**C. Primary Contact Other Than PI –** Complete only if the primary contact person is different than the PI

| Name (Last, First) | Net ID (e.g., NetID@uic.edu) |
|---|---|
| Phone Number | UIC E-mail Address |

☐ Principal Investigator grants this personnel access to OPRS Live for this protocol

**D. Include a copy of the current Appendix P as part of the Continuing Review.** The names of key research personnel no longer on the study should be crossed out.

**III. Conflict of Interest (COI) Disclosure**

## APPENDIX B


**University of Illinois at Chicago**
**Research Information and Parental Permission for Participation in Biomedical Research**

"Evaluation of Silver Diamine fluoride in reduction of plaque and salivary oral bacteria in children with early childhood caries (ECC)"

Your child is being asked to participate in a research study. Researchers are required to provide a consent form such as this one to tell you about the research, to explain that taking part is voluntary, to describe the risks and benefits of participation, and to help you to make an informed decision. You should feel free to ask the researchers any questions you may have.

Principal Investigator Name and Title: Sahar Alrayyes, DDS, Pediatric Dental Resident
Department and Institution: Pediatric Dentistry, UIC College of Dentistry
Address and Contact Information: 801 S Paulina St (MC 850), Chicago IL 60612, 312-996-7532
Emergency Contact Name and Information: Sahar Alrayyes, UIC College of Dentistry, Department of Pediatric Dentistry, salray1@uic.edu or at (312)996-6414

**Conflict of Interest**

Your health care provider may be an investigator on this research protocol, and as an investigator, is interested in both your clinical welfare and in the conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your child's care from a clinician who is not associated with this project. Your child is not obligated to participate in any research project offered by your clinician. Your child's participation in this research study is voluntary and you do not have to participate. The decision to not participate will not affect your child's clinical care now or in the future.

**Why am I being asked?**

Your child has been asked to participate in the research because they have been identified by their provider as being healthy, between the ages of 2-6 years old, having dental cavities needing treatment requiring general anesthesia (and thus being placed on the general anesthesia waitlist). . This study will examine how the amount of oral bacteria of children changes in response to oral hygiene instructions and/or application of 2.26 fluoride varnish alone or with silver diamine fluoride (SDF) at 3 months intervals or 6 months interval. This study is conducted by Sahar Alrayyes of the Pediatric Dentistry Department at the University of Illinois at Chicago (UIC) and held at UIC Pediatric Dental Clinic.

Your child's participation in this research is voluntary. Your decision whether or not to participate will not affect your child's current or future dealings with the University of Illinois at

Chicago. **If you decide to participate, you are free to withdraw your child at any time without affecting that relationship.**

Approximately 150 subjects may be involved in this research at UIC.

**What is the purpose of this research?**

This research is being done to better understand whether SDF application on dental cavities, in addition to the gold standard practice of oral hygiene instructions and fluoride varnish, reduces oral microbial load, and to what degree it reduces it over three month application intervals vs 6 months interval.

**What procedures are involved?**

This research will be performed at UIC College of Dentistry, Department of Pediatric Dentistry.

You will need to come to the study site two more times over the next six months. The next visit will be in three months, and the last visit will be in six months.

Each of those visits will take about 30 minutes.

The study procedures consist of:

1. Complete an oral exam on your child to evaluate if they have any visible plaque
2. Using a cotton swab, collect a plaque sample from your child before his/her dental cleaning, which will be tested for any bacteria that causes cavities. A technology called ATP bioluminescence will be used for this test, which will be done chairside, with immediate results, and minimal to no discomfort. The ATP bioluminescence swab is not routinely done in dental clinics, but will be used for this research.
3. Collect a saliva sample from your child which will be tested for any bacteria that causes cavities. This is not routinely done in dental clinics, but will be used for this research.
4. Complete a prophylaxis (cleaning) and apply fluoride varnish. This the standard care at our clinic
5. Due to the active dental caries and extended wait time for your child to be treated under general anesthesia, we recommend that your child receive a Silver Diamine Fluoride application to halt the cavities till your child receives the treatment needed. This is not a covered procedure by your insurance and the out of pocket cost is $39. It is the standard practice at our clinic to recommend it to parents.
6. If you do not wish to pay the $39, we will apply only fluoride varnish at the initial visit, 3 months recall visit and 6 months visit.
7. Participants who are willing to pay for the Silver Diamine Fluoride will be identified by a study number, which is allocated to them at the time of study enrolment. All study data will be coded using only the participants' study numbers and not including any other personal identifiers. The key to the code (personal information matching participants

'study numbers) along with all participants' personal information and records will be kept confidential at all times. Only the research team will have access to the study documentation. Parental permission forms, and data collection sheets will be stored in a locked cabinet in the room 269-A at the Pediatric Dentistry Department of the College of Dentistry, UIC. All computerized records, including the key to the data coding, will be protected in an encrypted folder on a password protected UIC computer.

a. If your child was randomly assigned (similar to the flip of a coin) to receive Silver Diamine Fluoride application at the 6 months recall visit, he/she will receive another Silver Diamine Fluoride at the 6 months recall at no cost to you. At the 3 months recall visit, your child will receive an exam, cleaning and fluoride varnish application. At 6 months recall visit, your child will receive an exam, cleaning, fluoride varnish application and Silver Diamine Fluoride application-This is the current recommended treatment.
b. If your child was randomly assigned (similar to the flip of a coin) to receive Silver Diamine Fluoride application at the 3 months recall visits, he/she will receive a Silver Diamine Fluoride application at the 3 months recall visit and 6 months recall visit at no additional cost to you. At the 3 months recall visit, your child will receive an exam, cleaning and fluoride varnish application and Silver Diamine Fluoride application-. At 6 months recall visit, your child will receive an exam, cleaning, fluoride varnish application and Silver Diamine Fluoride application-. It is the current practice at UIC to recommend Silver Diamine Fluoride treatment at 6 months, but not at 3 months.

8. Using the data collection sheet, Dr. Sahar Alrayyes will report whether your child received fluoride varnish application alone or along with a SDF application on dental cavities during his/her initial exam, 3 month recall visit and 6 month recall visit.
9. We will collect all this data using your child's dental chart number to keep the data together. When the study is finished, we will remove your child's dental chart number from all research materials.

**What are the potential risks and discomforts?**

The research has these mild risks: the discomfort of swabbing the child's teeth for the ATP bioluminescence, the discomfort of collecting saliva, and the risk of loss of privacy of protected health information by including that information in a research project. Lastly, **black staining of the carious tissue where it is being applied from the formation of metallic silver from silver compounds.**

**Will I be told about new information that may affect my decision to participate?**

During the course of the study, you will be informed of any significant new research findings (either good or bad), such as changes in the risks or benefits resulting from participation in the research or new alternatives to participation, that might cause you to change your mind about

continuing in the study. If new information is provided to you, your consent to continue participating in this study may be re-obtained.

**Are there benefits to taking part in the research?**

There are no direct benefits to subjects for their participation in this research. The benefits of the research are limited to knowledge that may be gained, which may be benefit others in the future.

**What other options are there?**

You have the option to refuse to allow your child to participate in this research. Your child will still receive their dental exam, dental cleaning and fluoride varnish application alone or along with silver diamine fluoride application even if you choose not to participate.

**What about privacy and confidentiality?**

The people who will know that your child is a research subject are members of the research team, and, if appropriate, your child's dental personnel at the clinic. No information about your child during the research will be disclosed to others without your written permission, except:

- if necessary to protect your child's rights or welfare (for example, if your child is injured and need emergency care or when the UIC Institutional Review Board monitors the research or consent process); or
- if required by law.

Study information which identifies you and the consent form signed by you will be looked at and/or copied for examining the research by:

- Food and Drug Administration (FDA)
- the research co-investigators
- UIC Office for the Protection of Research Subjects, State of Illinois Auditors

A possible risk of the research is that your child's participation in the research or information about your child and their health might become known to individuals outside the research. Personal information, research data, and related records will be stored as coded data. Your child will be given an identifier number linked to their dental electronic health record number where that list will be kept in a locked drawer and a locked office of the Principle Investigator. The list will be destroyed at the end of the study to avoid any loss of privacy.

When the results of the research are published or discussed in conferences, no information will be included that would reveal your child's identity.

**Will health information about you be created, used or shared with others during this study?**

State and federal laws, including the Health Insurance Portability and Accountability Act (HIPAA), require researchers to protect your health information. This section of this form describes how researchers, with your authorization (permission), may use and release (disclose or share) your protected health information in this research study. By signing this form you are

authorizing Dr. Sahar Alrayyes and her research team to create, get, use, store, and share protected health information that identifies your child for the purposes of this research.

The health information includes all information created and/or collected during the research as described within this consent form and/or any health information in your child's medical record that is needed for the research and that specifically includes medical record number.

During the conduct of the research, the researchers may use or share your health information:

- With each other and with other researchers involved with the study;
- With law enforcement or other agencies, when required by law;
- With representatives of government agencies (i.e., Food and Drug Administration), review boards including the University of Illinois at Chicago Institutional Review Board, the University of Illinois Medical Center and its representatives, and other persons who watch over the safety, effectiveness, and conduct of research

**How will your health information be protected?**

The researchers agree to protect your health information and will only share this information as described within this research consent/authorization form.

When your health information is given to people outside of the research study, those agencies that receive your health information may not be required by federal privacy laws (such as the Privacy Rule) to protect it. They may also share your information with others without your permission, if permitted by laws that they have to follow.

**What are the costs for participating in this research?**

Covered by insurance: Initial and 6 months recall exams, Cleaning at initial and 6 months, fluoride varnish application at initial visit
Not covered by insurance (will be written off and not billed): 3 months recall exam, cleaning at 3 months recall visit, fluoride varnish at 3 and 6 months, and Silver Diamine Fluoride application at 3 and 6 months recall visit. ATP bioluminescence and Saliva collection. Tooth brushes, tooth pastes and floss
Not covered by insurance (will be submitted to patients for payment): Silver Diamine Fluoride at initial visit

**Will I be reimbursed for any of my expenses or paid for my participation in this research?**

You will offered $10 payment for participating in this study in this study at the initial, 3 months recall and 6 months recall visit

**Can I withdraw or be removed from the study?**

If you decide for your child to participate, you are free to withdraw your permission and discontinue participation at any time without affecting your child's future care at UIC.

Your Authorization for release of your child's health information for this research study expires at the end of the study, but can be canceled sooner if you decide to withdraw your permission.

You may change your mind and cancel this Authorization at any time. To cancel this Authorization, you must write to: Dr. Sahar Alrayyes, MC 850, Department of Pediatric Dentistry, 801 S. Paulina, Chicago, IL 60612.

If you cancel this Authorization, your child may no longer be allowed to take part in the research study. Even if you cancel this Authorization, the researchers may still use and disclose health information from your child that they have already obtained as necessary to maintain the integrity and reliability of the research and to report any adverse (bad) effects that may have happened to your child.

**Who should I contact if I have questions?**

Contact the researchers Dr. Sahar Alrayyes (312-996-6414), Dr. Evelina Kratunova (312-996-1984), or Dr. Christine Wu at (312) 996-7531 if you have any questions about this study or your child's part in it,

- if you feel you have had a research-related injury (or a bad reaction to the study treatment), and/or
- if you have questions, concerns or complaints about the research.

**What are my rights as a research subject?**

If you have questions about your child's rights as a research subject or concerns, complaints, or to offer input you may call the Office for the Protection of Research Subjects (OPRS) at 312-996-1711 or 1-866-789-6215 (toll-free) or e-mail OPRS at uicirb@uic.edu.

If you have questions or concerns regarding your privacy rights under HIPAA, you should contact the University of Illinois at Chicago Privacy Officer at Ph: (312) 996-2271.

**Remember**:

Your child's participation in this research is voluntary. Your decision whether or not to have your child participate will not affect their current or future relations with the University. If you decide to have your child participate, you are free to withdraw them at any time without affecting that relationship.

**Right to Refuse to Sign this Authorization**

You do not have to sign this Consent/Authorization. However, because your child's health information is required for research participation, your child cannot be in this research study if you do not sign this form. If you decide not to sign this Consent/Authorization form, it will only mean your child cannot take part in this research. Not signing this form will not affect your child's non-research related treatment, payment or enrollment in any health plans or your child's eligibility for other medical benefits.

If you have not already received a copy of the Notice of Privacy Practices, you should ask for one.

Your signature below indicates that you are providing both consent to have your child participate in the research study and authorization for the researcher to use and share your child's health information for the research.

**Signature of Subject or Legally Authorized Representative**

I have read (or someone has read to me) the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this research. I will be given a copy of this signed and dated form.

I am 18 years old or older, and I am the legal guardian of the child _____.
(name of child)

_____ _____
Signature of Parent / Guardian or Legally Authorized Representative of Subject — Date (must be same as Subject's)

_____
Printed name of Parent / Guardian or Legally Authorized Representative of Subject

Describe relationship to subject including the legal authority this individual has to act on behalf of the subject. (Check one below)

- ☐ Parent
- ☐ Medical Power of attorney/representative
- ☐ Legal guardian
- ☐ Health care surrogate
- ☐ Other; specify

_____

_____

_____

## APPENDIX C

OFFICE OF THE VICE CHANCELLOR FOR RESEARCH

UIC

Office for the Protection of Research Subjects (OPRS)
**Institutional Review Board**
FWA# 00000083

201 AOB (MC 672)
1737 West Polk Street
Chicago, IL 60612-7227
Phone: 312 996-1711
http://research.uic.edu/compliance/irb

**Appendix P: Co-Investigators/Other Key Research Personnel (Not PI or Faculty Sponsor)**
Version: 4.3; Date: 10/15/2018

**All investigators must disclose all real, apparent, or potential Significant Financial Interest (SFI) to the IRB. For more information, refer to *Investigator Conflict of Interest Disclosure Policy for Human Subjects*.**

| **Name (Last, First)** | **Degree(s)** | **Net ID** (e.g., NetID@uic.edu) |
|---|---|---|
| **LaMay, Austin** | **DMD** | **alamay2** |
| **Department** | **College** | **E-mail Address (if no Net ID)** |
| **Pediatric Dentistry** | **Dentistry** | **alamay2@UIC.EDU** |

**Role:**
- ☒ Co-Investigator
- ☐ Key Research Personnel (Describe)
- ☐ Administrative Coordinator*
- ☐ Other (Describe)

☒ Principal Investigator grants this personnel access to OPRS Live for this protocol

**(Note: The Administrative Coordinator role is limited to management of submission documents and IRB correspondence. Personnel who will be interacting with subjects or accessing identifiable data must be listed as Co-Investigators or Key Research Personnel and must meet the applicable investigator training requirements.)*

| **Name (Last, First)** | **Degree(s)** | **Net ID** (e.g., NetID@uic.edu) |
|---|---|---|
| | | |
| **Department** | **College** | **E-mail Address (if no Net ID)** |
| | | |

**Role:**
- ☐ Co-Investigator
- ☐ Key Research Personnel (Describe)
- ☐ Administrative Coordinator*
- ☐ Other (Describe)

☐ Principal Investigator grants this personnel access to OPRS Live for this protocol

**(Note: The Administrative Coordinator role is limited to management of submission documents and IRB correspondence. Personnel who will be interacting with subjects or accessing identifiable data must be listed as Co-Investigators or Key Research Personnel and must meet the applicable investigator training requirements.)*

## APPENDIX D

Permission for use of NHANES publications from HHS:

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## VITA

# Austin LaMay, DMD

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**Education:**

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|---|---|
| 2018 – Present | University of Illinois at Chicago – College of Dentistry<br>Pediatric Dentistry Residency, PGY2<br>Masters in Oral Sciences<br>*Projected Completion: June 2020* |
| 2014 – 2018 | Southern Illinois School of Dental Medicine<br>*Doctor of Dental Medicine* |
| 2011 – 2014 | Southern Illinois University-Edwardsville<br>Major: Biological Sciences |

**Board Examinations:**

NBDE Part I – Pass
NDBE Part II – Pass

**Licensure:**

CRDTS Licensure Exam – Pass
Illinois State Dental License
Illinois State Controlled Substance License

**Work Experiences:**

| | |
|---|---|
| 2018 – 2020 | University of Illinois at Chicago<br>*PGY-2 Pediatric Dental Resident*<br>Program emphasizes behavioral management, sedation, medically compromised individuals, hospital protocols, fourhanded dentistry, and orthodontics<br>Chicago, IL |

**Presentations:**

| | |
|---|---|
| 2020 | Evaluation of Silver Diamine Fluoride in Reduction of Plaque and Salivary Oral Bacteria in Children with Early Childhood Caries (ECC) |

*Presented at the UIC Clinic and Research Day, Chicago, IL*

2019 Antibacterial Effects of Silver Diamine Fluoride in Patients with Early Childhood Caries
*Presented at the UIC Clinic and Research Day, Chicago IL*

**Research:**

2018 - 2020 Evaluation of Silver Diamine Fluoride in Reduction of Plaque and Salivary Oral Bacteria in Children with Early Childhood Caries (ECC)
Mentor: Christine Da Wu PhD
University of Illinois at Chicago Department of Pediatric Dentistry Chicago, IL

**Honors and Awards:**

| | |
|---|---|
| 2018 | Inductee to Omicron Kappa Upsilon Dental Honor Society |
| 2018 | American Academy of Pediatric Dentistry Certificate of Merit |
| 2017 | Southern Illinois School of Dental Medicine Dean's Scholarship |
| 2014, 2018 | Inductee to Phi Kappa Phi National Honor Society |
| 2012-2014 | Dean's List Southern Illinois University Edwardsville |
| 2012 | Alpha Kappa Lambda – Beta Tau chapter Scholarship Award |
| 2011 | Distinguished Writers Award – Rock Valley College English Department |

**Affiliations:**

| | |
|---|---|
| 2014-Present | American Dental Association |
| 2014-Present | Illinois State Dental Society |
| 2014-Present | Chicago Dental Society |
| 2018-Present | Illinois Society of Pediatric Dentistry |
| 2017-Present | American Academy of Pediatric Dentistry |
| 2018-Present | Omicron Kappa Upsilon Dental Honor Society |