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Evaluating The Effects Of Silver Diamine Fluoride On Microbiome Analysis Of Carious Lesions

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EVALUATING THE EFFECTS OF SILVER DIAMINE FLUORIDE ON MICROBIOME ANALYSIS OF CARIOUS LESIONS

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MASTER OF SCIENCE IN DENTISTRY

ABSTRACT

Purpose: The purpose of this study was to evaluate changes in the oral bacterial flora before and after application of 38% Silver Diamine Fluoride (SDF, manufacturer and city/state) to carious lesions.

Methods: Fifteen children with at least one cavitated carious tooth and one healthy tooth were enrolled and following examination, a carious tooth was selected to receive SDF treatment. Prior to SDF treatment, two supragingival plaque samples were obtained (using sterile cotton swabs, then storage in transport fluid and frozen at -80oC until DNA extraction) at the first appointment, one carious tooth, another from a non-carious tooth. At the four-week post-intervention appointment, the same procedure was repeated (i.e., sample collections and additional SDF treatment). Using Qiagen DNeasy UltraClean Microbial Kit Redwood City, CA 94063, bacterial DNA was extracted, and submitted to the UAB Microbiome Center for analysis. The results of DNA sample analysis resulted in bacterial phyla, genus and in some cases species identification using 16S rDNA sequencing of the V4 region. t-tests were used for statistical comparison. Using the microbiome data, the most abundant bacteria identified overall, we compared for changes between pre and post SDF samples as well as for control (non-carious teeth) samples.

Results: was performed to detect if there is a significant difference in bacterial content and test show no significant difference in bacterial content before and after application, but over time, bacterial content decrease

Keywords: ___

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INTRODUCTION

Oral health is essential to all Americans' general health and well-being, and it is a window into the health of the body. Diseases that affect the entire body can first become apparent because of oral problems such as nutritional deficiencies or widespread infection [hhs.gov]. Many Americans who have the biggest obstacles to getting dental care continue to experience unnecessary pain and many oral health complications [hhs.gov]. The burden of oral disease restricts school, work, and home activities and often significantly diminishes life quality. In the past 50 years, significant progress has been made in understanding the common oral diseases—dental caries (tooth decay) and periodontal (gum) diseases—resulting in marked improvements in the nation's oral health [U.S. Department 2000].

Dental caries is among of the most common chronic diseases in children, it is about five times as common as asthma and seven times as common as hay fever [Benjamin 2010]. Dental caries cannot occur in the absence of dietary fermentable carbohydrates and, therefore, it has been characterized as a “dietobacterial” disease [Domenick T.ZeroDDS 2014]. Oral microorganisms produce acids from metabolism of carbohydrates that result in demineralization of enamel and ultimately dental caries.

According to the extended caries ecological hypothesis, the caries process consists of 3 reversible stages. The microflora on clinically sound (non-carious) enamel surfaces contains mainly streptococci and actinomyces; acidification is mild and infrequent; This is compatible with the equilibrium away from demineralization, and more toward remineralization balance,

i.e., mineral balance shifts toward net gain (dynamic stability stage). When sugar is relatively abundant (especially sucrose) frequently provided, acidification.

The mineral balance shift may enhance the acidogenicity of the bacterial flora to more aciduric species which may, over time, shift the demineralization, remineralization balance toward net mineral loss, leading to the initiation and progression of dental caries (acidogenic stage). The most common bacteria associated with this acidic shift associated with demineralization and dental caries are the mutans streptococci (i.e., Streptococcus mutans and Streptococcus sobrinus) [Krzyściak, W et al 2014]. At this stage, mutans streptococci and later, lactobacilli, as well as other potentially aciduric species, including Actinomyces, bifidobacteria, and yeasts, may become dominant. Many acidogenic and aciduric bacteria are involved in caries. Environmental acidification is the primary determinant of the phenotypic, genotypic changes in the microflora during caries (Takahashi 2011). In contrast, several studies have found species such as Capnocytophaga, Fusobacteria, Tannerella, Phorpyromonas, Abiotrophia, and non-mutans streptococci associated with healthy tooth surfaces (Lif Holgerson 2015).

Since untreated caries can lead to pain, sepsis, infection spread, and poor general health, early treatment of dental caries is essential. However, performing restorative treatment on young patients might be challenging due to the lack of maturity and cooperation. Dental and medical care providers are increasingly involved in early intervention to prevent tooth decay including non-surgical/medical management aimed at arresting caries in young children. The purpose of arresting caries is to slow or stop caries progression. This can also provide time to allow for patient maturity to cope with in-office dental procedures or scheduling of treatment under general anesthesia. Therefore, there is a significant interest in identifying a simple treatment to stop dental lesion progress after their occurrence. Over the years, various topical fluoride agents

have been used to prevent and manage dental caries, which in sequential order are stannous fluoride, acidulated phosphate fluoride and varnish containing fluoride. Fluoride inhibits enamel demineralization. The calcium fluoride that is deposited onto a tooth surface after fluoride therapy is not readily soluble and can act as a fluoride reservoir. This fluoride also can lower the critical pH value of hydroxyapatite crystal dissolution, or the pH value when demineralization occurs, from approximately 5.5 to 4.5 in the mouth.

Fluoride can be incorporated incrementally into fluorapatite crystals on the tooth surface, making the surface more resistant to acid dissolution. In addition to inhibiting demineralization, fluoride enhances enamel remineralization, increasing remineralization process's speed and mineral content of early carious lesions. The incorporation of fluoride also makes the deposited mineral less acid soluble [Sherry Shiqian Gao 2016].

Although fluoride's specific mechanism in caries prevention is not fully understood, topically applied fluorides influence tooth surfaces. Fluoride inhibits plaque metabolism, alters plaque composition, affects plaque the formation, and reduces plaque bacteria's ability to produce acid from carbohydrate metabolism (Gao 2020).

Thirty-eight percent silver diamine fluoride (SDF), a transparent liquid with a high concentration of silver (24–27 w/v%), and fluoride (5–6 w/v%). SDF was cleared by the Food and Drug Administration in 2014 as a treatment for sensitive teeth and used off-label to treat cavities in the United States since 2015. Clinical trials outside the United States have documented caries arrest. Moreover, the preventive benefits extend to un-affected teeth [Contreras, Violeta et al 2017]. Serum concentrations of fluoride and silver after topical application revealed no potential toxicity [Nelson, Suchitra et al 2020].

Silver diamine fluoride has been used to treat cavitated caries lesions for many decades in different parts of the world [Richards, Derek 2017]. The exact mechanisms of action are not well understood. Studies suggested that both fluoride ions and silver ions in SDF contribute to caries arrested reactions (Yamaha R 1972). Silver are ions likely to be primarily responsible for the antimicrobial action of silver diamine fluoride because they inhibit the growth of oral bacteria. Further, silver ions are known to denature enzymes that would breakdown collagenous dentin which is related to the cavitation of progressive dental caries [Richards, Derek 2017]. Mei, M. L. et study indicated that SDF inhibited the development of a multispecies biofilm composed such as S. mutans, S. sobrinus, L. acidophilus, Lactobacillus rhamnosus, and A. naeslundii on an SDF-treated dentine surface [Mei, M. L., et al.2018].

On the other hand, fluoride enhances mineral formation by forming fluorohydroxyapatite with reduced solubility (Milgrom, Peter, et al. 2018). Fluoride also inhibits matrix metalloproteinase activities and therefore inhibits dentine collagen degradation. The combination of silver and fluoride in an alkaline solution has a synergistic effect in arresting dentine caries (Mei, M. L., et al., .2018). This study aims to evaluate the changes of the oral bacterial flora on carious lesions before and after Silver Diamine Fluoride application.

METHODS

This study was conducted at the University of Alabama Birmingham School of Dentistry FINN clinic and Children's of Alabama Hospital dental clinic following approval from The University of Alabama Birmingham Institutional Review Board for Human Use (IRB-300000732).

Inclusion criteria involved thirty healthy children by medical history, between the ages of 2 to 7-year-old, having at least one tooth with untreated cavitated active caries lesion and a tooth with non - cavitation. The caries lesion had to be accessible to hardness assessment, SDF application, and plaque collection. For this study, exclusion criteria are any serious medical condition that could interfere with daily self-care activities, being on antibiotics within the two weeks before the baseline appointment, children with previous SDF application, silver allergy, pulp exposure and pulpitis.

All parents and caregivers were informed of the benefits/ risks of silver diamine fluoride (SDF). After obtaining written informed consent, participants were examined by the principal investigator for their eligibility for inclusion. At the first appointment, two supragingival plaque samples were obtained, one from the carious tooth, another from the non-carious tooth in the patient's mouth, SDF was applied in the carious site, and the plaque samples were frozen at -80 °C. At the following appointment, the same procedure was repeated. DNA extraction was done on plaque samples and sent for Microbiome Analysis to establish any changes in the plaque composition before and after the SDF application.

Plaque sample collection

Eligible study subjects had plaque collected from one randomly selected caries lesion that met the inclusion criteria and non-carious tooth. Plaque samples were collected from each patient before definitive treatment and SDF application at the first visit (baseline) and after a one-month recall visit to compare the microbial composition of bacterial sample pre and post SDF treatment. Bacterial samples from the subject's carious and non- caries tooth were collected with sterile cotton swabs via gently rubbing movement. A cotton swab with samples was placed into a test tube filled with 1ml of reduced transport median (Syed, S A, and W J Loesche 1972). Each lab test was labeled with a coded identifier, stored on ice, transported to the University of Alabama School of Dentistry laboratory, and frozen at -80ºC until DNA extraction was performed.

At the baseline appointment, after plaque collection, 38% Silver Diamine Fluoride (Advantage ArrestTM; Elevate Oral Care LLC, West Palm Beach, FL, USA) was applied to the carious lesion following the manufacturer's recommendation and allowing 1-3 minutes for the SDF to react with the lesion. Patients were instructed not to eat or drink for thirty minutes after treatment. They were also scheduled for one month to follow up appointments. At the follow-up visit (1-month post-SDF application), a second microbial sample was collected and frozen until analyzed, and then the principal investigator evaluated the lesion for hardness. Lesions were considered active if dentin was soft on gentle probing and inactive if dentin was hard on gentle probing.

Sample Analysis

DNA bacterial extraction was done using Qiagen DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany) following manufactures'' instructions. DNA quality and purity were

assessed using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc, Wilmington, DE). After 16s amplification, traditional PCR was performed to confirm the presence of specific oral bacteria, measure the DNA concentration, and conform DNA content. Microbiome analysis was performed by the UAB Microbiome

Center and bacterial identification using the 16S V4 region. Plaque microbial community was compared for changes between the two applications of Silver Diamine Fluoride

The statistical method used was linear regression using generalized estimating equations (GEEs). T-tests performed were the chi-square test by score statistics.

RESULTS

Samples were collected from a total of 30 patients; a number of 15 subgingival plaque samples from carious and non-carious were processed and analyzed. After the one-month application, 38% Silver Diamine Fluoride demonstrated clinical hardness on the carious lesion. The top twenty five most predominant microbial general levels in each group of sample was shown in the bar chart (Fig1); although, Streptococcus, Actinomyces, Neisseira show to be the three most abundant genera in each group there was no statistical significance. Fig 2 shows the most relative abundance of the top ten abundant OTUs at the genus level in each group, also showing streptococcus with 30% of abundance. A t- test performed to assess the difference in bacterial content showed no significant difference in bacterial content before and after application between the samples. The t-test also showed an overall decrease of bacterial content over time.

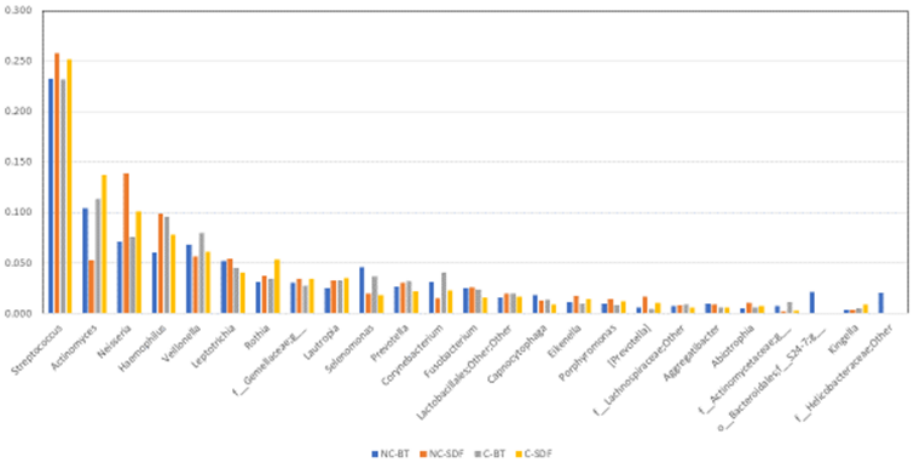

Figure 1. The top 25 genera detected in each group.

Figure 1. Twenty-five most predominant bacteria a genera level in each group. Streptococcus, Actinomyces, Neisseira, Haemophilus, veillonella, leptotrichia, Rothia, f Gemellacea, Lautropia, Corynebacteria, Fusobacterium, Lactobacillales, Capnoctophaga, Eikenella, Prophyromonas, f_ Lachospira, Aggregatibacter, Abiotrophia, f_Actinomyce, o_ Bacteroidal, Kingella, f_Helicobacteria

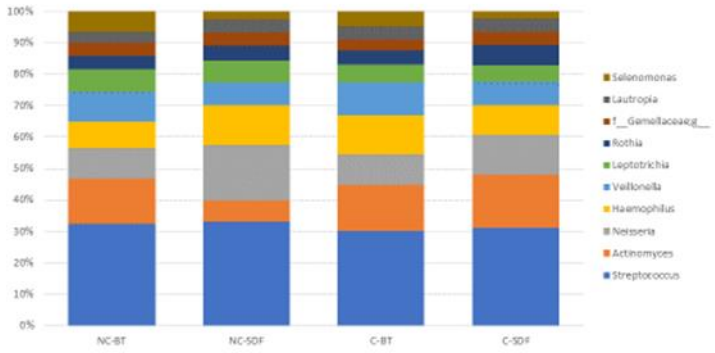

Figure 2. The relative abundance of the top 10 abundant OTUs at the genus level in each group.

Figure 2. Shows the most relative abundance of the top 10 abundant OTUs at the genus level in each group. In this figure we can see that the most abundant genus is streptococcus, actinomyces and Neisseria; streptococcus showing 30% of abundance however it did not reach statistical significance

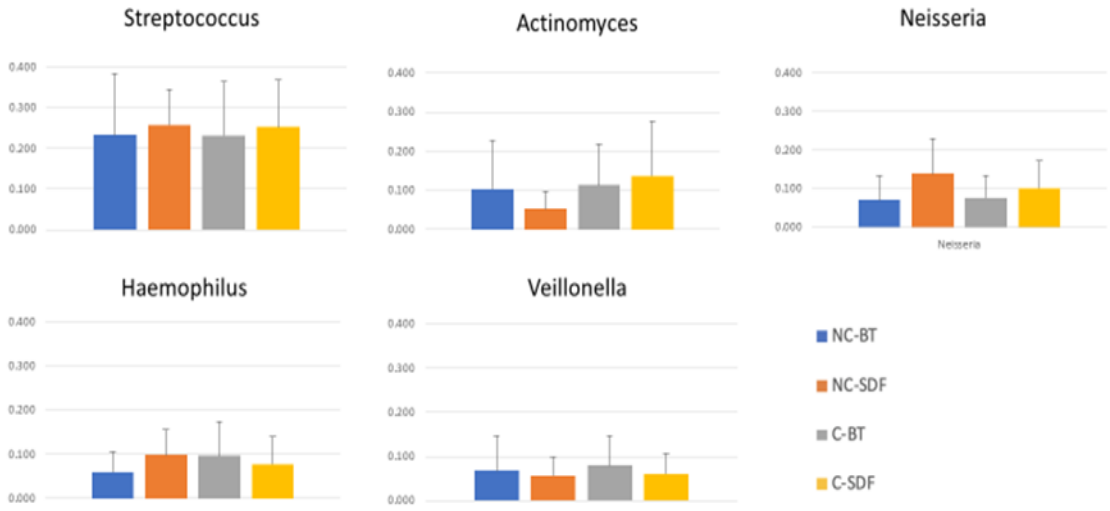

Figure 3. The top 5 abundant genera in each group.

Figure 3 shows the top 5 abundant genera in each group: streptococcus, actinomyces, Neisseria, Haemophilus, Veillonella.

DISCUSSION

The purpose of this study was to assess the effect of Silver Diamond Fluoride treatment on the microbial profile of dental plaque present on coronal carious lesions. We hypothesized that there would be changes in microbial content before and after 38% SDF application.

Unlike other fluoride products that mainly influence the prevention of new caries, 38% SDF can efficiently arrest the caries process; this was proved one more time by carious lesion hardness noted after the treatment of the fluoride product application. This study demonstrated that the Silver Diamine Fluoride does not change the bacterial content on carious lesions which had been proven in previous studies. The statistical analysis did not show a significant difference between the before and after applications. However, the statistical results may be due to changes in confounding factors that change plaque content before and after SDF application.

There were likely many factors that could have affected the results in this study, first in the sample collection after 1 month, the microbial content may have changed and recolonized after SDF application; second, the patient's oral hygiene before plaque collection on the first and second appointments may have compromised the result of this study. It has been hypothesized that oral hygiene and the use of fluoridated toothpaste affects the arresting action of SDF. In fact, it has been found that lesions with visible plaque and large lesions have a lower chance of being arrested [Fung et al., 2016]. In 2016, it was reported that caries lesions treated with SDF might reactivate within the year if salivary function and oral hygiene are poor [Deutsch, 2016].

In conclusion, although topical application of SDF showed clinical caries arrested by the hardness of the carious lesions after a single application. This study showed that there was no correlation between the clinical result and the microbial content. There was not a significant difference in the microbial abundance before and after application of the SDF. The t-test performed to assess the difference in bacterial content showed no significant difference in bacterial content between the samples before and after application of the SDF.

LIST OF REFERENCES

Benjamin, Regina M. “Oral Health: The Silent Epidemic.” Public Health Reports 125.2 (2010): 158–159.

Mei, M. L., et al. “Arresting Dentine Caries with Silver Diamine Fluoride: What’s Behind It?” Journal of Dental Research, vol. 97, no. 7, July 2018, pp. 751–758, doi:10.1177/0022034518774783.

Yamaha R, Nishino M, Yoshida S, Yokomizo I, Diamine silver fluoride and its clinical application. The journal of Osaka University Dental School 1972; 12:1-20.

Peng JJ, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: A review J Dent. 2012 Jul;40(7):531-41.

M.L. Mei, Q.-L. Li, C.-H. Chu, E.C.-M. Lo, L.P. Samaranayake, Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries, Ann. Clin. Microbiol. Antimicrob. 12 (2013)

Milgrom, Peter et al. “Topical silver diamine fluoride for dental caries arrest in preschool children: A randomized controlled trial and microbiological analysis of caries associated microbes and resistance gene expression.” Journal of dentistry vol. 68 (2018): 72-78. doi: 10.1016/j.jdent.2017.08.015

G.M. Knight, J.M. McIntyre, G.G. Craig, Mulyani, P.S. Zilm, N.J. Gully, Inability to form a biofilm of Streptococcus mutans on silver fluoride- and potassium iodidetreated demineralized dentin, Quintessence Int. 40 (2009) 155–161.

Rosenblatt A, Stamford TC, Niederman R Silver Diamine Fluoride: A caries “Silver- Fluoride bullet” J Dent Res. 2009 Feb;88(2):116-25.

Zander H, Smith H. Penetration of silver nitrate into dentin II Journal of dental research 1945; 24:121-8.

Thibodeau EA, Handyman SL, Marquis RE Inhibition and killing of oral bacteria by silver ions generated with low intensity direct current. J Dent Res 1978; 57:922-926

Craig GG, Powell KR, Copper MH. Caries progression in primary molars: 24-mpnth results from a minimal treatment program. Community Dentistry and Oral Epidemiology 1981; 9:260-5. Dentistry for Children. 1987:170-5

Suzuki T, Sobue S, Suginaka H. Mechanism of anti-plaque action of diamine silver fluoride. The journal of Osaka University Dental School 1976; 16:87-95.

Hiraishi N, You CKY, King NM, Tatami J, Tay FR. Antimicrobial efficacy of 3.8% silverdiamine fluoride and its effect on root dentin. Journal of Endodontics 2010; 36:1026-9

Spacciapoli P, Buxton D, Rothstein D, Friden P. Antimicrobial activity of silver nitrate against periodontal pathogens. J Periodontal Res. 2001 Apr;36(2):108-13.

Silvestry-Rodriguez N, Bright KR, Slack DC, Uhlmann DR, Gerba CP. Silver as a residual disinfectant to prevent biofilm formation in water distribution systems. Appl Environ Microbiol. 2008 Mar;74(5):1639-41.

Syed, S A, and W J Loesche. "Survival of human dental plaque flora in various transport media." Applied microbiology vol. 24,4 (1972): 638-44

https://www.hhs.gov/surgeongeneral/priorities/oral-health/index.html

U.S. Department of Health and Human Services (USDHHS). Healthy People 2010 (Conference Edition, in two volumes). Washington; 2000 Jan.

H, Mourao M, D, A, Dennison J, Yaman P, Paster B, J, Fontana M: Effect of Silver Diamine Fluoride Treatment on Microbial Profiles of Plaque Biofilms from Root/Cervical Caries Lesions

Lif Holgerson, Pernilla et al. "Maturation of Oral Microbiota in Children with or without Dental Caries." PloS one vol. 10,5 e0128534. 28 May. 2015, doi: 10.1371/journal.pone.0128534

Takahashi, N., and B. Nyvad. "The Role of Bacteria in the Caries Process: Ecological Perspectives." Journal of Dental Research, vol. 90, no. 3, Mar. 2011, pp. 294–303, doi:10.1177/0022034510379602.

Shah, Shalin et al. "Efficacy of Silver Diamine Fluoride as an Antibacterial as Well as Antiplaque Agent Compared to Fluoride Varnish and Acidulated Phosphate Fluoride Gel: An in Vivo study. (Original Research)." Indian Journal of Dental Research 24.5 (2013): 575–81. Web.

Gao, Sherry Shiqian, et al. "Caries remineralisation and arresting effect in children by professionally applied fluoride treatment - a systematic review." BMC Oral Health, vol. 16, no. 10, 2016. Gale Academic OneFile, https://link-gale-com.ezproxy3.lhl.uab.edu/apps/doc/A443251205/AONE?u=birm97026&sid=AONE&xid=29e86bbf. Accessed 18 Mar. 2020.

APPENDIX A

DESCRIPTIVE STATISTICS

Group	Frequency	Percent	Cumulative Frequency	Cumulative Percent
C	30	50.00	30	50.00
NC	30	50.00	60	100.00

Time	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	30	50.00	30	50.00
2	30	50.00	60	100.00

Group	Time	N Obs	Variable	N	Mean	Std Dev	Quartile Median	Range
C	1	15	Bacteria_1	15	0.2381	0.1323	0.2239	0.1865
			Bacteria_2	15	0.1108	0.1026	0.0700	0.1407
			Bacteria_3	15	0.0731	0.0556	0.0683	0.0954
			Bacteria_4	15	0.0918	0.0750	0.0705	0.1045
			Bacteria_5	15	0.0774	0.0657	0.0559	0.1279
			Bacteria_6	15	0.0449	0.0489	0.0284	0.0342
			Bacteria_7	15	0.0346	0.0386	0.0192	0.0440
			Bacteria_8	15	0.0323	0.0350	0.0160	0.0319
			Bacteria_9	15	0.0259	0.0202	0.0221	0.0174
			Bacteria_10	15	0.0355	0.0774	0.0168	0.0239
			Bacteria_11	15	0.0311	0.0379	0.0136	0.0696
			Bacteria_12	15	0.0386	0.0458	0.0215	0.0360
			Bacteria_13	15	0.0234	0.0303	0.0108	0.0332
			Bacteria_14	15	0.0205	0.0140	0.0164	0.0193
			Bacteria_15	15	0.0137	0.0131	0.0113	0.0211
			Bacteria_16	15	0.0093	0.0092	0.0052	0.0140
			Bacteria_17	15	0.0095	0.0119	0.0041	0.0121
		20						
			Bacteria_18	15	0.0043	0.0051	0.0030	0.0060
			Bacteria_19	15	0.0085	0.0158	0.0023	0.0056
			Bacteria_20	15	0.0055	0.0084	0.0026	0.0055
			Bacteria_21	15	0.0054	0.0062	0.0030	0.0059
			Bacteria_22	15	0.0107	0.0201	0.0024	0.0120

	Bacteria_23	15	0.0002	0.0002	0.0002	0.0002
	Bacteria_24	15	0.0046	0.0036	0.0026	0.0071
	Bacteria_25	15	0.0036	0.0038	0.0027	0.0023
	Bacteria_26	15	0.0037	0.0024	0.0039	0.0047
	Bacteria_27	15	0.0000	0.0000	0.0000	0.0000
	Bacteria_28	15	0.0041	0.0055	0.0018	0.0037
	Bacteria_29	15	0.0044	0.0059	0.0017	0.0040
	Bacteria_30	15	0.0035	0.0067	0.0005	0.0015
	Bacteria_31	15	0.0023	0.0023	0.0015	0.0020
	Bacteria_32	15	0.0022	0.0022	0.0018	0.0034
	Bacteria_33	15	0.0025	0.0066	0.0001	0.0023
	Bacteria_34	15	0.0017	0.0003	0.0017	0.0004
	Bacteria_35	15	0.0001	0.0002	0.0001	0.0002
	Bacteria_36	15	0.0009	0.0012	0.0004	0.0012
	Bacteria_37	15	0.0014	0.0032	0.0002	0.0009
	Bacteria_38	15	0.0000	0.0000	0.0000	0.0000
	Bacteria_39	15	0.0004	0.0002	0.0004	0.0003
	Bacteria_40	15	0.0012	0.0017	0.0008	0.0012
	Bacteria_41	15	0.0021	0.0055	0.0003	0.0007
	Bacteria_42	15	0.0010	0.0011	0.0006	0.0018
	Bacteria_43	15	0.0007	0.0006	0.0005	0.0008
	Bacteria_44	15	0.0000	0.0001	0.0000	0.0000
	Bacteria_45	15	0.0005	0.0004	0.0003	0.0007
	Bacteria_46	15	0.0002	0.0004	0.0000	0.0006
	Bacteria_47	15	0.0006	0.0013	0.0002	0.0003
	Bacteria_48	15	0.0006	0.0006	0.0004	0.0006
	Bacteria_49	15	0.0000	0.0000	0.0000	0.0000
	Bacteria_50	15	0.0004	0.0004	0.0002	0.0006
2	15	Bacteria_1	15	0.2558	0.1148	0.1987	0.1597
		Bacteria_2	15	0.1327	0.1339	0.0640	0.2305
		Bacteria_3	15	0.0967	0.0677	0.0948	0.1119
		Bacteria_4	15	0.0747	0.0587	0.0678	0.1117
		Bacteria_5	15	0.0590	0.0446	0.0561	0.0898
		Bacteria_6	15	0.0401	0.0487	0.0163	0.0649
		Bacteria_7	15	0.0535	0.0527	0.0324	0.0935
		Bacteria_8	15	0.0337	0.0469	0.0176	0.0281
		Bacteria_9	15	0.0324	0.0325	0.0162	0.0395
		Bacteria_10	15	0.0176	0.0385	0.0023	0.0103
		Bacteria_11	15	0.0222	0.0259	0.0113	0.0293
		Bacteria_12	15	0.0221	0.0187	0.0132	0.0370
		Bacteria_13	15	0.0151	0.0142	0.0111	0.0208
		Bacteria_14	15	0.0173	0.0139	0.0117	0.0165
		Bacteria_15	15	0.0087	0.0096	0.0027	0.0164
		Bacteria_16	15	0.0137	0.0162	0.0063	0.0252
		Bacteria_17	15	0.0132	0.0213	0.0028	0.0217

			Bacteria_18	15	0.0103	0.0166	0.0032	0.0167
			Bacteria_19	15	0.0060	0.0079	0.0039	0.0057
			Bacteria_20	15	0.0055	0.0063	0.0035	0.0065
			Bacteria_21	15	0.0072	0.0073	0.0053	0.0043
			Bacteria_22	15	0.0029	0.0094	0.0002	0.0006
			Bacteria_23	15	0.0006	0.0011	0.0001	0.0006
			Bacteria_24	15	0.0085	0.0150	0.0047	0.0086
			Bacteria_25	15	0.0054	0.0049	0.0035	0.0083
			Bacteria_26	15	0.0043	0.0042	0.0034	0.0063
			Bacteria_27	15	0.0000	0.0001	0.0000	0.0000
			Bacteria_28	15	0.0025	0.0024	0.0015	0.0028
			Bacteria_29	15	0.0027	0.0039	0.0006	0.0036
			Bacteria_30	15	0.0015	0.0017	0.0009	0.0013
			Bacteria_31	15	0.0025	0.0021	0.0012	0.0027
			Bacteria_32	15	0.0023	0.0020	0.0021	0.0020
			Bacteria_33	15	0.0003	0.0008	0.0001	0.0003
			Bacteria_34	15	0.0019	0.0005	0.0017	0.0008
			Bacteria_35	15	0.0035	0.0120	0.0002	0.0010
			Bacteria_36	15	0.0005	0.0006	0.0003	0.0007
			Bacteria_37	15	0.0006	0.0010	0.0001	0.0006
			Bacteria_38	15	0.0000	0.0000	0.0000	0.0000
			Bacteria_39	15	0.0025	0.0074	0.0005	0.0006
			Bacteria_40	15	0.0007	0.0009	0.0005	0.0008
			Bacteria_41	15	0.0003	0.0005	0.0000	0.0004
			Bacteria_42	15	0.0004	0.0005	0.0003	0.0004
			Bacteria_43	15	0.0009	0.0008	0.0004	0.0008
			Bacteria_44	15	0.0003	0.0008	0.0000	0.0002
			Bacteria_45	15	0.0006	0.0007	0.0003	0.0010
			Bacteria_46	15	0.0004	0.0009	0.0000	0.0003
			Bacteria_47	15	0.0007	0.0013	0.0003	0.0006
			Bacteria_48	15	0.0008	0.0010	0.0004	0.0008
			Bacteria_49	15	0.0019	0.0067	0.0000	0.0002
			Bacteria_50	15	0.0007	0.0014	0.0002	0.0007
N	1	15	Bacteria_1	15	0.2370	0.1525	0.2290	0.2208
			Bacteria_2	15	0.1012	0.1224	0.0742	0.0578
			Bacteria_3	15	0.0683	0.0612	0.0602	0.0776
			Bacteria_4	15	0.0577	0.0438	0.0426	0.0483
			Bacteria_5	15	0.0661	0.0752	0.0348	0.0776
			Bacteria_6	15	0.0516	0.0396	0.0416	0.0707
			Bacteria_7	15	0.0308	0.0358	0.0180	0.0389
			Bacteria_8	15	0.0248	0.0233	0.0156	0.0428
			Bacteria_9	15	0.0289	0.0227	0.0311	0.0298
			Bacteria_10	15	0.0451	0.0925	0.0148	0.0600
			Bacteria_11	15	0.0264	0.0278	0.0143	0.0456
			Bacteria_12	15	0.0300	0.0321	0.0222	0.0355

	Bacteria_13	15	0.0245	0.0174	0.0243	0.0232
	Bacteria_14	15	0.0167	0.0133	0.0189	0.0205
	Bacteria_15	15	0.0178	0.0203	0.0085	0.0186
	Bacteria_16	15	0.0110	0.0098	0.0106	0.0150
	Bacteria_17	15	0.0105	0.0125	0.0087	0.0162
	Bacteria_18	15	0.0056	0.0102	0.0014	0.0057
	Bacteria_19	15	0.0077	0.0099	0.0035	0.0074
	Bacteria_20	15	0.0091	0.0109	0.0042	0.0181
	Bacteria_21	15	0.0049	0.0045	0.0033	0.0078
	Bacteria_22	15	0.0069	0.0082	0.0041	0.0106
	Bacteria_23	15	0.0204	0.0781	0.0002	0.0002
	Bacteria_24	15	0.0036	0.0041	0.0035	0.0032
	Bacteria_25	15	0.0056	0.0063	0.0024	0.0101
	Bacteria_26	15	0.0031	0.0022	0.0030	0.0026
	Bacteria_27	15	0.0158	0.0610	0.0000	0.0000
	Bacteria_28	15	0.0049	0.0040	0.0037	0.0079
	Bacteria_29	15	0.0047	0.0060	0.0018	0.0069
	Bacteria_30	15	0.0050	0.0130	0.0010	0.0030
	Bacteria_31	15	0.0028	0.0023	0.0019	0.0035
	Bacteria_32	15	0.0022	0.0024	0.0014	0.0026
	Bacteria_33	15	0.0052	0.0186	0.0001	0.0004
	Bacteria_34	15	0.0023	0.0019	0.0018	0.0006
	Bacteria_35	15	0.0036	0.0129	0.0001	0.0002
	Bacteria_36	15	0.0041	0.0118	0.0004	0.0020
	Bacteria_37	15	0.0015	0.0023	0.0007	0.0022
	Bacteria_38	15	0.0043	0.0166	0.0000	0.0000
	Bacteria_39	15	0.0008	0.0010	0.0003	0.0010
	Bacteria_40	15	0.0009	0.0009	0.0007	0.0015
	Bacteria_41	15	0.0007	0.0009	0.0001	0.0011
	Bacteria_42	15	0.0007	0.0007	0.0003	0.0015
	Bacteria_43	15	0.0008	0.0005	0.0007	0.0009
	Bacteria_44	15	0.0022	0.0084	0.0000	0.0001
	Bacteria_45	15	0.0006	0.0004	0.0005	0.0006
	Bacteria_46	15	0.0010	0.0022	0.0001	0.0007
	Bacteria_47	15	0.0006	0.0013	0.0003	0.0004
	Bacteria_48	15	0.0005	0.0004	0.0003	0.0005
	Bacteria_49	15	0.0002	0.0004	0.0000	0.0001
	Bacteria_50	15	0.0003	0.0003	0.0002	0.0004
2	15Bacteria_1	15	0.2631	0.0873	0.2686	0.1310
	Bacteria_2	15	0.0507	0.0430	0.0455	0.0458
	Bacteria_3	15	0.1345	0.0878	0.1508	0.1662
	Bacteria_4	15	0.0938	0.0547	0.1017	0.0883
	Bacteria_5	15	0.0547	0.0415	0.0417	0.0636
	Bacteria_6	15	0.0536	0.0624	0.0291	0.0382
	Bacteria_7	15	0.0364	0.0442	0.0245	0.0419

Bacteria_8	15	0.0314	0.0352	0.0171	0.0517
Bacteria_9	15	0.0328	0.0236	0.0321	0.0337
Bacteria_10	15	0.0189	0.0328	0.0070	0.0270
Bacteria_11	15	0.0309	0.0381	0.0284	0.0291
Bacteria_12	15	0.0143	0.0119	0.0168	0.0155
Bacteria_13	15	0.0253	0.0160	0.0255	0.0259
Bacteria_14	15	0.0199	0.0096	0.0236	0.0187
Bacteria_15	15	0.0123	0.0117	0.0075	0.0140
Bacteria_16	15	0.0172	0.0158	0.0139	0.0127
Bacteria_17	15	0.0163	0.0264	0.0049	0.0086
Bacteria_18	15	0.0160	0.0391	0.0016	0.0140
Bacteria_19	15	0.0079	0.0135	0.0029	0.0062
Bacteria_20	15	0.0088	0.0072	0.0062	0.0148
Bacteria_21	15	0.0103	0.0122	0.0060	0.0112
Bacteria_22	15	0.0019	0.0039	0.0002	0.0018
Bacteria_23	15	0.0002	0.0002	0.0001	0.0002
Bacteria_24	15	0.0030	0.0033	0.0022	0.0025
Bacteria_25	15	0.0049	0.0043	0.0046	0.0087
Bacteria_26	15	0.0048	0.0033	0.0046	0.0041
Bacteria_27	15	0.0000	0.0000	0.0000	0.0000
Bacteria_28	15	0.0035	0.0036	0.0025	0.0050
Bacteria_29	15	0.0028	0.0028	0.0021	0.0026
Bacteria_30	15	0.0038	0.0060	0.0026	0.0032
Bacteria_31	15	0.0041	0.0035	0.0024	0.0037
Bacteria_32	15	0.0026	0.0018	0.0032	0.0027
Bacteria_33	15	0.0005	0.0012	0.0000	0.0001
Bacteria_34	15	0.0017	0.0004	0.0016	0.0005
Bacteria_35	15	0.0003	0.0004	0.0001	0.0003
Bacteria_36	15	0.0006	0.0007	0.0002	0.0009
Bacteria_37	15	0.0015	0.0020	0.0007	0.0016
Bacteria_38	15	0.0000	0.0000	0.0000	0.0000
Bacteria_39	15	0.0004	0.0002	0.0003	0.0003
Bacteria_40	15	0.0007	0.0007	0.0005	0.0011
Bacteria_41	15	0.0004	0.0006	0.0001	0.0006
Bacteria_42	15	0.0011	0.0011	0.0008	0.0016
Bacteria_43	15	0.0009	0.0005	0.0010	0.0008
Bacteria_44	15	0.0001	0.0001	0.0000	0.0000
Bacteria_45	15	0.0007	0.0006	0.0005	0.0010
Bacteria_46	15	0.0006	0.0013	0.0000	0.0012
Bacteria_47	15	0.0003	0.0005	0.0001	0.0002
Bacteria_48	15	0.0003	0.0002	0.0002	0.0002
Bacteria_49	15	0.0001	0.0001	0.0000	0.0000
Bacteria_50	15	0.0006	0.0008	0.0003	0.0007

APPENDIX B

INVESTIGATOR'S PROGRESS REPORT

Investigator's Progress Report

Form version July 30, 2015

☒ Continuing Review (Complete Items 1-12)
—OR—
☐ Final Report—all protocol-related activities are complete, including data analysis (Complete Items 1-11, and Item 13)

—FOR—

☒ Expedited Review
—OR—
☐ Convened (Full) Review

1. Dates

Today's Date	27-JAN- 2020	To help avoid delay, respond to all required items in the format provided, and include requested materials.
Starting Date of Project	19-Mar-2018	If previous approval expires before approval is officially re-issued by the Office of the IRB, all work on the protocol must cease.
Current IRB Expiration Date	24-JAN-2020	The IRB recommends applying for continuing review *4-6 weeks* before expiration of current approval. (See schedule.)

2. Principal Investigator (PI)

Name (with degree)	Karol F Bryant	**Blazer ID**	Kbryant5
Department	UAB Pediatric Dentistry	**Division**	
Office Address	3545 Grandview Pkwy # 127 Birmingham, Al 35243	**Office Phone**	8325771586
E-mail	kbryant5@uab.edu		
PI Contact who should receive copies of IRB correspondence (Optional)			
Name		**E-mail**	
Phone			

3. UAB IRB Protocol Identification

Protocol Number	300000732
Protocol Title	Evaluation the Effect of Silver Diamine Fluoride on Biofilm of Carious lesions
Study Sponsor(s)	UAB School of Dentistry, Department of Pediatric Dentistry
OSP Assigned Number (9 digits)	
Note. *If the source or amount of funding for this project has changed or a new OSP # has been assigned to the protocol, include the new or revised funding application and/or provide the new OSP Assigned Number:*	

4. Purpose

In two or three sentences, briefly summarize the purpose of this protocol, and related studies if applicable. Please use non-technical language, and write for adults with general knowledge rather than for specialists.

▶ The purpose of this protocol is to evaluate the changes of mouth bacteria on dental cavities before and after the application of Silver Diamine Fluoride

5. Screened, entered, or otherwise accessed by the UAB Investigator(s). Include numbers for individuals, specimens, data records, charts, etc., as applicable to the protocol.

5.a. Number screened for study entry since the start of the project?	80
5.b. Number entered in study since the start of the project? (See Total in 5.e.)	30
5.c. Number entered in study since the last IRB review?	20
5.d. What is the age range for all participants entered in the study since the start of the project (e.g., 18-65)?	0-14 year old

APPENDIX C

CONSENT FORM

CONSENT

Title of Research: Evaluation the Effect of Silver Diamine Fluoride on Biofilm of Carious Lesions

UAB IRB Protocol #: IRB-300000732

Principal Investigator: Karol F Bryant, DDS

Sponsor: UAB School of Dentistry, Department of Pediatric Dentistry

Purpose of the Research

We are asking your child to take part in a research study because your dentist has decided to use a fluoride treatment that uses Silver Diamine Fluoride (SDF). SDF is a non-invasive approach of dental caries treatment. We are interested in finding out if the use of SDF affects the bacteria in your mouth

The purpose of this research study is to evaluate the effect of Silver Diamine Fluoride (SDF) on small dental cavities on bacteria in your child's mouth. We will enroll 60 participants at UAB and Children's of Alabama.

Explanation of Procedures

If you agree to join the study your child will:

• Prior to the application of Silver Diamine Fluoride, we will collect two samples of the plaque in your child's mouth by gently rubbing a cotton swab in a cavity area and one on a non-cavity site.

• The rest of your appointment will be the regular standard of care for your child's treatment, including the application of the Silver Diamine Fluoride.

• You will be asked to allow us to collect samples from your child's mouth over 2 visits that will occur 4 weeks apart. Both visits are clinical visits. The additional samples will be collected at your child's next scheduled appointment.

• Prior to the clinical re-evaluation of dental cavities at 2nd visit, we will collect two samples of the plaque in your child's mouth by gently rubbing a cotton swab in a cavity area and one on a non-cavity site.

• The rest of your child's appointment will be regular standard of care for your child's treatment.

Risks and Discomforts
There are no known risks for your participation in this study except for the low potential for loss of confidentiality.

Benefits
Your child may not benefit from taking part in this study; however, your participation may help us to better understand how bacteria changes in your mouth with SDF treatment.

Alternatives
Your alternative is to not participate.

Confidentiality
Information obtained about you for this study will be kept confidential to the extent allowed by law. However, research information that identifies you may be shared with people or organizations for quality assurance or data analysis, or with those responsible for ensuring compliance with laws and regulations related to research. They include:

- the UAB Institutional Review Board (IRB). An IRB is a group that reviews the study to protect the rights and welfare of research participants.
- the Office for Human Research Protections (OHRP

The information from the research may be published for scientific purposes; however, your identity will not be given out.

Voluntary Participation and Withdrawal
Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in the study. If you decide not to be in the study, you will not lose any benefits. You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your relationship with this institution

Cost of Participation
There will be no cost to you for taking part in this study. All samples collected related to this study will be analyzed at no cost to you.
The costs of your standard medical care will be billed to you and/or your insurance company in the usual manner.

Payment for Participation in Research
You will not be paid for your participation in this research study.

Optional Research
Please note: This section of the consent form is about optional research that is being done with people who are taking part in this study. You may take part in this optional research if you want to. You can still be a part of this study even if you say no to taking part in any of the optional research.

You can say "yes" or "no" to each of the following studies. Please mark your choice for each study.

Storage of Specimens for Future Use

As part of this study, we would like to store some of the saliva specimens collected from you for future dental caries research. The future research may be conducted by the study doctor or by other researchers that obtain IRB approval for their research. The specimens will be labeled with a code that only the study doctor can link back to you. Results of any future research will not be given to you or your doctor. You do not have to agree to allow your specimens to be stored in order to be part of this study.

You may request at any time that your specimens be removed from storage and not be used for future research. If you decide you want your specimens removed, you may contact the study doctor. Once the request is received, and if your specimens have not already been used for another research, they will be destroyed. If you do not make such a request, your specimens will be stored indefinitely or until used.

Initial your choice below:

___ I agree to allow my specimens to be kept and used for future research on dental caries.

___ I do not agree to allow my specimens to be kept and used for future research.

Questions

If you have any questions, concerns, or complaints about the research or a research-related injury including available treatments, please contact the study doctor. You may contact Dr. Bryant at (832)577-1586

If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the UAB Office of the IRB (OIRB) at (205) 934-3789 or toll free at 1-855-860-3789. Regular hours for the OIRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday.

Legal Rights

You are not waiving any of your legal rights by signing this consent form

Signatures

Your signature below indicates that you have read (or been read) the information provided above and agree to participate in this study. You will receive a copy of this signed consent form.

_____

Signature of Parent or Guardian Date

Signature of Principal Investigator Reviewing Consent Document Date

Waiver of Assent

The assent of _____ (name of child/minor) was waived because of:

Age _____ Maturity _____ Psychological state of the child _____

APPENDIX D

UNIVERSITY OF ALABAMA AT BIRMINGHAM AUTHORIZATION FOR USE/DISCLOSURE OF PROTECTED HEALTH INFORMATION (PHI) FOR RESEARCH

UNIVERSITY OF ALABAMA AT BIRMINGHAM
AUTHORIZATION FOR USE/DISCLOSURE OF PROTECTED HEALTH INFORMATION (PHI) FOR RESEARCH

Participant Name: _____
UAB IRB Protocol Number: IRB300000732
Research Protocol: Evaluation the Effect of Silver Diamine Fluoride on Biofilm of Carious Lesions
Principal Investigator: Karol F Bryant, DDS
Sponsor: UAB School of Dentistry

What is the purpose of this form? You are being asked to sign this form so that UAB may use and release your protected health information for research. Participation in research is voluntary. If you choose to participate in the research, you must sign this form so that your protected health information may be used for the research.

Why do the researchers want my protected health information? The researchers want to use your protected health information as part of the research protocol listed above and as described to you in the informed consent.

What protected health information do the researchers want to use? All medical information, including but not limited to information and/or records of any diagnosis or treatment of disease or condition, which may include sexually transmitted diseases (e.g., HIV, etc.) or communicable diseases, drug/alcohol dependency, etc.; all personal identifiers, including but not limited to your name, social security number, medical record number, date of birth, dates of service, etc.; any past, present, and future history, examinations, laboratory results, imaging studies and reports and treatments of whatever kind, including but not limited to drug/alcohol treatment, psychiatric/psychological treatment; financial/billing information, including but not limited to copies of your medical bills, and any other information related to or collected for use in the research protocol, regardless of whether the information was collected for research or non-research (e.g., treatment) purposes.

Who will disclose, use and/or receive my protected health information? All Individuals/entities listed in the informed consent documents, including but not limited to, the physicians, nurses and staff and others performing services related to the research (whether at UAB or elsewhere); other operating units of UAB, HSF, UAB Highlands, Children's of Alabama, Eye Foundation Hospital, and the Jefferson County Department of Health, as necessary for their operations; the IRB and its staff; the sponsor of the research and its employees and agents, including any CRO; and any outside regulatory agencies, such as the Food and Drug Administration, providing oversight or performing other legal and/or regulatory functions for which access to participant information is required.

How will my protected health information be protected once it is given to others? Your protected health information that is given to the study sponsor will remain private to

the extent possible, even though the study sponsor is not required to follow the federal privacy laws. However, once your information is given to other organizations that are not required to follow federal privacy laws, we cannot assure that the information will remain protected.

How long will this Authorization last? Your authorization for the uses and disclosures described in this Authorization does not have an expiration date.

Can I cancel this Authorization? You may cancel this Authorization at any time by notifying the Principal Investigator, in writing, referencing the research protocol and IRB Protocol Number. If you cancel this Authorization, the study doctor and staff will not use any new health information for research. However, researchers may continue to use the protected health information that was provided before you cancelled your authorization.

Can I see my protected health information? You have a right to request to see your protected health information. However, to ensure the scientific integrity of the research, you will not be able to review the research information until after the research protocol has been completed.

Signature of participant: _____Date:_____

or participant's legally authorized representative: _____ Date: _____

Printed Name of participant's representative: _____

Relationship to the participant: _____