The effect of silver diamine fluoride (SDF) on bacteria involved in root or cervical carious lesions using HOMINGS technology. A clinical study

Heba Mitwalli, DDS

Version 2.0

11 November 2016

Thesis Committee:

Margherita Fontana, DDS, PhD, Chair

Joseph Dennison, DDS, MS

Peter Yaman, DDS, MS

Bruce Paster, PhD, Department of Microbiology, The Forsyth Institute, Cambridge, MA

Introduction:

The World Health Organization recommends that countries urgently need to adopt strategies for improving the oral health of elderly populations and recognizes that there is a universal need for prevention of oral diseases such as root caries.[1] Root caries is common in institutionalized elders and periodontal patients with exposed root surfaces. Effective prevention methods are needed.[2] The use of fluoride releasing agents as a means of preventing dental caries has long been recognized, and amongst these agents silver diamine fluoride (SDF) has received considerable attention as a caries-controlling agent of choice.[3]

Clinical trials have demonstrated that silver diamine fluoride (SDF) can arrest caries.[4-6] However, the mechanism of action is not yet fully known.[7] Previous studies on the mechanisms of SDF have mostly focused on limited antimicrobial effects and mineral changes in tooth structure.[7-9] SDF is used in many countries and has been approved in the USA as "Advantage Arrest" manufactured by Elevate Oral Care company.

SDF contains high concentrations of silver and fluoride ions which can inhibit the growth of multi-species cariogenic biofilms.[10] The concentration

of 38% silver diamine fluoride (SDF) has been documented to arrest dental caries by reducing the demineralization process.[11 -16] SDF is a clear liquid. Several studies in children reported that in rare cases SDF might cause gingival/mucosal irritation, which heals spontaneously within 48 hours.[11,17-19] Most studies recommend using SDF once a year to arrest caries, but applications every 6 months, or once every 2-3 years have also been tested. Furthermore, the need for maintaining salivary function and oral hygiene has been emphasized to avoid reactivation of treated caries. In 2016, Deutsch reported that caries lesions might reactivate within the year if salivary function and oral hygiene is poor.[20]

A literature review of SDF suggested that 38% SDF can be an effective agent in preventing new caries and arresting dental caries in the primary teeth of children.[21] Another review concluded that SDF is an effective, efficient, and equitable caries-control agent.[22] Milgrom and Chi (2011) advocated SDF therapy as an important prevention-centered caries management strategy during critical early childhood periods.[23] The clinical trial by dos Santos et al. (2012) concluded that 38 % SDF was better than interim restorative treatment with a glass ionomer for arresting cavitated lesions in early childhood caries (ECC).[24]

A randomized clinical study used a 38% SDF solution among institutionalized elderly subjects and showed that it was more effective in preventing new root caries lesions than giving oral hygiene instruction (OHI) alone (Tan et al., 2010).[3] Another clinical study reported for the first time that synergetic applications of SDF solution and oral health education (OHE) annually was more effective than giving OHI alone in preventing and arresting root caries among community-dwelling elderly subjects. There was a trend of less new root caries development among those who received synergetic applications of SDF and OHE compared to those who received SDF alone.[25]

Since root caries is caused by plaque bacteria that adhere to exposed root surfaces (Berry et al., 2004), providing instructions on how to improve oral hygiene would theoretically be beneficial.[26] Investigating the effects of SDF agents on plaque bacteria will allow better understanding of SDF's mechanism of action on root caries arrest.

The International Caries Detection and Assessment System (ICDAS) will be used to score carious lesions. The ICDAS criteria include early and later stage caries severity, and instructions for standard examination processes. ICDAS includes scores from 0 (sound) to 6 (extensive distinct cavity with visible dentin).

Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) utilizes the speed and efficiency of next generation sequencing combined with the refinement of species-level identification. Nearly 600 oral bacterial taxa are identified at the Species-level and Genus-level identification of remaining sequences for 129 genera. HOMINGS allows identification and comparison of bacterial associations in oral health and disease, including different types of periodontitis, caries, gingivitis, ventilator-associated pneumonia, endodontic and odontogenic lesions, abscesses, and halitosis. Also, HOMINGS can determine the efficacy of therapies/treatments, e.g., mouth rinses, antibiotic treatment, scaling and root planning, and laser or periodontal surgery. HOMINGS will be utilized for microbial analysis in this study.

Research Question:

What is the mechanism of action of silver diamine fluoride (SDF) on bacteria involved in root or cervical carious lesions using human oral microbe identification microarray (HOMINGS) technology?

Objective:

To **assess** the effect of SDF treatment on a large array of microorganisms present in root or cervical carious lesions using HOMINGS technology. This will be accomplished by assessing microbial deposits on root or cervical caries lesions before and 1 month after SDF application.

Rationale:

Older people that have caries-active disease experience root caries at a rate which is at least as great as that of coronal caries among adolescents.[27]

The use of fluoride releasing agents as a means of preventing dental caries has long been recognized, and amongst these agents silver diamine fluoride (SDF) has received considerable attention as a caries-arresting agent of choice.[28]

Silver diamine fluoride outperforms other anticaries medicaments such as chlorhexine, NaOCl-based gel (Carisolv), and an enzyme-based gel (Papacarie) in killing cariogenic bacteria in dentinal tubules, and results in less aversive taste and texture responses than to fluoride varnish.[29]

38% SDF arrests dental caries producing bacteria such as *Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Actinomyces naeslundii* by reducing the demineralization process. It minimizes the loss of mineral content and slows down collagen I

destruction. Furthermore, it contains high concentrations of silver and fluoride ions, which may inhibit the growth of multi-species cariogenic biofilms. [10]

Safety is an important aspect, which should be considered in the clinical use of SDF solution. An important review considered silver diamine fluoride (SDF) as a safe, effective, efficient, and equitable caries-preventive agent that appears to meet the criteria of the WHO Millennium Goals and the US Institute.[28, 30] The effect of SDF in preventing dentine collagen degradation from bacterial collagenase challenge has also been well documented.[31] The germicidal action of the SDF preparation in arresting active caries and contributing to disinfection of root canals has also been documented.[32]

There are 3 components of SDF: silver, amine, and fluoride. Silver alone and the more complex silver nitrate have been used for millennia for medical applications. Fluoride is now widely used in a multitude of applications for caries prevention. Unlike the application of fluoride among children, the use of higher concentrations of fluoride among the elderly is acceptable and cannot cause fluorosis. [33]

Hypothesis:

H_0: there is no inhibitory effect of bacteria by 38% SDF

H_A: there is an inhibitory effect of bacteria by 38% SDF

Materials and Methods:

Setting: General Dentistry clinics of the University of Michigan School of Dentistry.

Approval from the Internal Review Board Med (IRB) of the University of Michigan will be obtained prior to implementation.

Study Subjects and Screening:

Inclusion Criteria:

- Generally healthy adult participants 18 years of age and older.
- At least one tooth with active (soft) cavitated lesions of ICDAS score 5 or 6 near the gingival margin (coronal cervical caries at the CEJ or exposed root surfaces with soft active carious lesions exposing dentin).

Exclusion Criteria:

- Adult participants who suffer from serious life-threatening medical diseases that interfere with basic daily self care activities.
- Patients taking antibiotics within the last two weeks.
- Patients using chlorhexidine or fluoride mouth washes within the last two weeks.
- Pregnant or breastfeeding women.
- Teeth with arrested (hard) cervical or root caries lesions.
- Teeth that have been diagnosed with an abscess or an irreversible pulpitis, or are mobile.

Generally healthy adult participants 18 years of age and older with at least one tooth with active cavitated lesions of ICDAS score 5 or 6 near the gingival margin (coronal cervical caries at the CEJ or exposed root surfaces with soft active carious lesions exposing dentin) will be recruited by one of the researchers after the IRB approval in the General Dentistry Clinic at the University of Michigan. Other providers in the General Dentistry Clinic, or other departments, will be informed of the eligibility criteria by the study PI personally, via word of mouth, and via study flyers or emails, and can refer potential subjects to the study team. This could occur either at a patient appointment, or via a review of the patient chart (please see request for Waiver of Consent). The PI will approach potential subjects in person or via phone call to assess interest in study participation. If interested, a study appointment will be scheduled and Informed Consent performed.

At the study appointment, patients will be provided with verbal and written information describing the purpose of the study and potential side effects (lesions will turn black). They will be informed that all information is confidential and that they can freely withdraw at any time during their participation. They will have the opportunity to ask questions related to the study. After obtaining written informed consent, study subjects will be examined by the study PI. Status of the cervical or root caries lesions will be assessed by visual inspection and aided by tactile detection using a probe. Decay- missing- filled teeth (DMFT) index will be assessed for each patient at the initial visit. We will also use ICDAS sensitivity and specificity criteria scores 5 and 6. Score 5 is a distinct cavity with visible dentin; frank cavitation involving less than half of a tooth surface. Score 6 is an extensive distinct cavity with dentin; cavity is deep and wide involving more than half of a tooth surface.

Food particles obscuring visual inspection of root or cervical surfaces will be removed. Two surfaces per tooth (buccal and lingual) will be examined clinically for root surface caries. Individualized oral hygiene instructions will be provided to each participant, focusing on effective brushing with a manual toothbrush, and use of fluoride toothpaste will be recommended.

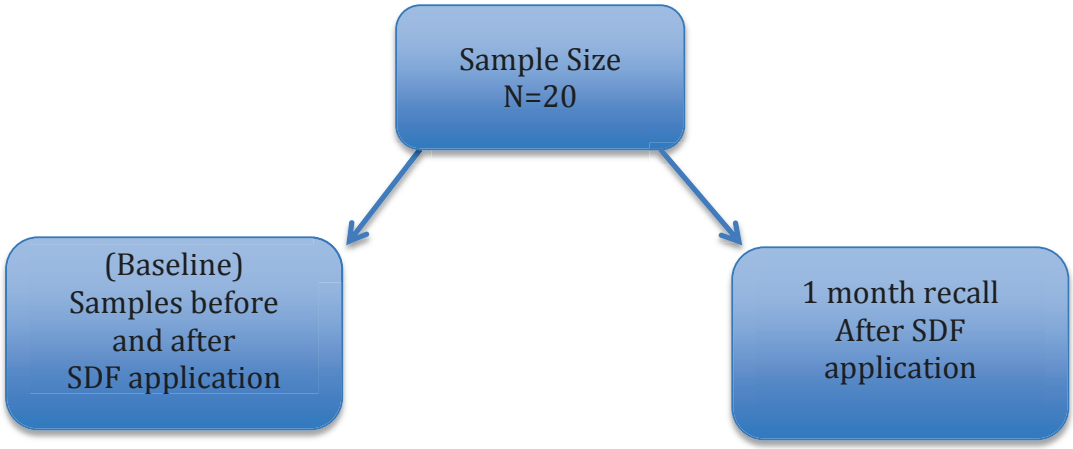
Treatment:

Eligible study subjects will have plaque collected from 1 randomly selected caries lesion that meets the inclusion criteria. Before applications of the study agent, a piece of gauze will be used to dry the teeth. Bacterial samples from subjects' root or cervical carious lesions will be collected with sterile Gracey curettes via a gentle scraping movement.[34, 35] Samples will be collected at the beginning of each appointment, but following consent and eligibility review. Samples will be frozen until analyses. The SDF solution will then be applied onto the exposed root surfaces of participants by means of a disposable microbrush following the manufacturer's recommendation by using their specialized Advantage Arrest applicator. The applicator will not absorb the product as it applies, and there is no risk of leaving flock portions of the applicator on the site. The surface tension of the material being applied holds the liquid on the applicator until it contacts the application site. The participants will be instructed not to eat or drink for one hour after treatment. Patients will be asked to return 1 month later. At this time, the lesions will be assessed for changes in hardness and color, and then a second microbial sample will be collected as described below and frozen until analyses.

Sample Size:

A sample including 20 subjects is decided to constitute the study sample. Samples will be collected from each patient at the first visit and at the one month recall visit.

An additional 2 test plaque samples will be collected for calibration purposes. These will be collected either from subjects who will also participate in the main project or 2 additional subjects. Plaque sampling on the two test subjects will be for calibration purposes, to ensure the sampling technique yields samples with sufficient volume to reliably extract bacterial DNA.



Patient Participation:

Test Sample Collection (2 subjects):

1/Consent form and Eligibility review

2/Questionnaire

3/Examination and diagnosis for root surface caries (photographs when possible)

4/Bacterial sample collection

1st appointment:

1/Consent form and Eligibility review

2/Questionnaire

3/Examination and diagnosis for root surface caries (Pre-SDF photographs when possible)

4/Bacterial sample collection

5/SDF application

6/Post-SDF application photograph

2nd appointment (one month recall):

1/Questionnaire

2/Examination and diagnosis for root or cervical surface caries to confirm if caries still exist (Photographs when possible)

3/Bacterial sample collection

Outcome Measures and its Assessment:

Patients will be seen twice, at baseline and at one-month recall, to compare the microbial composition of bacterial samples at the two different stages. Also, questionnaires will be completed by the patients at baseline and after one month to collect information on tooth brushing behavior, use of fluoride toothpaste, frequency of sweet snack intake between meals. Patients will also be asked about the acceptability of the treatment. Two eligible subjects with multiple eligible lesions will be seen for plaque collection calibration to ensure the plaque sample technique yields adequate volume to extract bacterial DNA for analysis. These subjects can also participate in the baseline and one-month recall, if the subject elects.

Incentives:

1/Participants will be given a toothbrush and toothpaste at the initial visit, and $10 for their initial participation. We will pay for patient parking for the visit.

2/Fifteen dollars at the one-month recall research clinical visit. We will pay for patient parking at the visit.

3/At the one month recall, if active caries still exists or if the patient does not like the color, patients will be offered an appropriate restoration at our expense at the General Dentistry Clinic at the University of Michigan.

4/Two subjects will also participate in a test plaque sample collection visit. These subjects will receive $18 for this visit and will receive compensation for patient parking.

Collection and determination of the bacteria involved in the carious lesions:

Gracey curettes will be used to collect plaque samples. Plaque samples will be coded, which will allow the samples to be connected to the overall study data. These samples will be used to identify the types of bacteria involved in the carious lesions and bacteria arrested after the application of the testing material after one month. The microbial composition of the samples will be assessed using human oral microbe identification microarray (HOMINGS). After the bacterial collection, the samples from the cervical or root carious lesions will be suspended in tubes containing 150 µl of TE (Tris, EDTA) Buffer. The bacterial samples will be stored at −80 °C. Prior to shipment to Forsyth for HOMINGS analyses, genomic DNA will be

purified using the Master Pure Gram Positive DNA Purification kit (Epicentre Biotechnologies, Madison, WI, USA), with modifications (http://mim.forsyth.org). DNA samples will be sent on dry ice to Forsyth Institute. Per Forsyth Institute and the University of Michigan Office of Technology Transfer, a Material Transfer Agreement (MTA) will not be required prior to sharing of the samples.

HOMINGS:

At the Forsyth Institute, microbial profiles will be generated from image files of scanned HOMINGS microarrays (https://homings.forsyth.org). In brief, concentration levels of approximately 600 oral taxa will be determined by microarray hybridization using a fluorescent readout reverse-capture method.[36]

Fluorescently labeled sample microbial DNA will be captured by 16S rRNA-based probes attached to glass slides. The fluorescent intensity for each probe will be scanned, normalized and scaled as previously reported.[36] Signals of 2× backgrounds will be considered to be negative and assigned a HOMINGS level score of 0. Positive hybridization signals will be categorized into five levels, with 1 indicating a signal that was just detectable, and 5 indicating maximum signal intensity.[37]

Statistical Analysis:

Data will be analyzed using SPSS Pc+ version 22.0 statistical software. Descriptive statistics (mean, standard deviation, frequencies and percentages) will be used to describe the quantitative and categorical variables. Appropriate parametric statistical tests (one way analysis of variance followed by Tukey's test) and non-parametric statistical tests (Pearson's chi-square and Krushal Wallis test) will be used depending on the type and distribution of variables. A p-value ≤ 0.05 will be used to report the statistical significance of results.

Future Research:

Data and any samples remaining following HOMINGS analysis may be retained for future analysis/research. Subjects will provide or deny permission for their samples and data from this project to be used in future research in the informed consent document.

References:

1. Petersen PE, Yamamoto T: Improving the oral health of older people: the approach of the WHO global oral health programme. Community Dent Oral Epidemiol 2005; 33:81–92.

2. Tan HP, Lo EC, Dyson JE, Luo Y, Corbet EF: A randomized trial on root caries prevention in elders. Journal of Dental Research 2010; 89:1086–1090.

3. Rosenblatt A, Stamford TC, Niederman R: Silver diamine fluoride: a caries 'silver-fluoride bullet'. Journal of Dental Research 2009; 88:116–125.

4. Llodra JC, Rodriguez A, Ferrer B, Menardia V, Ramos T, Morato M. Efficacy of silver diamine fluoride for caries reduction in primary teeth and first permanent molars of schoolchildren: 36-month clinical trial. Journal of Dental Research 2005; 84:721–4.

5. Chu CH, Lo EC, Lin HC. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. Journal of Dental Research 2002; 81:767–70.

6. Zhi QH, Lo EC, Lin HC. Randomized clinical trial on effectiveness of silver diamine fluoride and glass ionomer in arresting dentine caries in preschool children. Journal of Dentistry 2012; 40:962–7.

7. M.L. Mei, L. Ito, Y. Cao, Q. Li, C.H. Chu, E.C.Lo.The inhibitory effects of silver diamine fluoride on cysteine cathepsins. Journal of Dentistry 2013; 42 (2014) 329–335.

8. Liu BY, Lo EC, Li CM. Effect of silver and fluoride ions on enamel demineralization: a quantitative study using micro- computed tomography. Australian Dental Journal 2012; 57:65– 70.

9. Lou YL, Botelho MG, Darvell BW. Reaction of silver diamine [corrected] fluoride with hydroxyapatite and protein. Journal of Dentistry 2011; 39:612–8.

10. Mei ML, Li QL, Chu CH, Lo EC, Samaranayake LP. Antibacterial effects of silver diamine fluoride on multi- species cariogenic biofilm on caries. Annals of Clinical Microbiology and Antimicrobials 2013; 12:4.

11. Chu C, Lo E, Lin H. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. Journal of Dental Research 2002; 81(11): 767–70.

12. Braga M, Mendes F, De Benedetto M, Imparato J. Effect of silver diamine fluoride on incipient caries lesions in erupting permanent first molars: a pilot study. Journal of Dentistry for Children 2009; 76(1): 28–33.

13. Liu BY, Lo ECM, Chu CH, Lin HC. Randomized trial on fluorides and sealants for fissure caries prevention. Journal of Dental Research 2012; 91:753.

14. Llodra J, Rodriguez A, Ferrer B, Menardia V, Ramos T, Morato M. Efficacy of silver diamine fluoride for caries reduction in primary teeth and first permanent molars of schoolchildren: 36-month clinical trial. Journal of Dental Research 2005; 84(8): 721–4.

15. Lo E, Chu C, Lin H. A community-based caries control program for pre-school children using topical fluorides: 18-month results. Journal of Dental Resarch 2001; 80(12): 2071–4.

16. Yee R, Holmgren C, Mulder J, Lama D, Walker D, van Palenstein HW. Efficacy of silver diamine fluoride for arresting caries treatment. Journal of Dental Research 2009; 88(7): 644–7.

17. Llodra JC, Rodriguez A, Ferrer B, Menardia V, Ramos T, Morato M. Efficacy of silver diamine fluoride for caries reduction in primary and first permanent molars of school children, 36 month clinical trial. Journal of Dental Research 2005; 84:721-4.

18. Craig GG, Knight GM, McIntyre JM. Clinical evaluation of diamine

silver fluoride/potassium iodide as a dentine-desensitizing agent. A pilot study. Australian Dental Journal 2012; 57:308-11.

19. Rosenblatt A, Stamford T, Neiderman R. Silver diamine fluoride: a caries "silver bullet." Journal of Dental Research 2009; 88:116-25.

20. Deutsch, A., An alternate technique of care using silver fluoride followed by stannous fluoride in the management of root caries in aged care. Special Care in Dentistry, 2016; 36: 85–92. doi: 10.1111/scd.12153

21. Chu C, Lo E. Promoting caries arrest in children with silver diamine fluoride: a review. Oral Health and Preventive Dentistry 2008;6(4):315–21.

22. Rosenblatt A, Stamford T, Niederman R. Silver diamine fluoride: caries 'silver-fluoride bullet'. Journal of Dental Research 2009; 88(2): 116–25.

23. Milgrom P, Chi DL. Prevention-centered caries management strategies during critical periods in early childhood. Journal of the California Dental Association 2011; 39(10): 735–41.

24. dos Santos VE, de Vasconcelos F, Ribeiro AG, Rosenblatt A. Paradigm shift in the effective treatment of caries in schoolchildren at risk. International Dental Journal 2012; 62(1): 47–51.

25. Zhang W, McGrath C, Lo ECM, Li JY. Silver diamine fluoride and education to prevent and arrest root caries among community- dwelling elders. Caries Research Journal 2013;47:284-90.

26. Berry TG, Summitt JB, Sift EJ Jr .Root caries. Operative Dentistry Journal 2004; 29:601-607.

27. Beck J: The epidemiology of root surface caries. Journal of Dental Research 1990;69:1216–1221.

28. Rosenblatt A, Stamford TC, Niederman R. Silver diamine fluoride:

caries ‘‘silver-fluoride bullet’’. Journal of Dental Research 2009; 88:116–25.

29. Hamama HH, Yiu CK, Burrow MF. Effect of silver diamine fluoride and potassium iodide on residual bacteria in dentinal tubules. Australian Dental Journal 2015;60(1):80-87. doi:10.1111/ adj.12276.

30. Hobdell M, Petersen PE, Clarkson J, Johnson N. Global goals for oral health 2020. Journal of Dent2003; 53:285-8.

31. Mei ML, Ito L, Cao Y, Li QL, Lo EC, Chu CH. Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation. Journal of Dentistry 2013; 41:809–17.

32. Yamaga 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.

33. Gotjamanos T: Safety issues related to the use of silver fluoride in paediatric dentistry. Austrlian Dental Journal 1997;42:166–168.

34. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. Journal of Clinical Microbiology. 2005;43:5721–5732.

35. Mougeot JL, Stevens CB, Cotton SL, Morton DS, Krishnan K, Brennan MT, Lockhart PB, Paster BJ, Mougeot FK. Concordance of HOMIM and HOMINGS technologies in the microbiome analysis of clinical samples. Journal of Oral Microbiology. 2016;8.

36. Colombo AP, Boches SK, Cotton SL, Goodson JM, Kent R, Haffajee AD, Socransky SS, Hasturk H, Van Dyke TE, Dewhirst F, Paper BJ. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. Journal of Periodontology 2009; 80: 1421–1432.

37. Toiviainen A, Jalasvuori H, Lahti E, et al. Impact of orally administered

lozenges with Lactobacillus rhamnosus GG and Bifidobacterium animalis subsp. lactis BB-12 on the number of salivary mutans streptococci, amount of plaque, gingival inflammation and the oral microbiome in healthy adults. Clinical Oral Investigations Journal 2015; 19:77-83. doi: 10.1007/s00784-014-1221-6.