Effect of Light on the Antibacterial Property of Silver Diamine Fluoride

Nawaporn Chanratchakool* Wichaya Chavengvorakul* Supakorn Taweesedt*
Chutima Trairatvorakul Panida Thanyasrisung*****

Abstract

Objective: The study aimed to evaluate the antibacterial effect of shortened time and light curing on the biofilm of silver diamine fluoride (SDF) applied lesions.

Materials and methods: Twenty dentin specimens (3 x 3 x 2 mm^3) were prepared from twenty primary molar teeth with caries extended to the middle third of dentin. The specimens were randomly allocated into 4 groups (n = 5/group); 1) distilled water (DW, negative control), 2) SDF 10 seconds, 3) SDF 10 seconds with light curing (LC) for 20 seconds and 4) SDF 1 minute (AAPD recommendation) and then were treated following the assigned group. Treated specimens were individually placed in 24-well plates with *Streptococcus mutans (S. mutans)* culture (approximately 6 x 10^8 colony-forming-unit (CFU)/mL) and incubated at 37°C, 5% CO_2 for 24 hours to form biofilm. The biofilm was removed from the specimens by a pipette tip and underwent serial 10-fold dilutions. The diluted solutions were then plated on agar. After incubation, the colonies were counted and presented as CFU/mL.

Results: There was no difference in the number of bacteria that remained on the specimens treated with SDF for 10 seconds, SDF for 10 seconds with LC, and SDF for 1 minute (0, 284 ± 284, and 0 CFU/mL, respectively; mean ± standard error). However, all three groups had significantly fewer bacterial counts than the DW treatment (8.39 x 10^6 ± 4.94 x 10^6 CFU/mL), ($p < 0.05$).

Conclusion: The antibacterial activity of shortening the duration of SDF application with or without light curing was not different from the conventional 1-minute application.

Keywords: Antibacterial agents, Biofilms, Dental curing lights, Silver diamine fluoride, *Streptococcus mutans*

**Faculty of Dentistry, Chulalongkorn University, 34 Henri Dunant Road, Wang Mai, Pathum Wan, Bangkok 10330. Thailand.*
***Department of Pediatric Dentistry, Faculty of Dentistry, Chulalongkorn University, 34 Henri Dunant Road, Wang Mai, Pathum Wan, Bangkok 10330. Thailand.*
****Department of Microbiology and Center of Excellence on Oral Microbiology and Immunology, Faculty of Dentistry, Chulalongkorn University, 34 Henri Dunant Road, Wang Mai, Pathum Wan, Bangkok 10330, Thailand.*

Introduction

Dental caries is still one of the most serious oral health issues in children. According to the latest national Oral Health Survey, 75.6% of Thai children age 5 years old suffer from dental caries with an average dmft of 4.5 (1). One of the reasons that contribute to the high prevalence of dental caries is the shortage of dentists and difficult access to services in remote areas. Conventional treatments for dental caries are sealant and restoration. Such approaches are not practically applied in rural areas, which are mostly managed by dental hygienists.

Recently, a non-restorative treatment that simply applies silver diamine fluoride (SDF) to lesions without drilling has been developed (2). Silver diamine fluoride is a liquid substance that is used to arrest dental caries. The material contains two active ingredients which are silver and fluoride. Silver has an inhibition effect on bacterial DNA and amino acids with a consequence of inhibition of biofilm formation. In addition, a high concentration of fluoride promotes remineralization and formation of calcium fluoride on the tooth surface which acts as a reservoir of fluoride ions for further remineralization (3). When aerosol-generating operations are avoided during the coronavirus pandemic, minimally invasive methods are becoming more prevalent (4). The use of silver diamine fluoride (SDF) is recommended since it is efficient at arresting caries, is painless, and does not need cavity excavation (5). Because of these advantages, SDF was used to prevent caries, which helps to improve the quality of life of patients with limited cooperation or access to dental treatments, such as young children and the elderly (6,7).

The existing evidence has shown that there are no significant differences between the effectiveness of annual application of SDF solution and that of the annual application of a flowable high fluoride-releasing glass ionomer in arresting active dentine caries in primary teeth (8). However, the disadvantage of SDF is an esthetic concern from the blackening of the applied area as a result of the silver phosphate (9). SDF reacts with hydroxyapatite and forms calcium fluoride, silver phosphate and ammonium hydroxide. Silver phosphate is reduced by light to form black metallic silver precipitation which explains the blackening of the applied lesion. The blackening and hardness of the lesion after SDF application is considered a caries arrestment (9). Recent studies have shown that a dental curing light has no effect on the penetration of silver ion precipitation into dentinal tubules but does increase silver ion precipitation on carious lesions (10,11), which leads to a shorter operation time.

The current recommendation of SDF application time by the American Academy of Pediatric Dentistry (AAPD) is 1 minute per lesion (12) which might not be applicable in uncooperative or young children. Ten seconds of application of SDF was used in a study (13). This may account for the lower caries arrest rate than other SDF studies (14,15). Therefore, rather than waiting for natural light, LED light curing is used to enhance exposure to light (16) to reduce application time. However, light curing is not officially recommended since its effect on SDF properties, such as antibacterial activity, is still unknown. Therefore, this study aims to evaluate the antibacterial effect of shortened time and light curing on the biofilm of SDF applied lesions.

Materials and Methods

The study was approved by the Human Research Ethics Committee (HREC-DCU 2020-087) and Institutional Biosafety Committee (DENT CU-IBC 033/2020), Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. The inclusion criteria were primary molar teeth with visible caries extended into dentin which involves occlusal surfaces. Teeth with mechanical fractures were excluded from this experiment.

Specimen preparation

The primary molars used in this study were extracted due to irreversible pulpitis with a lesion depth from the outer to the middle third of the dentin. Twenty dentin blocks (3 x 3 x 2 mm^3) were sectioned from the active carious area and stored in 0.9% normal saline. The dentin blocks were randomly allocated into 4 groups (n = 5/group, Fig. 1); group 1: DW (control) group 2: SDF 10 sec, group 3: SDF 10 sec + LC 20 sec and group 4: SDF 1 min. Specimens in group 1 were rubbed with a micro brush for 10 seconds with 5 µL of DW, whereas SDF was applied according to the manufacturer's instruction in the SDF-treated groups. Briefly, 5 µL of SDF (Saforide®: Toyo Seiyaku Kesei Co., Japan) was pipetted and applied directly to each specimen, then rubbed for 10 seconds (group 2 and 3) and 1 minute (group 4) using a micro brush. For group 3, additional LED light (XL3000, 3M ESPE, St. Paul, MN, USA) curing was done for 20 seconds. The dentin blocks were sterilized by a plasma sterilization system before incubation with *S. mutans* to form biofilms (Fig. 1).

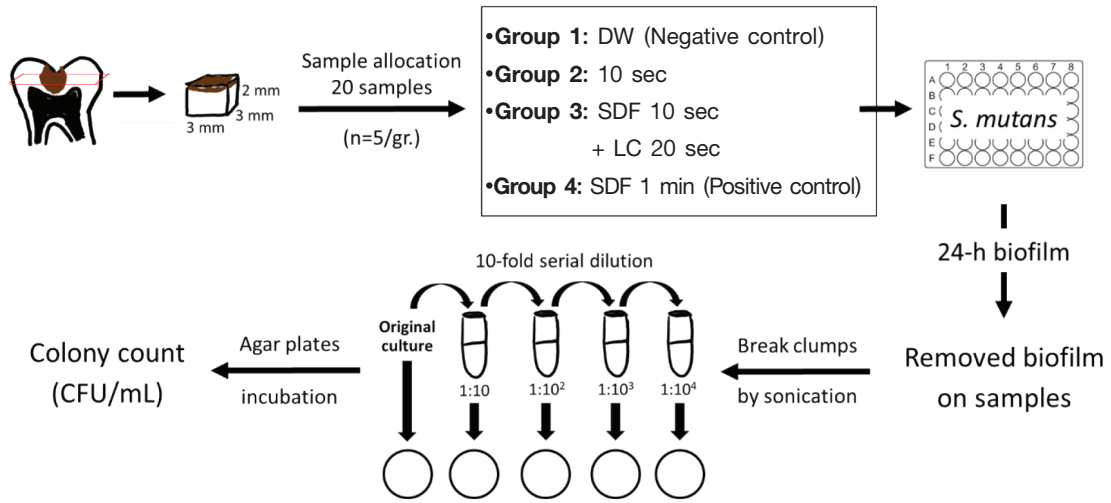

Fig 1. Experimental flowchart.

Determination of antibacterial activity

The experiment protocol was modified from the previous study (17). *S. mutans* UA159 were inoculated in 10 mL of Brain Heart Infusion (BHI) broth (HiMedia Laboratories, Mumbai, India) and incubated at 37°C, 5% CO_2 for approximately 4 hours to reach the log-phase (approximately 6 x 10^8 CFU/mL). The medium was replaced with BHI broth containing 5% sucrose, and 1 mL of culture was added to each well of 24-well plates. The dentin specimens were then individually placed in 24-well plates and incubated at 37°C, 5% CO_2 for 24 hours to form biofilm. Then, the specimens were rinsed with phosphate buffer saline (PBS). A sterile pipette tip was used to scrape the biofilm from the specimens, which was then suspended in PBS (18). To break biofilm clumps, the suspensions were sonicated for 5 minutes in an ultrasonic bath (Elma, Germany) and serially diluted 10-fold. The diluted solutions were then plated on BHI agar in duplicate and incubated at 37°C, 5% CO_2 overnight. The colonies were counted and presented as CFU/mL (Fig. 1).

Statistical analysis

The analysis was conducted using IBM SPSS Version 28.0 software (IBM Corporation, Armonk, New York, USA). The normality of data was tested by Shapiro-Wilk test. Kruskal-Wallis test followed by Bonferroni correction test was used. A p-value < 0.05 was considered statistically significant.

Results

The number of bacteria that remained on the specimens was quantified as CFU/mL. The DW-treated group had the highest bacteria left on the specimens (8.39 x 10^6 ± 4.94 x 10^6 CFU/mL), whereas the SDF 10 seconds, SDF 10 seconds with LC, and SDF 1 minutes-treated groups had 0, 284 ± 284, and 0 CFU/mL, respectively (Table 1). There were no differences in bacterial numbers between SDF-treated groups regardless of the shortened time or light curing, but all were significantly different from DW (a control group).

Table 1 Bacterial count of each treatment.

Treatment (n = 5/group)	CFU/mL (Mean ± SE)
DW	8.39 x 10^6 ± 4.94 x 10^{6a}
SDF 10 sec	0^b
SDF 10 sec + LC 20 sec	284 ± 284^b
SDF 1 min	0^b

The difference in superscript letters is significantly different (p < 0.05) by Kruskal-Wallis and Bonferroni correction tests.

DW = distilled water, SDF = silver diamine fluoride, LC = light curing, SE = standard error, CFU = colony forming unit

Discussion

The purpose of this study is to compare the antibacterial activity of SDF in terms of shortening vs. conventional application times, as well as light curing vs. non-light curing techniques. The results revealed that applying SDF for 10 seconds, 10 seconds with light curing, or 1 minute provided no difference in antibacterial activity against *S. mutans* but was significantly better than DW treatment ($p < 0.05$).

Antibacterial effect of SDF is contributed mainly by silver ions (Ag^+). Light exposure accelerates the precipitation of silver ions (Ag^+) released from SDF to silver compounds (9), which may decrease their antibacterial activity. However, we found that there was no differences in antibacterial efficacy between light curing and non-light curing SDF. This discovery might be explained by the fact that different silver compounds, such as silver chloride, silver phosphate, and silver oxide, have different solubilities (9). Some of them may be partly dissolved in water, releasing silver ions. The released silver ions may still have antibacterial properties against bacteria on the surface. Nevertheless, our experimental design was unable to demonstrate the antibacterial activity of SDF against bacteria inside the dentinal tubules.

There was an outlier value in the application of SDF for 10 seconds with the light curing group, which might be attributed to the fact that the dentin samples in this investigation were obtained from affected dentin of different teeth. As a result, the pattern on each surface may differ, affecting *S. mutans* adherence. An alternative method is the formation of artificial caries on dentin to create caries of identical depths and surfaces on sound dentin.

In this study, *S. mutans* was used to assess the antibacterial activity of SDF since the bacteria were related to dental caries, particularly in young children (19). However, there are multiple species of microbes that contribute to the formation of dental caries in a real situation. Therefore, clinical research should be carried out to completely assess the bactericidal activity of light cured SDF in the oral cavity.

Nevertheless, SDF achieves caries arrestment not only through antibacterial activity but also through remineralization, which should be further investigated to better understand the influence of dental light curing on overall SDF properties. Moreover, understanding the mechanism of light cured SDF may help in the improvement of this approach.

Conclusion

Shortening the duration of SDF application with or without light curing procedure had no different effect on antibacterial activity against *S. mutans* comparing to the standard 1-minute application. Reduced operation time while maintaining antibacterial efficacy would be useful in the treatment of uncooperative young children, special needs patients, and the elderly. However, clinical investigations on its effectiveness in remineralization and antimicrobial activity are required.

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References

1. Bureau of Dental Health. The 8th national oral health survey of Thailand 2017. Nonthaburi: Department of Health, Ministry of Public Health; 2018.

2. van Strijp G, van Loveren C. No removal and inactivation of carious tissue: non-restorative cavity control. Monogr Oral Sci. 2018;27:124-36.

3. Seifo N, Robertson M, MacLean J, Blain K, Grosse S, Milne R, et al. The use of silver diamine fluoride (SDF) in dental practice. Br Dent J. 2020;228(2):75-81.

4. Al-Halabi M, Salami A, Alnuaimi E, Kowash M, Hussein I. Assessment of paediatric dental guidelines and caries management alternatives in the post COVID-19 period. A critical review and clinical recommendations. Eur Arch Paediatr Dent. 2020;21(5):543-56.

5. Greenwall-Cohen J, Greenwall L, Barry S. Silver diamine fluoride - an overview of the literature and current clinical techniques. Br Dent J. 2020;228(11):831-8.

6. Renugalakshmi A, Vinothkumar TS, Hakami FB, Salem RM, Qadri AA, Harbosh ZM, et al. Impact of silver diamine fluoride therapy on oral health-related quality of life of uncooperative preschool children: a prospective study. Oral Health Prev Dent. 2021;19(1):93-9.

7. Grandjean ML, Maccarone NR, McKenna G, Muller F, Srinivasan M. Silver Diamine Fluoride (SDF) in the management of root caries in elders: a systematic review and meta-analysis. Swiss Dent J. 2021;131(5):417-24.

8. 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. J Dent. 2012;40(11): 962-7.

9. Mei ML, Lo ECM, Chu CH. Arresting dentine caries with silver diamine fluoride: what's behind it?. J Dent Res. 2018;97(7):751-8.

10. Toopchi S, Bakhurji E, Loo CY, Hassan M. Effect of light curing on silver diamine fluoride in primary incisors: a microscopic *ex vivo* study. Pediatr Dent. 2021;43(1):44-9.

11. Lau L, Quock RL, Wu DI, Harrington DA, Patel SA, Barros JA. Effect of surface preparation and light curing on penetration of silver particles from 38% silver diamine fluoride in dentin of primary teeth: An *in vitro* evaluation. Am J Dent. 2021;34(1):44-8.

12. Crystal YO, Marghalani AA, Ureles SD, Wright JT, Sulyanto R, Divaris K, et al. Use of silver diamine fluoride for dental caries management in children and adolescents, including those with special health care needs. Pediatr Dent. 2017;39(5):135-45.

13. Duangthip D, Chu CH, Lo EC. A randomized clinical trial on arresting dentine caries in preschool children by topical fluorides--18 month results. J Dent. 2016;44:57-63.

14. Yee R, Holmgren C, Mulder J, Lama D, Walker D, van Palenstein Helderman W. Efficacy of silver diamine fluoride for Arresting Caries Treatment. J Dent Res. 2009;88(7):644-7.

15. Santos VE, Vasconcelos Filho A, Targino AG, Flores MA, Galembeck A, Caldas AF, et al. A new "silver-bullet" to treat caries in children--nano silver fluoride: a randomised clinical trial. J Dent. 2014;42(8):945-51.

16. McDonald J, Alrayyes S, Bedran-Russo A, Garcia MC. Evaluating the effectiveness of light cured SDF and its penetration: an *in vitro* study [Ph.D.]: University of Illinois at Chicago; 2018.

17. Yu OY, Zhao IS, Mei ML, Lo ECM, Chu CH. Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions. J Dent. 2018;78:65-71.

18. Kunze B, Reck M, Dotsch A, Lemme A, Schummer D, Irschik H, et al. Damage of *Streptococcus mutans* biofilms by carolacton, a secondary metabolite from the *myxobacterium Sorangium cellulosum.* BMC Microbiol. 2010;10: 199. doi: 10.1186/1471-2180-10-199.

19. Zhang L, Sun T, Zhu P, Sun Z, Li S, Li F, et al. Quantitative analysis of salivary oral bacteria associated with severe early childhood caries and construction of caries assessment model. Sci Rep. 2020;10(1):6365. doi: 10.1038/ s41598-020-63222-1.

Corresponding author:
Assoc.Prof.Dr. Panida Thanyasrisung
Department of Microbiology and Center of Excellence on Oral Microbiology and Immunology, 34 Henri Dunant Road, Wang Mai, Pathum Wan, Bangkok 10330, Thailand.
Tel and Fax: 02-2188680
E-mail: panida.t@chula.ac.th

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