Original Article

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Silver and fluoride penetration and biofilm suppression on root dentin following various silver diamine fluoride application strategies

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Objectives: The aim of this study was to evaluate silver and fluoride penetration and their effects on biofilm, using different silver diamine fluoride (SDF) applications.

Materials and Methods: Thirty caries-free maxillary premolars were sectioned into 1 mm-thick root dentin slabs. The area of 2x2 mm root dentin window was made with nail varnish. A total of 40 specimens were prepared for element analysis, and 20 were sterilized for biofilm assessment. Specimens were divided into four groups based on SDF application: Group 1: distilled water (control), Group 2: SDF 10 seconds, Group 3: SDF 10 seconds + light curing 20 seconds, and Group 4: SDF 60 seconds. Specimens underwent energy dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) for silver and fluoride penetration from 0-500 µm analysis. The remaining 20 specimens were divided into four groups according to SDF applications and incubated for multispecies biofilm (*Streptococcus mutans*, *Lactobacillus rhamnosus*, and *Actinomyces naeslundii)* at 24 hours after SDF application, then analyzed using confocal laser scanning microscopy with live/dead staining. Biovolume and cell viability were quantified. Two-way ANOVA was used for silver and fluoride penetration. One-way ANOVA was used to analyze biovolume and cell viability.

Results: At the surface (0 µm depth), groups 3 and 4 showed significantly higher atomic percentages of silver (At%Ag: $p<0.001$), while no significant differences were observed at other depths and application methods. All experimental groups (Groups 2–4) exhibited higher atomic percentages of fluorine (At%F) than the control, but no clear trend was identified among them. Biofilm assessments indicated that SDF applications reduced biovolume and cell viability ($p<0.001$), with more significant reductions in light-cured and prolonged application time groups.

Conclusions: This study provides key insights into how light curing can enhance silver retention and biofilm suppression within a shorter application time.

Keywords: biofilm, caries prevention, root caries prevention, silver diamine fluoride

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Introduction

Dental biofilms are complex assembly of microorganisms adhered to surfaces, encased in a self-produced extracellular matrix that provides protection against environmental stresses [1]. Understanding and controlling biofilm formation is essential for improving oral health outcomes and providing effective dental care.

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Recently, silver diamine fluoride (SDF) has emerged as a promising alternative in pediatric dentistry [2] and management of root caries [3] due to its dual action of arresting carious lesions and exhibiting broad-spectrum antimicrobial properties [4]. SDF contains both silver, which possesses potent antibacterial qualities [5], and fluoride, which aids in remineralization [6]. In addition, the effectiveness of SDF on the prevention of dentinal caries in elderly people has been proposed [7].

Currently, the limitation of how different concentrations and application methods of SDF may be interesting because it may impact its efficacy. The application mode—whether through direct application, incorporation into dental materials, or delivery via various techniques—may further influence the efficacy of fluoride and SDF [8]. Recent findings suggest that a 60-second application of SDF yields optimal results [9], though this duration may be challenging for children, older adults, and individuals with special needs. Light curing is being explored to shorten the application time, with previous research indicating that exposure to natural light enhances silver precipitation [10]. Recent studies have examined the role of light curing in silver diamine fluoride (SDF) application, demonstrating its ability to enhance silver ion penetration and precipitation within carious dentin while contributing to increased dentin hardness. These findings suggest that light curing may optimize the physicochemical properties of SDF, potentially improving its clinical efficacy [11, 12]. However, its impact of penetration and retention on sound dentin for root caries prevention and biofilm growth remains inadequately studied [13].

Therefore, the aim of this study was to evaluate silver and fluoride distribution, along with their effects on biofilm, using different SDF application methods to determine the better technique for caries prevention on root dentin. The null hypotheses were: i) there is no significant difference in silver and fluoride distribution among different SDF application techniques and various depths of root dentin, ii) there is no significant difference in biovolume and cell viability of biofilm on root dentin surface among different SDF application techniques.

Materials and Methods

Ethical approval for this study was obtained from the ethics committee in human research (Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board; MU-DT/PY-IRB) (COE. MU-DT/PY-IRB 2025/002.0801).

The investigation was divided into two parts: 1. Qualitative element analysis, and 2. Biofilm analysis. Specimens of each part were investigated independently.

Specimens Preparation and Intervention

Tooth sample collection and preparation

Thirty maxillary premolars that were free from caries and cracks were used in this study. The teeth were preserved in 0.1% thymol solution and used within 6 months. The root dentin specimens were obtained by separating the root from the crown and cutting parallel to the tooth's long axis using a low-speed diamond saw (Isomet™; Buehler, Evanston, IL, USA). Two specimens from each tooth's mesial and distal parts were collected, then shaped into 1 mm-thick and not exceeding 5x5 mm rectangular slabs. The specimens were polished using silicon carbide papers (800, 1,000, 1,200, 2,000, 4,000, and 5,000 grit: Buehler, Buehler Ltd, Lake Bluff, IL, USA) and diamond paste (6, 3, 1, and 0.25 microns: DP-Paste, Struers A/S, Copenhagen, Denmark) to standardized smooth surface undergoing ultrasonic cleaning (Crest Ultrasonics,

New Jersey, USA) between steps and after the final polish [14].

Sixty polished specimens were prepared. Specimens were coated with nail varnish (Revlon Nail Enamel, Revlon, NY, USA), leaving a 2 × 2 mm window of exposed dentin for evaluating silver and fluoride distribution. Twenty specimens for biofilm analysis were sterilized in a UV chamber for 90 minutes per side [15].

Treatment with silver diamine fluoride

Sixty specimens were divided into four groups by different SDF (Topamine, Dentalife Pty Ltd, Australia) application technique: distilled water (control), SDF 10 seconds, SDF 10 seconds + light curing 20 seconds, and SDF 60 seconds (n = 10 per group for element analysis, n = 5 for biofilm analysis). Group 1 specimens were treated with 5 µL of distilled water and rubbed with a microbrush for 10 s. Groups 2–4 received 5 µL of SDF, then rubbed for 10 seconds (groups 2 and 3) or 60 seconds (group 4). Group 3 had an additional 20 seconds of LED light curing (Bluephase G2, Ivoclar-Vivadent, Schann, Liechtenstein) at 1,200 mW/cm² metered by radiometer (Bluephase Meter II, Ivoclar-Vivadent, Schann, Liechtenstein). All specimens were rinsed with deionized water for 10 seconds immediately after SDF intervention and stored at 37°C for 24 hours [16].

Specimens Investigation and Analysis

1. Qualitative element analysis

To evaluate silver and fluoride penetration, forty specimens underwent energy dispersive X-ray spectroscopy (EDX: X-Max 20, Oxford Instruments, Abingdon, UK) and line scan analysis using a scanning electron microscope (SEM: JSM 6610LV, JEOL, Tokyo, Japan). After drying in a desiccator for 24 hours, specimens were embedded in self-cure epoxy resin (EpoxiCure® 2: Buehler, Leinfelden-Echterdingen, Germany) and left for 8 hours to polymerize, then cross-sectioned at the window area for element distribution analysis and polished per preparation protocols.

Specimens were sputter-coated with gold (23 mA) using a sputter coater (K500X Sputter coater, SPI Supplies, West Chester, PA, USA) and examined under SEM at 5 kV with magnifications of 30,000x [17]. Line scanning was used to track silver and fluoride atomic percentage. Three random lines per specimen drawn manually in the software, scanning 500 µm deep at 100 µm intervals, were used to quantify the presence of silver and fluoride. The mean atomic percentage of fluoride (F), silver (Ag), calcium (Ca), and phosphorus (P) was used for analysis.

2. Biofilm analysis

Multispecies Biofilm Incubation

Saliva samples from three healthy donors were centrifuged, diluted in PBS (1:10), sterilized through a 0.2 µm filter, and stored at 4°C. Twenty dentin specimens were coated with 600 µm sterile-filtered saliva by immersing in a 24-well plate for 16 hours at 37°C to induce pellicle formation before biofilm development [18]. This procedure was taken 24 hours after the SDF intervention.

Multispecies biofilm was created from a culture sample using *Streptococcus mutans*, *Lactobacillus rhamnosus*, and *Actinomyces naeslundii*, cultured in BHI agar and broth. Bacterial suspension was diluted to 1×10^8 CFU/ml (0.5 McFarland) and mixed equally (1:1:1). Twenty Saliva-coated specimens were incubated with bacterial suspensions in a 5% CO_2 chamber at 37°C for 24 hours, then washed with PBS and transferred to new plates [18].

Biofilms on 20 specimens were assessed using confocal laser scanning microscopy (Leica DMi8, Leica Microsystems, Singapore). Specimens were stained with a Live/Dead

BacLight™ Bacterial Viability kit (Molecular Probes, Eugene, USA), incubated for 20 minutes, and analyzed at 400X magnification. Each specimen was analyzed with 3 randomized positions. Biovolume and cell viability were quantified using Comstat 2.1 software [19].

Statistical analysis

The results for elemental analysis were calculated and summarized, including the mean and standard deviation. The normality of the distribution was assessed using the Shapiro-Wilk test, while the homogeneity of variance was evaluated through Levene's test. For the analysis of factors involving two variables, namely the depth of scan and application methods, a two-way analysis of variance (ANOVA) was conducted to examine the effects of the two independent variables on the mean scores and the interaction effects between them with $\alpha = 0.05$. Post-hoc comparisons were performed using Duncan's test to identify differences among specific groups.

In the biofilm analysis, the normality of the distribution was also assessed with the Shapiro–Wilk test, while the homogeneity of variance was evaluated with Levene's test. A one-way ANOVA was then employed to evaluate variations across the groups, with statistical significance level $\alpha = 0.05$. Subsequently, post-hoc comparisons were conducted using Duncan's test to detect differences among groups.

Results

1. Qualitative element analysis

Elemental analysis was conducted to determine the atomic percentage (At%) of four elements: calcium (At%Ca), phosphorus (At%P), silver (At%Ag), and fluorine (At%F). The mean (SD) values of At%Ag and At%F for each group are presented in the table 1 and table 2 respectively.

Silver was not detected in the control group (Table 1). At a surface (depth of 0 μm), Groups 3 and 4 demonstrated a significantly higher atomic percentage of silver (At%Ag) compared to the other groups. No significant differences in At%Ag were observed across other groups or depths. Regarding the atomic percentage of fluorine (At%F), all experimental groups (Groups 2–4) exhibited higher values than the control group (Table 2). Although some variations were present among the experimental groups, no clear trend was identified.

Table 1 Means and standard deviations for the silver atomic % (At%Ag) of different SDF application methods at vary depth of scan.

	At%Ag Mean (Standard deviation)					
	Depth of scan (μm)					
	0	100	200	300	400	500
Group 1: Control	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}
Group 2: SDF 10 s	0.40 (0.42)^{a}	0.23 (0.20)^{a}	0.15 (0.15)^{a}	0.08 (0.15)^{a}	0.36 (0.94)^{a}	0.01 (0.16)^{a}
Group 3: SDF 10 s + LC	1.32 (2.00)^{b}	0.17 (0.19)^{a}	0.08 (0.11)^{a}	0.03 (0.04)^{a}	0.02 (0.02)^{a}	0.01 (0.02)^{a}
Group 4: SDF 60 s	1.01 (0.85)^{b}	0.16 (0.20)^{a}	0.10 (0.16)^{a}	0.16 (0.23)^{a}	0.09 (0.11)^{a}	0.09 (0.14)^{a}

Same superscript letters indicate no statistically significant difference ($p>0.05$).

Table 2 Means and standard deviations for the Fluoride atomic % (At%F) of different SDF application methods at vary depth of scan.

	At%F Mean (Standard deviation)					
	Depth of scan (μm)					
	0	100	200	300	400	500
Group 1: Control	6.55 (0.85)^{a}	6.38 (0.73)^{a}	6.37 (0.43)^{a}	6.95 (1.00)^{a}	7.25 (1.56)^{a}	6.33 (0.92)^{a}
Group 2: SDF 10 s	10.74 (0.92)^{b,c}	10.46 (0.79)^{b,c}	10.10 (0.49)^{b,c,d,e,f}	9.91 (1.27)^{b,c,d,e,f}	10.52 (0.71)^{b,c}	10.36 (1.28)^{b,c,d,f}
Group 3: SDF 10 s + LC	10.87 (1.66)^{c}	10.05 (0.84)^{b,c,d,e,f}	9.94 (0.77)^{b,c,d,e,f}	10.23 (1.52)^{b,c,d,f}	10.07 (1.22)^{b,c,d,e,f}	9.85 (0.56)^{b,c,d,e,f}
Group 4: SDF 60 s	9.36 (1.19)^{d,e,f}	9.20 (1.25)^{e,f}	9.77 (0.48)^{b,d,e,f}	9.20 (1.05)^{e,f}	9.08 (1.08)^{e}	9.13 (0.93)^{e}

Same superscript letters indicate no statistically significant difference (p>0.05).

2. Biofilm analysis

Mean (SD) of biovolume (μm^3), cell viability (%) of each group are presented in Table 3:

The results of biofilm analysis are demonstrated in Table 3. Significant differences in biovolume and cell viability were observed in all groups except for Groups 3 and 4 (p<0.001). Groups 3 and 4 exhibited the lowest biovolume, measuring 5.79 (0.44) μm³ and 5.57 (0.55) μm³, respectively, as well as the lowest cell viability, recorded at 76.55% (4.48) and 74.91% (1.75), respectively. Furthermore, all experimental groups (Groups 2–4) demonstrated significantly lower biovolume and cell viability compared to the control group (p<0.001). Representative confocal laser microscopy images of biofilm are provided in Figures 1 and 2.

Table 3 Means and standard deviations for the biofilm biovolume and %cell viability of different SDF application

	Mean (Standard deviation)	
	Biovolume (μm^3)	Cell viability (%)
Group 1: Control	11.21 (1.23)^{a}	97.03 (1.62)^{a}
Group 2: SDF 10 s	8.61 (1.03)^{b}	85.56 (6.75)^{b}
Group 3: SDF 10s + LC 20s	5.79 (0.44)^{c}	76.55 (4.48)^{c}
Group 4: SDF 60s	5.57 (0.55)^{c}	74.91 (1.75)^{c}
p-value of ANOVA	<0.001	<0.001

Same superscript letters indicate no statistically significant difference among columns (p>0.05)

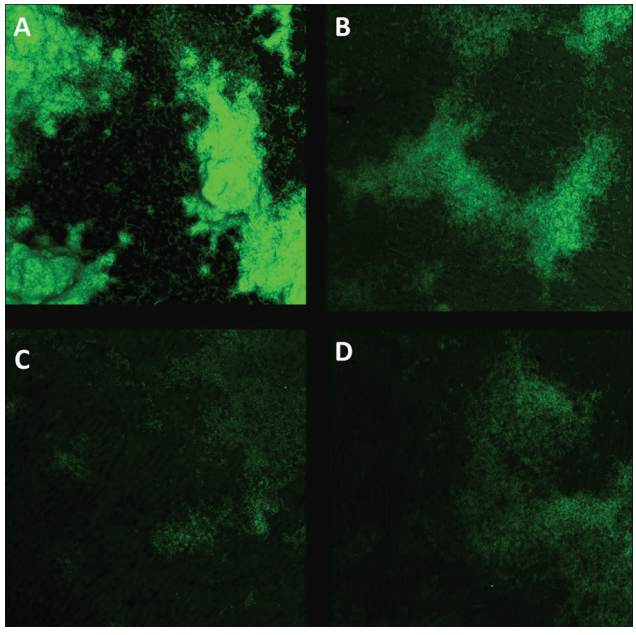

Figure 1 Biofilms under confocal laser microscopy: A-Control, B-SDF 10 s, C-SDF 10 s + LC 20 s, D-SDF 60 s

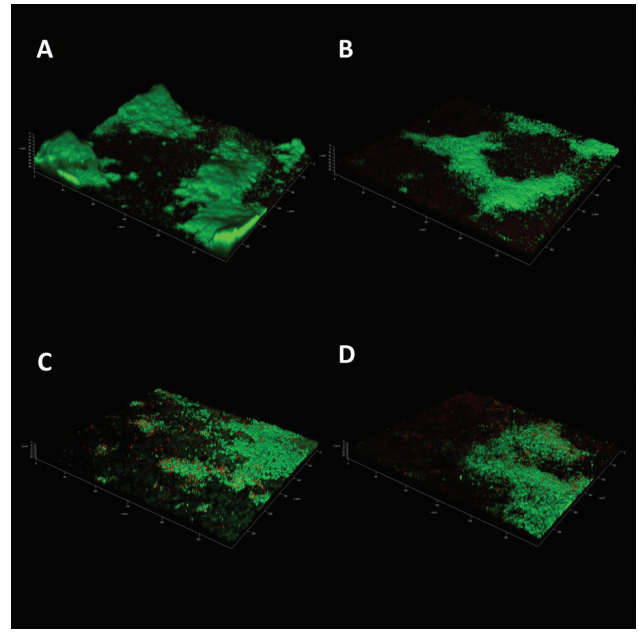

Figure 2 Constructed 3D images of biofilms. *Green = live cells, Red = dead cells.* A-Control, B-SDE 10 s, C-SDF 10 s + LC 20 s, D-SDF 60 s

Discussion

This study evaluated silver and fluoride distribution of dentin with different silver diamine fluoride (SDF) applications, focusing on fluoride deposition, silver retention & penetration, and its biofilm suppression effect. The findings emphasize the significance of application time and light curing in optimizing SDF efficacy. Therefore, the null hypotheses were both rejected ($p<0.05$).

Elemental analysis confirmed differences in fluoride and silver incorporation among the groups. The 10-second application with 20-second light curing (Group 3) exhibited high surface (0 µm depth) silver levels comparable to the 60-second application (Group 4). This indicates that light curing enhances silver retention and precipitation by accelerating the reduction reaction of silver in silver complex compound in SDF solution, forming more insoluble silver compounds (Ag_3PO_4 and Ag_2O) [20] in comparable quantity to 60-second application (group 4). This highlights the potential efficiency of light curing in improving silver deposition without requiring prolonged application. For the fluoride level, all experimental groups were found to be significantly higher than those in the control group at every scanning depth. This phenomenon can be attributed to the smaller ionic diameter of fluoride (72 pm), compared to silver ion (144 pm), which facilitates its diffusion throughout the experimental specimens [21], irrespective of the specific method of SDF application. Consequently, specimens treated with SDF exhibited notably elevated fluoride levels compared to untreated controls.

Biofilm assessments demonstrated significant reductions in biovolume and percentage cell viability in groups 2, 3, and 4 compared to the control (Group 1). Significantly lower biovolume and cell viability were observed in groups 3 and 4, indicating that surface silver compound

precipitation and retention may increase the biofilm suppression. This corresponds with the silver ion precipitation (Table 1). The higher silver compound might play a role by disrupting bacterial cell walls, cellular respiration, and microbial DNA [22], achieving favorable results compared with a shorter application time (Group 2). This efficiency could be beneficial for clinical applications where minimizing treatment time in special needs patients or the elderly without compromising effectiveness is essential.

The results indicate that a 10-second application combined with light curing can provide an effective prevention alternative to an optimum 60-second application. This approach may improve silver retention while optimizing antibacterial effects, making treatment more practical in clinical settings when facing cases with limitations of co-operation or treatment time. The ability to achieve biofilm inhibition in a shorter time frame highlights the importance of refining SDF protocols for root caries prevention to balance efficacy with convenience.

It is crucial to consider the broader implications of optimizing SDF application techniques. Since SDF can still stain sound dentin, SDF application consideration needs a delicate balance between maximizing antimicrobial efficacy and addressing esthetic concerns. While light curing enhances silver precipitation and biofilm suppression, its potential impact on staining patterns requires further investigation. However, this study is focusing on root caries prevention in high-risk groups, such as special needs and elderly patients; esthetic factors are not a primary focus of the analysis.

Understanding how variations in application duration and curing methods influence the longevity of silver and fluoride incorporation in dentin could provide valuable insights for refining clinical caries prevention protocols. Therefore, future research should focus on in vivo study, long-term silver retention and stability, patient-centered considerations, and strategies to mitigate unintended discoloration, ensuring that SDF remains both effective and aesthetically acceptable in clinical caries prevention practice.

Conclusion

The 10-second SDF application with light curing demonstrated similar silver precipitation and antibacterial effect compared to the traditional SDF application at 60 seconds.

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