EFFECT OF LIGHT CURING ON THE PENETRATION DEPTH OF SLIVER DIAMINE FLUORIDE IN DENTIN OF CARIOUS PRIMARY TEETH (IN VITRO STUDY)

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ABSTRACT

INTRODUCTION: Silver Diamine Fluoride (SDF) is introduced as a light-sensitive antibacterial and anti-caries agent that demonstrates efficacy in halting the progression of caries. This study was designed to investigate how dental light curing influences the extent of silver diamine fluoride (SDF) penetration, dentin hardness, and the deposition of silver and fluoride ions within the dentin of decayed primary molars.
METHODS: Silver diamine fluoride (SDF) was administered to 24 carious primary molars with deep carious lesions not pulpally involved. These teeth were categorized into two groups: (1) the control group, which was not subjected to light-curing, and (2) the test group, which was subjected to light-curing. The evaluation of penetration depth and ion precipitation was conducted using Energy-Dispersive X-ray Spectroscopy (EDX), while dentin hardness in sound dentin was assessed through the Vickers hardness test.
RESULTS: In both sets of samples, SDF exhibited penetration extending beyond the carious lesion and into healthy dentin. However, the depth of penetration into sound dentin was greater in the control group when contrasted with the test group. The presence of silver ions precipitated in infected dentin was more pronounced when exposed to dental curing light compared to the control group. Dentin hardness of sound dentin was higher in the test group than in the control group. There was no statistically significant difference in fluoride ions precipitation between both groups (P =0.124).
CONCLUSIONS: The utilization of a dental curing light alongside silver diamine fluoride treatment for carious lesions results in enhanced silver ion precipitation within the infected dentin, elevated dentin hardness, and reduced penetration of SDF into healthy dentin.
KEYWORDS: Energy Dispersive X-Ray, Silver Diamine Fluoride.
RUNNING TITLE: Effect of light curing on silver diamine fluoride.

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INTRODUCTION

Silver diamine fluoride ($AgF[NH3]_2$) is a transparent solution characterized by its alkaline pH. Its primary constituents include silver, fluoride, and ammonia. Fundamentally, silver serves as an antimicrobial agent, ammonia plays a role in stabilizing the solution, and fluoride contributes to the process of remineralization (1). The typical concentration used is 38%, which equates to 44,800 parts per million (ppm) of fluoride and 255,000 ppm of silver (2). At such elevated concentrations, these two elements exhibit a synergistic effect, manifesting as bactericidal action against cariogenic microorganisms, support for mineralization, prevention of tooth hard tissue demineralization, and reduction in the degradation of the organic portion of dentin (3).

Silver diamine fluoride is proved to be an efficient, secure, and equitable product. It affords the practitioner the opportunity to circumvent invasive procedures, dental local anesthesia, and dental drills, all of which tend to provoke anxiety and fear in dental patients. For young individuals with behavioral challenges that are difficult to be managed in a standard clinical environment, the availability of SDF treatment can potentially obviate the need for either sedation or general anesthesia, thereby reducing the associated risks (4).

While the utilization of SDF for halting dental caries in primary and permanent teeth is not a recent development in the field of dentistry, it has been extensively employed in Japan since the 1970s and has gained traction in other countries such as Brazil, Argentina, China, and Australia. Remarkably, it was not until 2014 that SDF saw broader adoption in the United States, following the approval by the Food and Drug Administration (FDA) for its use in addressing tooth hypersensitivity and, in an off-label capacity, for the management of caries arrest (5).

Five indications for SDF treatment were listed by Horst et al.: (1) high caries risk; (2) treatment challenged by behavioral or medical management; (3) patients with carious lesions that cannot be treated in a single visit;(4) difficulty to treat dental carious lesions; and (5) patients without access to dental care. In patients undergoing treatment for cancer and patients with salivary impairment, the SDF application might help decrease the need for traditional restorations and reduce the financial cost (6).

Although efforts have been made to standardize the SDF application protocol, additional studies are required to identify the optimum application method for better and more successful results(6). Previous studies justified the use of light curing by the fact that it may accelerate precipitation of silver ions onto dentin and significantly alter penetration depth (7).

Suzuki et al. (3) indicated that the fluoride and silver ions contained in SDF can permeate the enamel to a depth of approximately 25 µm. Chu et al. (8) observed the penetration of silver ions into intact, healthy dentin at a range of 50 to 200µ m. Recently, Li et al. (9) demonstrated that extracted carious primary incisors exhibited penetration depths of 744±448.9 µm. Unexpectedly, the depth of penetration increased significantly, reaching the pulp chamber in deep carious lesions. Fung et al. (2) found that primary anterior teeth had higher rates of caries arrest compared to posterior teeth. According to Crystal et al. (10), anterior teeth exposed to natural light may undergo shorter SDF soaking time and experience more active silver precipitation.

The aim of this in vitro study was to assess the extent of silver diamine fluoride (SDF) penetration, the hardness of dentin, and silver ions deposition at various depths of dentinal carious lesions in primary posterior teeth following the application of SDF, with and without the application of dental light-curing.

METHODS

This in vitro study was conducted in the Department of Pediatric Dentistry, Alexandria University, Alexandria, Egypt. The approval of the Research Ethics Committee, Faculty of Dentistry Alexandria University was obtained before beginning the study (IRB NO.001056 - IORG 0008839).

Twenty four extracted primary molars with cavities extended into dentin were selected based on the following inclusion criteria: extracted as aresult of dental caries reaching the dentin with cavitation and diagnosed as code five following the guidelines of the caries diagnostic criteria ICDAS II (11) (The International Caries Detection and Assessment System).The exclusion criteria were: teeth exhibiting advanced dental caries reaching the pulp as determined clinically or radiographically; classified as code six, according to the ICDAS II, teeth with previous restorations; teeth subjected to prior SDF applications and teeth displaying developmental anomalies.

The sample size was estimated assuming 5% alpha error and 80% study power. According to Toopchi et al. (7), the mean±SD penetration depth of SDF was higher in the control group (130±50 µm) than in the test group (60±10µm). Based on the difference between two independent means using the highest SD = 50 µm. To ensure adequate power, the minimum sample size was calculated to be 10 samples per group, increased to 12 samples to make up for processing errors. Total sample size = number per group x number of groups= 12 x 2 = 24 samples. The software Sample size was based on Rosner's method (12) calculated by G*Power 3.1.9.7 (13).

The collected teeth were cleaned of soft tissue with gauze immediately after extraction and stored in an artificial saliva solution (14) before the begining of the study .

All teeth were mounted in acrylic cylinders and were randomly divided into two groups using computer randomization. The control group: consisted of twelve teeth treated with SDF without light curing (38 percent Ag $[NH_3]_2F$; Advantage Arrest, USA), and the test group included twelve teeth with SDF application in addition to the use of dental light-curing for 20 seconds (Polywave LED, Ivoclarvivdent, USA) light with a high-intensity output of 2,000 mW/cm^2.

All teeth were dried with oil-free air for one minute, succeeded by application of SDF to the carious lesion utilizing a disposable microbrush. The applied SDF was permitted to air-dry without any rinsing with water. The experiment was repeated two times with two weeks apart for both groups. Throughout the entire duration of the study, the teeth were preserved in laboratory-prepared artificial saliva and placed in an incubator at 37˚C to resemble the oral environment.

Two weeks after the final application, samples were sliced vertically into two halves mesiodistally or bucco-lingually, based on the site of the lesions by a metal disc. The specimens were placed in distilled water (15) after sectioning in order to maintain the mineral contents of dentin. Then, they were swabbed with alcohol to be analyzed by EDX for quantitative chemical analysis to measure the fluoride and silver ions precipitation in different layers of the lesions. The depth of SDF penetration was measured using a stereomicroscope and quantitative EDX analysis. Images were captured and analyzed using Toup View software. Based on the different structures present in the images provided by Stereomicroscope, three different layers with different structural patterns were detected in each sample, which included (Figure 1): Layer one: the outermost layer, representing infected dentin showing the most bacteria accumulation and demineralization; Layer two, the subsurface layer, which includes the affected dentin with a harder and more solid structure; and Layer three, representing sound dentin below the decay in which SDF has penetrated. Depth of penetration measurement was taken by tracing the silver ions through EDX analysis until they became absent. Three measurements were taken, two mm, apart along the base of the lesion. The mean of these measurements was calculated for each sample and compared within the two groups. The quantitative Vickers hardness test was performed using an indenter with a static load of 25 g applied for 30 s on the sound dentin layer (layer three) around the infected dentin (16). The mean of the final numbers was determined and compared with other samples.

Statistical analysis

Normality was checked using Shapiro Wilk test and Q-Q plots. Dentin hardness and depth of penetration were normally distributed while silver and fluoride ions precipitation was not normally distributed. Mean, standard deviation, median, inter quartile range (IQR), and minimum and maximum values were used for data presentation. Comparison between groups regarding dentin hardness and penetration depth was done using an independent t-test. Differences between silver and fluoride ions between groups were analyzed using Mann Whiteny U test whereas differences between sound, affected and infected dentin within each group were assessed using the Fridman test and followed by post hoc test with Bonferroni correction. All tests were two-tailed, and the significance level was set at p value≤0.05. Data were analyzed using IBM SPSS version 23, Armonk, NY, USA.

RESULTS

The average depth of SDF penetration into the affected dentin was higher in the control group (480.00–570.00 µm) than in the test group (400.00 – 488.00µm; P<0.0001). SDF reached all three layers (infected, affected, and sound dentin) in both the test and control groups in all samples (Table 1), (Figure 3).

Precipitation of silver ions:

The precipitation of silver ions at the infected dentin was higher in the test group (20.80 ± 9.25 percent) than in the control group (9.15 ± 3.33 percent; P=0.002; Figure 2). Silver ion precipitation did not show a significant difference between the affected dentin and the sound dentin in both groups, specifically at the second layer. The precipitation at the second layer was 4.62 ± 4.07 percent and 4.42 ± 4.10 percent for the test group and control group, respectively (P=0.564), and precipitation depths at the third layer were 0.88 ± 1.11 percent and 1.44 ± 1.65 percent in test group and control group, respectively (P=0.083) (Table 2) , (Figure 4).

Fluoride ions precipitation at the outermost infected dentin was higher in the test group (0.59 ± 0.65percent) than in the control group (0.35 ± 0.24percent). The precipitation of fluoride ions at the affected dentin and sound dentin was almost similar for both groups. The precipitation at the second layer were 0.58 ± 0.47 percent and 0.49 ± 0.49 percent for the test group and control group, respectively, and precipitation depths at the third layer were 0.50 ± 0.32 percent and 0.70 ± 0.45 percent in the test group and control group, respectively. No significant difference was detected for either the second or third depths (P=0.603 and 0.326) respectively (Figure 5).

Vickers hardness test within the sound dentin was higher in the test group (69.00-88.00kgf/mm²) than the control group (53.00-66.00kgf/mm²; P<0.0001) (Table 3), (Figure 6).

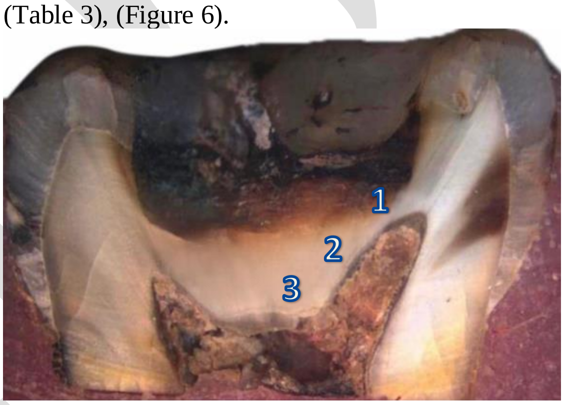
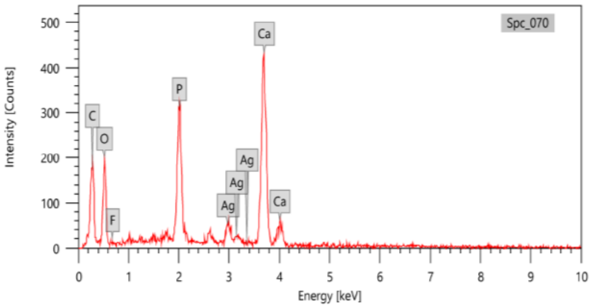


Figure (1): The penetration depth of silver diamine fluoride (SDF)in the test group. Vertical cross-section under the stereomicroscope (0.8× magnification) showing SDF staining in three different layers: 1) infected dentin; 2) affected dentin; and 3)sound dentin.

A



B

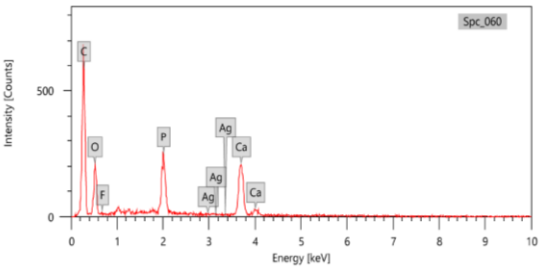


Figure (2): Chemical EDX analysis showing higher silver (Ag) precipitation in the infected dentin of the carious lesion in the test group (A) compared to the control group (B).

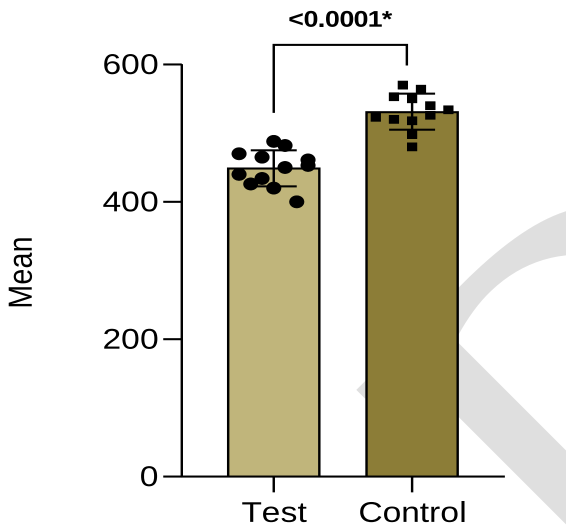


Figure (3): Comparison of penetration depth between the study groups

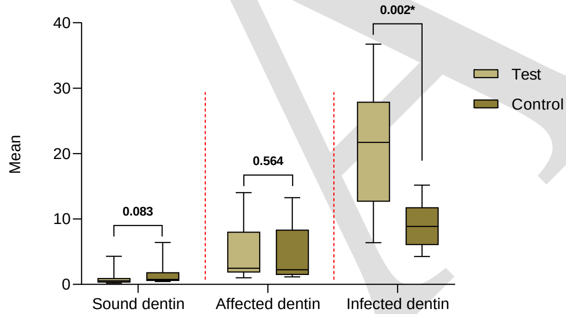


Figure (4): Comparison of silver ions precipitation into cavitated carious lesions between the study groups

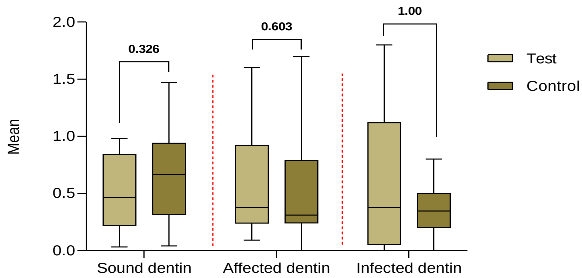


Figure (5): Comparison of fluoride ions precipitation into cavitated carious lesions between the study groups

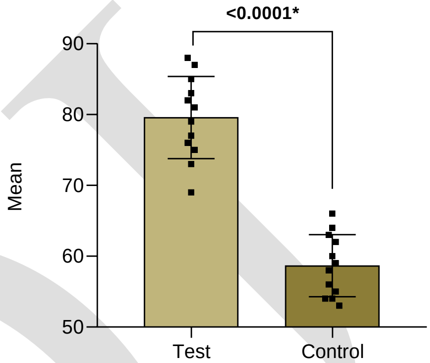


Figure (6): Comparison of sound dentin hardness between the study groups

Table (1): Comparison of penetration depth between the study groups

	Test group (n=12)	**Control group (n=12)**
Mean ± SD	449.08 ± 26.19	531.33 ± 26.31
Median (IQR)	451.50 (40.75)	530.00 (33.75)
Min – Max	400.00 – 488.00	480.00 – 570.00
F Test (p value)	7.676 (<0.0001*)	

*Statistically significant difference at p value≤0.05

Table (2): Comparison of silver ions precipitation into cavitated carious lesions between the study groups

		Test group (n=12)	**Control group(n=12)**	**U test (p value)**
Sound Dentin	Mean ± SD	0.88 ± 1.11	1.44 ± 1.65	1.732 (0.083)
	Median (IQR)	0.57 (0.60)	0.75 (1.21)	
	Min – Max	0.18 – 4.31	0.45 – 6.41	
Affected Dentin	Mean ± SD	4.62 ± 4.07	4.42 ± 4.10	0.577 (0.564)
	Median (IQR)	2.47 (6.14)	2.25 (4.10)	
	Min – Max	1.02 – 14.02	1.15 – 13.25	
Infected Dentin	Mean ± SD	20.80 ± 9.25	9.15 ± 3.33	2.944 (0.002*)
	Median(IQR)	21.71(15.18)	8.88 (5.70)	
	Min – Max	6.38 – 36.74	4.28 – 15.17	
Fridman test (p value)		24.00 (<0.0001*)	22.167 (<0.0001*)	
Pairwise comparisons		p_1= 0.043*, p_2<0.0001*, p_3=0.043*	p_1= 0.042*, p_2<0.0001*, p_3=0.124*	

*Statistically significant difference at p value≤0.05, p1: comparison between sound dentin and affected dentin, p2: comparison between sound dentin and infected dentin, p3: comparison between affected dentin and infected dentin

Table (3): Comparison of sound dentin hardness between the study groups

	Test group (n=12)	Control group (n=12)
Mean ± SD	79.58 ± 5.81	58.67 ± 4.38
Median (IQR)	80.00 (9.25)	58.50 (8.50)
Min – Max	69.00 – 88.00	53.00 – 66.00
F Test (*p* value)	9.965 (<0.0001*)	

*Statistically significant difference at p value≤0.05

DISCUSSION

The application method for SDF is continuously advancing in attempts to achieve optimal clinical results. This in vitro study reveals that incorporating an additional dental light-curing step for 20 seconds following SDF application will reduce the depth of penetration of SDF into sound dentin while improving hardness in the infected dentin.

This study concluded that dental light curing causes increased silver ions precipitation in the infected dentin layer of the carious lesion. Among the various underlying layers, the infected dentin layer absorbs the greatest amount of light. According to Babaahmadi et al. (17) natural light functions as a catalyst, triggering the conversion of the silver solution into silver ions and nanoparticles. Utilizing dental light curing as an initiating factor accelerates this reduction process, resulting in a swifter decrease and reduced timeframe for SDF penetration. This could potentially elucidate the heightened precipitation of silver ions within the carious dentin layer of the caries lesion.

The precise chemical interaction between the decayed lesion and SDF is not fully understood; previous research indicated that the dark color of the lesion could result from the creation of silver phosphate (18). Seto et al. (19) documented the creation of silver microwires filling the demineralized spaces caused by the caries process. Mei et al. (20) proposed that the interaction of silver with oxygen, phosphorus, and sulfur contributes to the development of silver phosphate, silver oxide, and silver sulfide. When subjected to light, these compounds could potentially give rise to the formation of metallic silver nanoparticles (9-21). The antibacterial properties of silver nanoparticles and their potential pros and cons require additional exploration, but these aspects fall outside the scope of this study (17-22).

The authors found no significant difference in the amount of silver ions within the affected and sound dentin layers among the research groups. This observation could suggest that the dental curing light did not deeply penetrate the inner layers of the decayed lesion beyond the surface of the infected dentin.

The penetration depth in the current study ranged from 480.00 – 570.00 µm for the control group and 400.00 – 488.00µm for the test group. As dental light curing speeds up the reduction of the silver solution (17), its omission would allow the solution additional time to permeate the decayed lesion and release the ions at a more gradual pace, preceding any reduction. This could potentially explain the increased depth of penetration observed in the control group in contrast to the test group. Subjecting the solution to light expedites the reduction process and consequently results in reduced penetration depth. Yamaga et al. (18) reported 20 to 100 µm penetration in SDF-treated dentin. Toopchi et al. (7) reported a 50-180 µm depth of penetration in carious primary incisors. Chu and Lo (8) reported a depth of penetration of 25 to 200 µm in SDF-treated carious lesions. Li et al. (9) was able to achieve a significantly greater level of penetration depth in carious primary incisors, recording an average depth of penetration reaching 744 µm. The dissimilarities in outcomes could potentially be attributed to variations in the utilized teeth, sample preparation techniques, and the approach employed for measurements. Due to disparities in methodology, direct comparisons cannot be established between the findings of this research and those of earlier investigations. It is important to acknowledge that the depth of penetration observed in in-vitro studies could potentially overestimate the actual depth of penetration achievable in real clinical scenarios, owing to sample desiccation and the absence of external positive pressure in non-extracted teeth. Investigating SDF penetration in primary teeth with carious lesions, Li et al. (9) observed significant silver deposition within the deepest demineralized lesions. These researchers deduced that the extent of SDF penetration is influenced by the extent of demineralization. This factor might contribute to the differences observed in measurements across various studies.

The Vickers hardness measurement exhibited higher values within the test group compared to the control group. The elevated dentin hardness signifies a more robust and structurally sound tooth composition within the carious lesion, acting as a deterrent to the progression of the caries (8). Elevated dentin hardness signifies the process of remineralization and halting of caries progression. By impeding the extension of bacteria and additional demineralization, the efficacy of SDF in arresting decay is enhanced. The elevated hardness could potentially be elucidated through the reduction of silver ions, resulting in the formation of insoluble silver sulfide. This compound is a compact, dark solid that exhibits insolubility in all solvents (9). The observation made by Seto et al. (19) regarding the presence of silver microwires led to their hypothesis that these microwires could potentially be responsible for the elevated dentin hardness. Due to the in vitro nature of this research, it proves challenging to entirely avert sample dehydration prior to conducting the microhardness assessment, consequently impacting the resultant microhardness measurements.

Considering that SDF penetration was observed beneath the carious lesion in all 24 samples, controlling the depth of penetration for SDF could potentially mitigate its diffusion into the pulp. This is particularly relevant for deep carious lesions that are in close proximity to the pulp. Comparable investigations concerning primary incisors affected by caries have demonstrated that in cases of deep carious lesions, SDF can reach the pulp (9). Crystal et al. discourage the application of SDF on exposed pulp and suggested meticulous follow-up when utilizing SDF for deep carious lesions (23). The researchers concluded that the use of SDF in close proximity to the pulp can be considered safe based on their findings.

The enhanced hardness measurements observed during dental light curing may also offer benefits for superficial carious lesions that do not extend close to the pulp. The elevated hardness levels could potentially contribute to the mitigation of subsequent acid attacks and the recurrence of caries. Microhardness serves as an indirect gauge of mineral concentration within dentin, wherein the mineral content acts as a deterrent against additional acid penetration. Additional in vivo studies are needed to validate the clinical significance of these observations.

Although the results from this in vitro study demonstrate positive results, such as heightened hardness, reduced depth of penetration, and elevated levels of silver ions within the infected dentin layer of carious lesions, additional investigations are required to explore whether incorporating light curing during the application of SDF will affect bonding and retention of overlying materials.

CONCLUSIONS

1. The utilization of a dental curing light following the application of silver diamine fluoride for carious dentinal lesions results in an increased silver ions precipitation within the infected dentin and a decrease in SDF penetration.
2. Light-curing following SDF treatment also increases the hardness of infected dentin.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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