ORIGINAL ARTICLE

Effect of Curing Light with Different Intensities on the Penetration of Silver and Fluoride Ions and Dentin Hardness in Primary Carious Molars Following Silver Diamine Fluoride Application: A Comparative Microscopic *Ex Vivo* Study

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Abstract

Background: A paradigm shift from surgical to medical approach for caries management has popularized silver diamine fluoride (SDF) as a preventive and interim caries arrest medicament. Few studies conducted have explored the effect of curing light on SDF's microbial property, its penetration, and effect on dentin. However, there is a research gap regarding the effect of different intensities of curing light on SDF performance.

Aim: To determine the effect of different curing light intensities on SDF penetration depth and dentin hardness in carious lesions of primary molars.

Materials and methods: Silver diamine fluoride was applied on 30 extracted carious primary molars. Teeth were randomly allocated into three groups—(1) control group, no light curing after application of SDF; (2) light curing of SDF with low intensity (1000 mW/cm^2); and (3) light curing of SDF with high intensity (2500 mW/cm^2). A scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis was performed to check ion penetration after 1 week, and a Vickers hardness test was used to assess dentin hardness of both infected and affected dentin layers at 1-week and 1-month intervals. Based on the distribution of data, parametric and nonparametric tests were applied. Statistical Package for the Social Sciences (SPSS) version 26 was used for statistical analysis. The level of significance was set at 5%.

Results: Silver diamine fluoride penetrated beyond the carious lesion in all three groups. The mean silver ion precipitation in infected dentin in group III (16.90 ± 0.68) was maximum, whereas it was found to be minimum in group II (7.31 ± 0.63). The mean fluoride ion precipitation in affected dentin in group III (4.06 ± 0.41) was highest and least in group II (3.09 ± 0.58). A considerable increase in mean dentin hardness of infected dentin was observed in all three groups (214.00 ± 89.06, 218.00 ± 75.17, 231.00 ± 98.86, respectively; $p < 0.001$) after 1 month.

Conclusion: Applying SDF to carious lesions using a high-intensity dental curing light induced more silver ion precipitation in infected dentin and increased its hardness.

Keywords: Curing light, Dental caries, Fluorides, Silver diamine fluoride, Tooth remineralization.

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Introduction

Dental caries is defined as an infectious process associated with tooth structure disintegration. Treatment of dental caries entails restorative procedures, root canal treatments, or extractions, all of which can be traumatic, especially for children. Preventive measures such as fluoride application, pit and fissure sealant placement, noninvasive techniques, and the use of silver diamine fluoride (SDF) are strongly considered in order to overcome these issues. SDF, in particular, is gaining popularity because of its role in caries prevention and inhibition.[1]

Silver diamine fluoride, also candidly known as "the silver fluoride bullet," was introduced into the field of dentistry around the 1900s in countries such as Japan, Mexico, and Australia as a method of caries prevention. Various *in vivo* and *in vitro* studies conducted at the end of the 20th and beginning of the 21st centuries documented the effectiveness of silver fluoride compounds in arresting caries lesions. Although SDF is used off-label to treat caries in the United States, the Food and Drug Administration (FDA) granted it "breakthrough therapy designation" for arresting dental cavities and approved the first product in August 2014 as a treatment for dentinal hypersensitivity in adults.[2]

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Silver diamine fluoride [$Ag(NH_3)_2F$], is a colorless alkaline solution containing silver and fluoride, which forms a complex with ammonia.[3] SDF is a mixed heavy-metal halide coordination complex rather than a simple salt of silver, ammonium, and fluoride ions. According to reports, SDF emits two to three times the amount of fluoride as sodium fluoride, stannous fluoride, or acidulated phosphate fluoride, all of which are common ingredients in fluoride foams, gels, and varnishes.[4]

The fluoride component of SDF helps slow down demineralization and promote remineralization, which strengthens the tooth structure. This is one of the four main ways that SDF functions. The silver component functions as an antibacterial agent, killing bacteria while simultaneously preventing the growth of new biofilm.[5] The use of SDF is painless and does not require cavity excavation.[5] Because of these advantages, SDF is used to prevent caries, which improves the quality of life for patients with limited cooperation or access to dental treatments, such as young children. The effectiveness of SDF depends on various factors like concentration (38 or 12%), application time (1–3 minutes), frequency of application, the addition of salts like potassium iodide, and exposure to dental curing light.[6]

According to Crystal and Niederman,[7] there may be a correlation between less SDF soaking time and increased active silver precipitation when anterior teeth are exposed to natural light. When SDF is exposed to light, it exhibits both lesion darkening and an increase in surface hardness. Lesion depth also decreased following SDF treatment, which may have been caused by calcium and phosphate aggregation on the surface of the lesion.[8] The use of dental light curing acts as an initiating agent, resulting in a significant reduction in application time and a shorter period for SDF penetration.[9]

However, there is a dearth of studies in the literature that assess the effect of different intensities of dental curing lights on silver, fluoride ions, and dentin hardness after SDF application. Therefore, the goal of this study was to determine the impact of various curing light intensities on the penetration of silver, fluoride ions, and dentin hardness in primary tooth carious lesions after SDF application.

The null hypothesis proposed was that the overall penetration of silver, fluoride ions, and dentin hardness after SDF treatment will not be significantly affected by the intensity of dental curing light.

Materials and Methods

This *ex vivo* comparative microscopic study was conducted in the Department of Pediatric Dentistry in accordance with CRIS guidelines for *in vitro* studies.

Sample Size Calculation

To calculate the sample size for the present study, the mean percentage scores of silver ion precipitation between control and test groups in infected dentin observed by Toopchi et al.[9] were considered. The mean percentage score for the control group was 9.28 ± 3.53, and for the test group, it was 24.61 ± 14.74. Substituting the mean difference of 15.33 in the formula[10] at a 95% confidence interval (two-sided) and a power of study of 80%, the estimated sample size per group was 8. This figure was rounded to 10. Hence, the total sample size for 3 groups for the present study was 30 (10 per group).

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2 / \kappa)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_2 = \frac{(\kappa * \sigma_1^2 + \sigma_2^2)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

Randomization and Blinding

To eliminate allocation bias, all extracted teeth were collected in one container and divided into three groups by another clinician using a simple random lottery approach.

Methodology

A total of 30 multi-rooted primary teeth with deep dentinal caries, more than one-third root resorption, nonrestorable, and indicated for extraction were selected. Teeth with previous restorations, previous SDF applications, and developmental anomalies were excluded.

The collected teeth were cleaned of soft tissue with gauze immediately after extraction and stored in an artificial saliva solution before the start of the study. The 30 teeth were randomly divided into three groups, each consisting of 10 teeth, using simple random assignment. The control group (group I) consisted of teeth with SDF [38% $Ag(NH_3)_2F$; Kids-e-dental, India] application only (Fig. 1 group II included teeth with SDF application in addition to the use of low-intensity (1000 mW/ cm^2) dental curing light (Woodpecker i-LED Plus, Guilin, Guangxi) for 20 seconds after SDF application. Group III included teeth with SDF application followed by exposure to high-intensity (2500 mW/cm^2) dental curing light (Woodpecker i-LED Plus, Guilin, Guangxi) for 3 seconds. Before SDF application, all teeth were dried with oil-free air for 1 minute, followed by 1 minute of SDF application to the carious lesion using a disposable microbrush (Fig. 1). The SDF was allowed 1 minute to dry after being applied, without rinsing with water. After SDF application and during the entire study period, samples were stored in artificial saliva made in the lab [potassium dihydrogen phosphate (KH_2PO_4) (544 mg/L) + sodium fluoride (NaF) (103 mg/L) + potassium chloride (KCl) (224 mg/L) + magnesium chloride ($MgCl_2$) (19 mg/L)] and placed in an incubator at 37°C to resemble the oral environment. Samples underwent thermocycling (250 cycles between 5 and 55°C), with a 30-second immersion period in each bath and a 10-second transfer period. The silver particles continued to penetrate within the carious lesion after 17–32 days of SDF application;[11] and hence, our study evaluated the microhardness of samples at 1 week. After 1 week, samples were sliced vertically with a thickness of 2 mm mesiodistally or buccolingually with a diamond disk bur. Fixation of samples was done using 4% formaldehyde, followed by placement of samples in a hot air oven at 37°C for 1 day for drying.

The slices were platinum-coated and prepared to be analyzed by a scanning electron microscopy (SEM) (JEOL 7600F FESEM, Japan) at a magnification of 145×. SEM images were recorded by INCA software (Oxford INCA software, ETAS, Japan) to perform chemical analysis [energy dispersive X-ray (EDX) analysis]. Chemical analysis

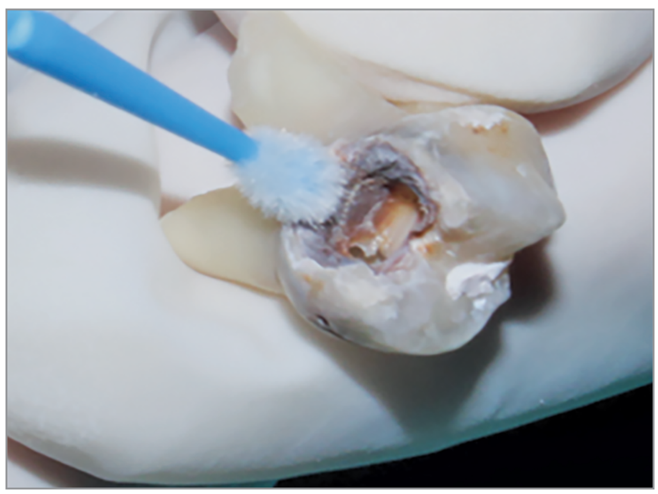


Fig. 1: Silver diamine fluoride application to the carious lesion using a disposable microbrush

was used to measure the fluoride and silver ion precipitation in different layers of the lesions.

The Vickers hardness test was performed using a microhardness indenter machine (Micromet 2100, Buhler) for all three layers of dentin—(1) infected dentin, (2) affected dentin, and (3) sound dentin after 1-week and 1-month intervals. Three loads in randomly selected different areas of infected, affected, and sound dentin were conducted, and the measurements were calculated based on the following formula:

$$HV = 0.102\ F/S = 0.102\ (2F\sin\ \theta/2)/d^2 = 0.1891\ F/d^2$$

The mean of the final numbers was determined and compared with other samples.

Statistical Analysis

After collection of data, the data were coded and entered into Microsoft Excel 2019. The Shapiro–Wilk test was applied to check whether the data were normally distributed or not. Except for silver ion precipitation, all variables showed $p > 0.05$, suggesting the data were normally distributed. Based on the distribution of data, parametric and nonparametric tests were applied. The descriptive analysis was presented in mean and standard deviation. A one-way analysis of variance (ANOVA) test was performed for the comparison of mean differences among the groups. *Post hoc* Tukey's test was applied for multiple comparisons if the result of one-way ANOVA was significant. The mean of silver ion precipitation was compared using the Kruskal–Wallis test. *Post hoc* Mann–Whitney *U* test was applied for multiple comparisons if the result of the Kruskal–Wallis test was significant. An independent *t*-test was used to compare the mean between two groups. Statistical Package for the Social Sciences (SPSS) (SPSS version 26, IBM Corporation, Armonk, New York) was used for statistical analysis. The level of significance was set at 5%.

Results

Precipitation of Silver and Fluoride Ions

Fluoride ion precipitation at the infected dentin was highest in group I (7.83 ± 1.17, Fig. 2), followed by group II (5.38 ± 0.64, Fig. 3) and least in group III (3.53 ± 0.43, Fig. 4) (p <0.001). In affected dentin layer, the highest concentration was observed in group III (4.06 ± 0.41), followed by group I (4.00 ± 0.88) and least in group II (3.09 ± 0.58) ($p = 0.004$) (Table 1).

Silver ion precipitation at the infected dentin was the highest in group III (17.86 ± 0.84, Fig. 4) followed by group I (16.90 ± 0.68, Fig. 2) and least in group II (7.31 ± 0.63, Fig. 3) ($p < 0.001$). Silver ion precipitation in the affected dentin was the highest in group I (21.05 ± 0.73) and least in group II (6.98 ± 0.76) ($p < 0.001$) (Table 1).

Dentin Hardness

After 1 week, dentin hardness in the sound dentin layer was the highest in group III (752.00 ± 85.63), followed by group II (657.00 ± 121.90), and least in group I (548.00 ± 132.52) ($p = 0.04$).

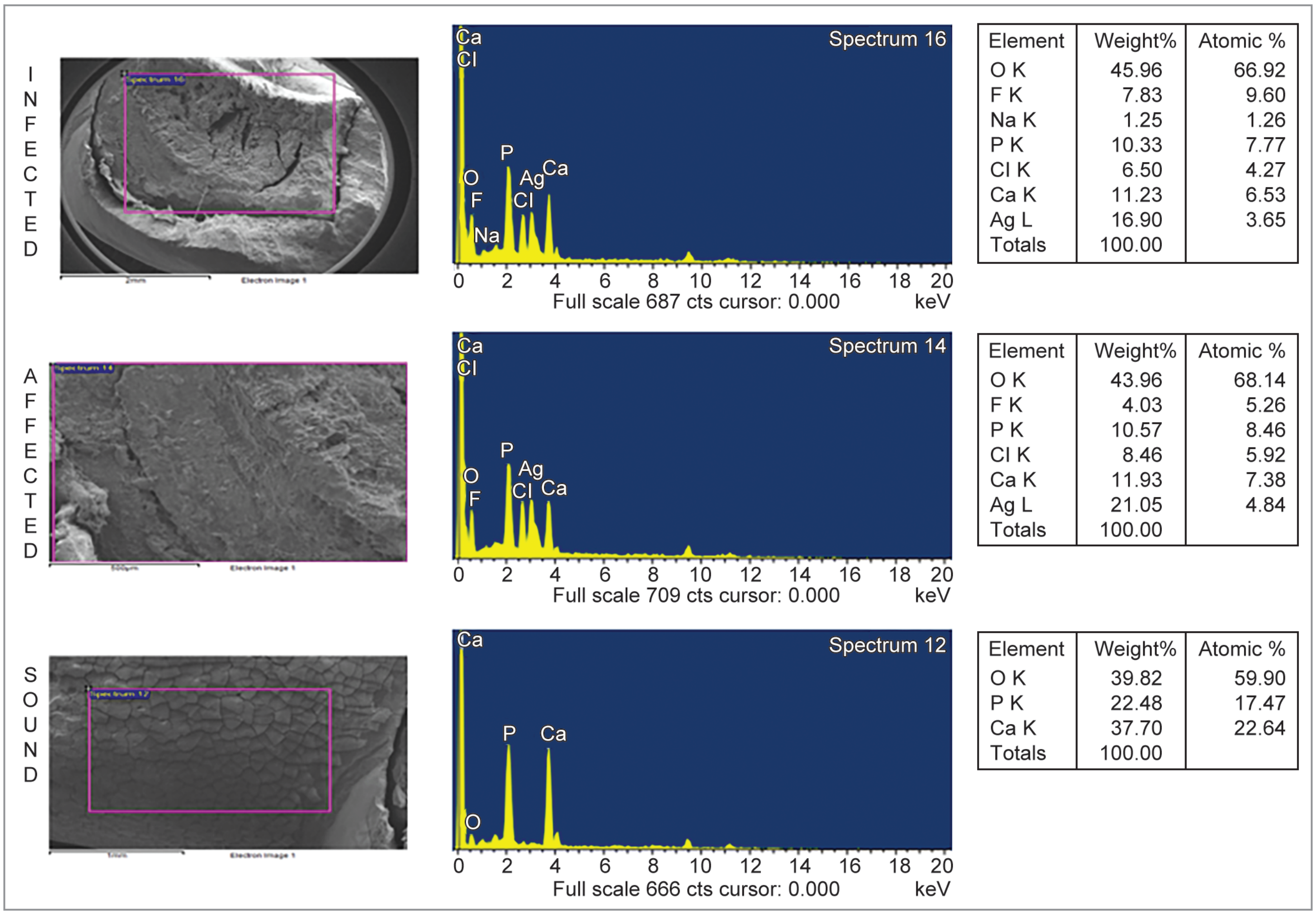
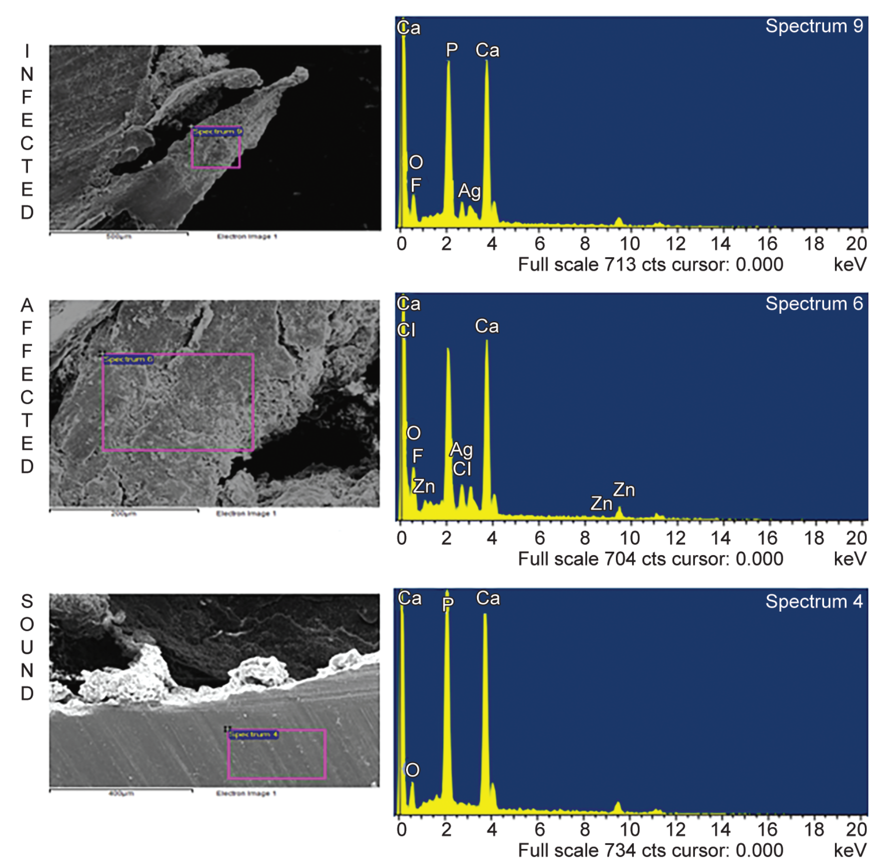


Fig. 2: Scanning electron microscopy images with EDX analysis for group I

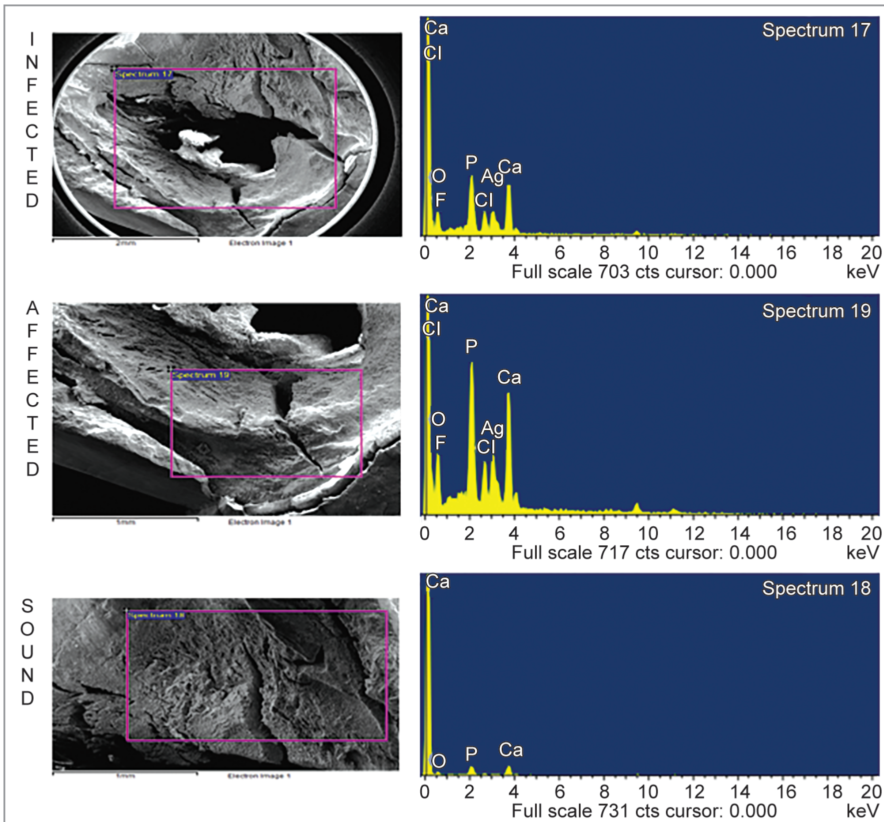


Element	Weight%	Atomic %
O K	34.60	54.37
F K	5.38	7.12
P K	20.27	16.46
Ca K	32.44	20.35
Ag L	7.31	1.70
Totals	100.00	

Element	Weight%	Atomic %
O K	46.93	67.30
F K	3.19	3.85
P K	15.53	11.51
Cl K	3.23	2.09
Ca K	23.87	13.67
Zn K	0.27	0.10
Ag L	6.98	1.48
Totals	100.00	

Element	Weight%	Atomic %
O K	39.05	58.98
P K	24.13	18.82
Ca K	36.81	22.19
Totals	100.00	

Fig. 3: Scanning electron microscopy images with EDX analysis for group II



Element	Weight%	Atomic %
O K	39.32	62.62
F K	3.53	4.73
P K	13.39	11.01
Cl K	6.57	4.72
Ca K	20.34	12.93
Ag L	16.86	3.98
Totals	100.00	

Element	Weight%	Atomic %
O K	39.73	62.67
F K	4.06	5.40
P K	14.24	11.61
Cl K	5.66	4.03
Ca K	19.71	12.41
Ag L	16.60	3.88
Totals	100.00	

Element	Weight%	Atomic %
O K	55.68	73.78
P K	17.87	12.23
Ca K	26.45	13.99
Totals	100.00	

Fig. 4: Scanning electron microscopy images with EDX analysis for group III

No statistically significant difference was noticed for affected and infected dentin after 1 week (Table 2).

A statistically significant ($p < 0.001$) increase in dentinal hardness of infected dentin was observed in all three groups after 1 month when compared with 1 week (214.00 ± 89.06, 218.00 ± 75.17, 231.00 ± 98.86 for groups I, II, and III, respectively) (Table 3).

Discussion

The SDF application protocol continues to evolve in attempts to achieve the best clinical outcome. According to this *ex vivo* investigation, the concentration of silver ions and hardness in the infected dentin were enhanced by performing a second step of dental light curing with high intensity for 3 seconds following the application of SDF.

Table 1: Comparison of concentration of fluoride and silver ions precipitations among groups in three different layers of dentin

Layers of dentin	*Groups*	*Mean ± standard deviation (SD)*	*95% CI*	*p-value*
Fluoride ions precipitations[a]				
Affected dentin	Group I (*n* = 10)	4.00 ± 0.88	3.37–4.63	0.004*
	Group II (*n* = 10)	3.09 ± 0.58	2.68–3.50	
	Group III (*n* = 10)	4.06 ± 0.41	3.77–4.35	
Infected dentin	Group I (*n* = 10)	7.83 ± 1.17	6.99–8.67	<0.001**
	Group II (*n* = 10)	5.38 ± 0.64	4.92–5.84	
	Group III (*n* = 10)	3.53 ± 0.43	3.22 -3.84	
Silver ions precipitations[b]				
Affected dentin	Group I (*n* = 10)	21.05 ± 0.73	20.53–21.57	<0.001**
	Group II (*n* = 10)	6.98 ± 0.76	6.43–7.53	
	Group III (*n* = 10)	16.60 ± 0.87	15.98–17.22	
Infected dentin	Group I (*n* = 10)	16.90 ± 0.68	16.42–17.38	<0.001**
	Group II (*n* = 10)	7.31 ± 0.63	6.86–7.76	
	Group III (*n* = 10)	17.86 ± 0.84	16.26–17.45	

Data were presented as mean ± SD. Mean were compared by using [a], one-way ANOVA, [b], Kruskal–Wallis; *, $p < 0.05$ significant; **, $p < 0.001$ highly significant

Table 2: Comparison of hardness test (kgf/mm^2) at 1st week among groups in three different layers of dentin

Layers of dentin	*Groups*	*Mean ± SD*	*95% CI*	*p-value*
Sound dentin	Group I (*n* = 10)	559.20 ± 179.09	431.08–687.31	0.04*
	Group II (*n*= 10)	637.93 ± 185.72	505.08–770.78	
	Group III (*n* = 10)	748.00 ± 108.37	670.48–825.52	
Affected dentin	Group I (*n* = 10)	235.93 ± 83.16	176.44–295.42	0.43
	Group II (*n* = 10)	249.98 ± 93.16	183.34–316.62	
	Group III (*n* = 10)	289.00 ± 101.74	216.22–361.78	
Infected dentin	Group I (*n* = 10)	34.60 ± 17.21	22.29–46.91	0.71
	Group II (*n* = 10)	36.89 ± 12.07	28.25–45.53	
	Group III (*n* = 10)	39.89 ± 12.69	30.81–48.97	

Data were presented as mean ± SD. Mean were compared by using one-way ANOVA; *, $p < 0.05$ significant

Table 3: Comparison of hardness test (kgf/mm^2) at 1 week and after 1 month in three different layers of dentin

Layers	*At 1 week*	*After 1 month*	*95% CI*	*p-value*
Group I				
Sound	559.20 ± 179.09	548.00 ± 132.52	−136.81–159.21	0.87
Affected	235.93 ± 83.16	250.00 ± 92.01	−96.46–68.32	0.72
Infected	34.60 ± 17.21	214.00 ± 89.06	−239.66 to −119.13	<0.001**
Group II				
Sound	637.93 ± 185.72	657.00 ± 121.90	−166.66–128.52	0.79
Affected	249.98 ± 93.16	275.95 ± 82.61	−108.69–56.75	0.52
Infected	36.89 ± 12.07	218.00 ± 75.17	−231.69 to −130.53	<0.001**
Group III				
Sound	748.00 ± 108.37	752.00 ± 85.63	−95.52–87.52	0.93
Affected	289.00 ± 101.74	294.00 ± 94.53	−97.26–87.26	0.91
Infected	39.89 ± 12.69	231.00 ± 98.86	−257.33 to −124.89	<0.001**

Data were presented as mean ± SD. Mean were compared by using *t*-test, CI, confidence interval, **, $p < 0.001$ highly significant

Natural light acts as a catalyst to reduce the silver solution, producing silver ions and nanoparticles.[12] Compared to the deeper dentinal layers, the superficial infected dentin layer absorbs most of the light. Since the use of dental light curing acts as an initiating agent, rapid reduction and shorter time allowed for the penetration of SDF might explain the increase in precipitation of silver ions in the infected dentin layer of the carious lesion.

Although prior research revealed that the creation of silver phosphate is responsible for the lesion's black hue, the exact chemical reaction between the carious lesion and SDF is still unknown.[13] The production of silver microwires through dentinal tubules destroyed by the carious process was described by Seto et al.[10] Silver interaction with oxygen, phosphorus, and sulfur results in the creation of silver phosphate, silver oxide, and silver sulfide, according to Mei et al.[5] When these substances are exposed to light, metallic silver nanoparticles may develop, giving the darker hue.

The microhardness test was preferred to assess dentinal hardness as it is an indirect method of determining dentin mineral content, which reflects the amount of remineralization that has taken place as a result of SDF application.[14] The silver particles continue to penetrate within the carious lesion after 17–32 days of SDF application,[11] and hence our study evaluated the microhardness of samples at 1-week and then at a 1-month interval. Due to its high sensitivity in detecting different elements in tissues, EDX spectroscopy (EDX analysis) was selected for recording ion penetration in this study.

Since dental light curing speeds up the reduction of silver solution,[12] not utilizing the curing light would give the solution more time to penetrate the carious lesion and release the ions more gradually before reduction could take place. The deeper penetration in the control group compared to the test group may be explained by this. Dentin hardness was not performed immediately after SDF application because the water content of dentin, at a level of approximately 12% by weight and 25% by volume, can lead to uncontrolled artifacts due to tissue contraction as moisture would be inescapable during testing of the samples.[14]

The test group had a higher Vickers hardness rating than the control group. An increase in dentin hardness indicates a stronger and more stable tooth structure surrounding the carious lesion, which stops it from progressing.[14] An increase in dentin hardness is a sign of remineralization and the arrest of caries. The ability of the SDF to stop the degradation is improved by preventing the spread of bacteria and subsequent demineralization. The application of SDF to tooth structure results in the release of highly reactive silver ions that form silver phosphate (Ag_3PO_4) and silver oxide (Ag_2O). Conversely, the proteins (collagen) reduce the other part of the silver ions, which remain as metallic silver that is attached to the proteins (a phenomenon known as the silver-protein complex). A small amount of the silver chloride is reduced to produce metallic silver, but silver phosphate crystals and oxide are unstable and are substituted by silver chloride, which is less soluble in acids.[15] Seto et al.[10] claimed that the hardening of SDF-arrested caries is caused by the silver reaction rather than traditional fluoride-mediated remineralization. Additionally, the dark stain formation and color change of the tooth structures that were noticed following the procedure are caused by the silver compounds previously described. The clinical appearance of a coal-black color indicates that caries has been arrested.[8]

In superficial carious lesions that aren't close to the pulp, the higher hardness values seen with dental light curing can be beneficial. It's possible that the enhanced hardness will lessen future acid attacks and the development of caries.[3] The mineral content of the dentin limits further acid penetration, and microhardness is an indirect indicator of dentin mineral content.[14]

In the study conducted by Toopchi et al.,[9] a 2000 mW/cm^2 intensity of dental curing light for 40 seconds after SDF application was used, which induced more silver ion precipitation in infected dentin and also showed a significant increment in the hardness of infected dentin. In our study, with a 2500 mW/cm^2 intensity of curing light for 3 seconds, we found maximum saturation in infected dentin along with the highest dentinal hardness.

To establish the clinical implications of these findings, more *in vivo* research is required. To confirm the long-term effect of SDF, studies with longer follow-up and larger sample sizes are needed in the future. Further studies are needed to investigate whether the addition of a curing light to the application of SDF will provide superior clinical outcomes in terms of arresting carious lesions. Additional research is required to fully understand the antibacterial action of silver nanoparticles along with its potential benefits and drawbacks.

Conclusion

The following inferences can be drawn from the findings of this study:

- Applying high-intensity curing light after SDF application resulted in increased saturation of the silver concentration in infected dentin compared to the control group.
- Light curing of SDF with a high intensity curing light increased dentinal hardness and caused deeper penetration of fluoride.

Based on these findings, it is recommended to use a high-intensity curing light after SDF application as it allows for a greater radius of caries preventive action of SDF.

Authors Contributions

Bhatt Rohan: Conception of idea and preparation of the manuscript; Patel Megha: Review of the manuscript; Thakkar Aakanxa: Conducted the study; Patel Chhaya: Adding scientific content; Makwani Disha: Review of the manuscript; Patel Foram: Compilation of the data.

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