Research Article

Caries Research

Caries Res
DOI: 10.1159/000496210

Received: July 10, 2018
Accepted after revision: December 12, 2018
Published online: February 26, 2019

Assessment of the Silver Penetration and Distribution in Carious Lesions of Deciduous Teeth Treated with Silver Diamine Fluoride

Yihong Li[a] Yingyi Liu[a, b] Walter J. Psoter[c] Olivia M. Nguyen[a] Timothy G. Bromage[d] Marc A. Walters[e] Bin Hu[d] Sasan Rabieh[d] Fancy C. Kumararaja[a]

[a]Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY, USA; [b]Department of Cariology, Endodontology, and Operative Dentistry, Peking University School and Hospital of Stomatology, Beijing, China; [c]University of Rochester Medical Center School of Medicine and Dentistry, Rochester, NY, USA; [d]Department of Biomaterials and Biomimetics, New York University College of Dentistry, New York, NY, USA; [e]Department of Chemistry, New York University College of Arts and Science, New York, NY, USA

Keywords

Early childhood caries · Silver diamine fluoride · Deciduous tooth · Enamel · Dentin

Abstract

The aim of this study was to determine the effects of 38% silver diamine fluoride (SDF) on carious lesions of human deciduous teeth. Ten extracted deciduous incisors with caries were collected and treated with SDF. After the treatment, the teeth were sectioned through the center of the carious lesion. The extent of sliver precipitation was examined using quantitative backscattered electron scanning electron microscopy (qBSE-SEM), energy-dispersive X-ray spectroscopy (EDX), and micro-computed tomography (micro-CT). The qBSE-SEM images revealed that the silver particles could penetrate through the pellicle complex, along with the rod sheaths into the demineralized enamel rods and the dentinal tubules, and form silver-enriched barriers surrounding the carious lesions at depths up to 2,490.2 µm (mean 744.7 ± 448.7 µm) within the dentinal tubules of the carious lesions, but less likely in the sound enamel. The EDX spectrum analysis revealed that carbon, oxygen, phosphorus, chlorine, silver, and calcium were the main elements detected in the lesions treated with SDF. Additionally, sodium, magnesium, aluminum, silicon, zinc, sulfur, and fluorine were detected as the minor elements within the SDF precipitation "zone." The micro-CT analysis further showed that in the deep cavitated lesions, the silver precipitation could be observed in the pulp chamber. These findings provide new evidence defining the SDF mode of action for arresting caries and suggest that the application of a highly concentrated SDF solution on deciduous teeth should be used with caution for various carious lesions.

Introduction

Clinical studies have demonstrated that topical treatment with 38% silver diamine fluoride (SDF) is useful in arresting dental caries among preschool children world-

Prof. Yihong Li
Department of Basic Science and Craniofacial Biology
New York University College of Dentistry, 345 E. 24th Street
New York, NY 10010 (USA)
E-Mail yihong.li@nyu.edu

wide [Lo et al., 2001; Chu et al., 2002; Llodra et al., 2005; Yee et al., 2009; Zhi et al., 2012]. The treatment effectiveness is approximately 68% (range 31–79%) [Rosenblatt et al., 2009; Peng et al., 2012; Gao et al., 2016; Horst et al., 2016]. As the treatment is inexpensive, minimally invasive, and available for off-label use in pediatric dental clinics, it has been rapidly adopted by dental clinicians in the United States since 2015. However, the estimated general failure rate of the treatment was 20–34% [Gao et al., 2016]. In vivo evidence defining the SDF mode of action for arresting or failing to arrest caries is lacking.

In the 1970s, Yamaga et al. [1972] suggested that the principal chemical reactions between SDF {$[Ag(NH_3)_2F]$} and the tooth component hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ involved the formation of an impermeable layer of silver phosphate (Ag_3PO_4) and calcium fluoride (CaF_2) on the treated tooth surfaces. Although some of the hypotheses were tested in laboratory studies [Suzuki et al., 1974], most of the previous observations were based on in vitro experiments using sound dental blocks, artificial mouths, or animal models [Mei et al., 2015, 2017; Rossi et al., 2017]. Limited is known regarding the characterization of SDF-tooth interactions and the mode of action of SDF against carious lesions in deciduous teeth.

The aim of this study was to assess the silver penetration and distribution in carious lesions of deciduous teeth treated with 38% SDF. We hypothesized that the degree of silver penetration was correlated with the degree of demineralization of the enamel and dentin and that the precipitation and solidification of a high concentration of silver in the depth of lesions facilitated caries arrest. Understanding the mechanisms of SDF will allow us to establish scientifically sound SDF treatment regimes in order to provide a better caries arrest agent and the best possible care for not only the special-needs patient population, but also for general use in caries management.

Materials and Methods

Sample Collection and Preparation

This study was an ex vivo study approved by the Institutional Review Boards of New York University School of Medicine. Ten deciduous incisors that had been newly extracted due to caries were collected from the Pediatric Dental Clinic of New York University College of Dentistry. The teeth without caries removal or excavation procedure were disinfected immediately with 0.1% thymol after the extraction and transferred to the microbiology lab within 24 h. The teeth were rinsed with phosphate-buffered saline using a 10-mL sterile syringe following an aseptic process protocol and air-dried for no more than 4 h. The 38% SDF was dispensed into a disposable dental dappen dish and applied directly onto the entire tooth surface including carious lesion using a microbrush applicator (Henry Schein, Inc., Melville, NY, USA) that was saturated with the solution, following the manufacturer's instructions (Advantage Arrest; Elevate Oral Care, West Palm Beach, FL, USA). All teeth were air-dried overnight, dehydrated, and embedded in polymethylmethacrylate resin, according to hard tissue preparation protocols for further analysis via electron microscopy [Boyde, 1984].

Backscattered Electron Scanning Assay of Deciduous Incisors

The embedded incisors were sectioned with a Buehler Isomet 1000 diamond saw (Buehler, Lake Bluff, IL, USA) through the center of each carious lesion. The surfaces were polished, washed, and air-dried. Each specimen was imaged using a Zeiss EVO-50 scanning electron microscope in a quantitative backscattered electron mode (qBSE-SEM) (Carl Zeiss SMT Ltd., Cambridge, UK). All images were obtained in a 500-nm-wide field with a pressure of 50 Pa, an accelerating voltage of 15.0 kV, a filament current of 1.965 A, a beam current of 600 pA, and a working distance of 9 mm without a conductive coating. Halogenated dimethacrylate standards were used to calibrate the specimens, characterize the scope of silver precipitation (location, depth, and range), and examine the alterations of enamel and dentinal tubule microstructural features in the tooth regions with and without caries [Boyde et al., 1995]. BSE detection, contrast, and brightness were set to capture the images of enamel and dentin cross-sections within a broad dynamic range of 0–255 Gy for semiquantitative comparisons. Images were recorded using the ImageJ IJ 1.46r software [Schneider et al., 2012].

Energy-Dispersive X-Ray Spectroscopy Assay of Deciduous Incisors

Following qBSE-SEM, the sectioned tooth surfaces were examined by energy-dispersive X-ray spectroscopy (EDX) to determine the anatomical localization of silver and the elemental spectrum at targeted areas. The same Zeiss EVO-50 field emission SEM equipped with an EDX spectrometer (Carl Zeiss SMT Ltd.) was used at the same accelerating voltage of 15.0 kV. EDX image maps were analyzed using the ImageJ IJ 1.46r software [Schneider et al., 2012].

Micro-Computed Tomography Scanning Assay of Deciduous Incisors

The teeth treated with SDF solution were scanned using a high-resolution micro-computed tomography (micro-CT) scanner (SkyScan 1172; Bruker, Kontich, Belgium). Each specimen was wrapped in sterile phosphate-buffered saline-soaked gauze, fixed in a customized holder for a "large" sample, and scanned in "in air" setting. Images were acquired using an 11-Mp digital detector. The X-ray source was set to 100 kV and 100 μA. An image voxel size of 9.01 μm and a 0.5-mm aluminum filter were used with a 0.30 rotation step and 42% beam hardening correction. In each scan, 848 to 1,331 images were captured, visualized, and reconstructed with the CT-Analyser software version 1.13 and the CT-Volume software version 2.0 (Bruker). The penetration of silver particles into the enamel or dentinal tubules was examined. Microphotographs were obtained for comparisons.

Measurement of Silver Penetration

Initially, a demineralized region with silver penetration was identified based on the changes in the enamel and dentin mineral

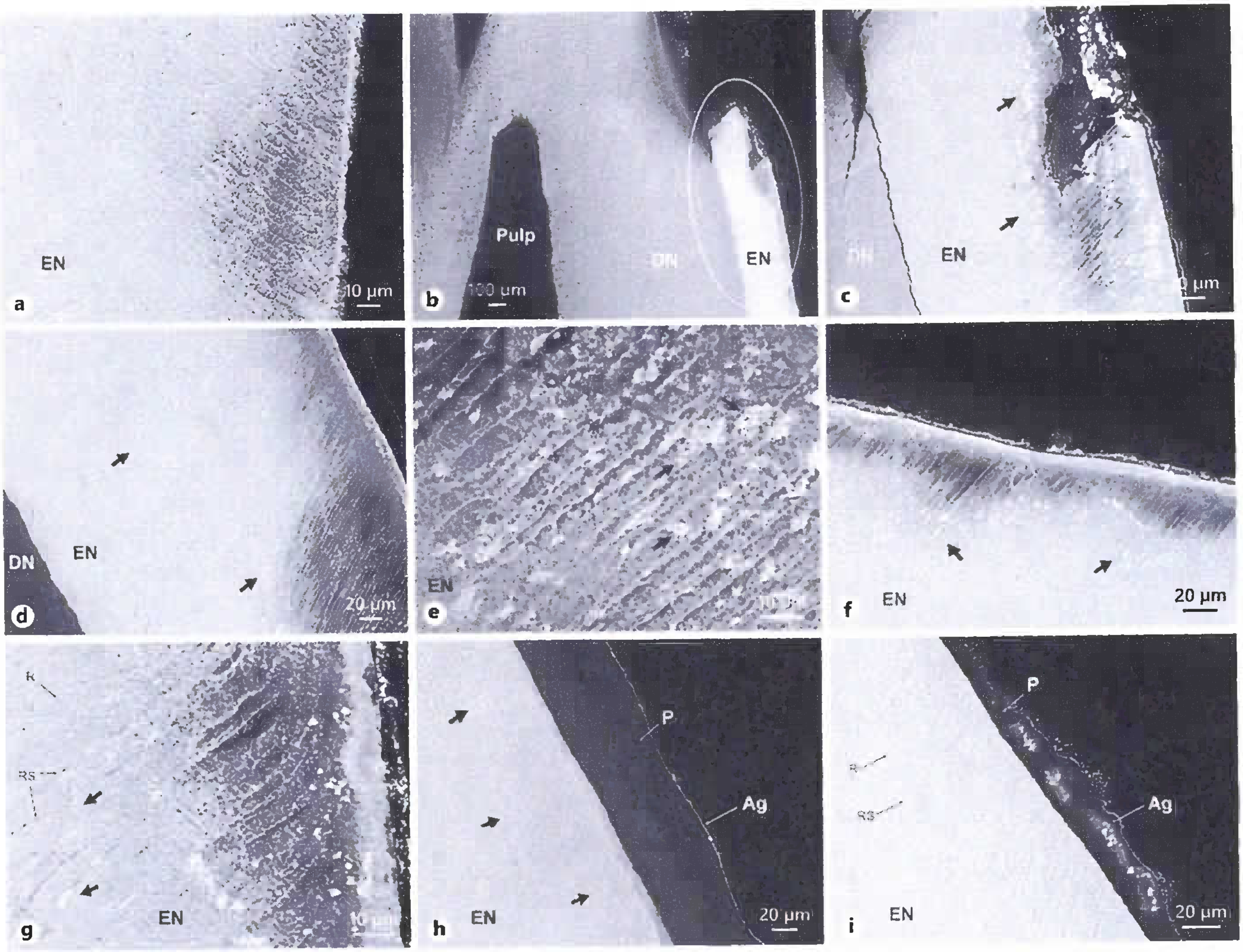


Fig. 1. Silver penetration into the enamel treated with 38% SDF. In the qBSE-SEM micrographs, the black and white images represent the differences in mineralization density. The whitest spots indicate silver particle deposition (arrows) in the enamel. **a** Carious lesion without SDF treatment. Enamel demineralization is evident. **b** Enamel carious lesion (circled) treated with SDF. **c** Magnified view of the silver particles deposited in the demineralized enamel. **d** Distribution of silver particles in the enamel. **e** Magnified view of the precipitation of silver particles in demineralized enamel rods. **f** A dense and bright silver-enriched zone is evident at the deepest carious lesion. **g** Magnified view of the deepest silver-enriched zone. Silver precipitation extends through enamel rods and rod sheaths. **h** Silver penetration through the pellicle into the demineralized enamel. **i** Silver deposited in the pellicle, but the penetration was not evident on sound enamel. Ag, silver particles; DN, dentin; EN, enamel; P, pellicle; qBSE-SEM, quantitative backscattered electron scanning electron microscopy; R, enamel rod; RS, rod sheath; SDF, silver diamine fluoride.

density and silver deposition detected. Within each of those regions, on average, 46 points were selected and labeled from the surface of the carious lesion to the deepest silver particles detected. The depths of silver penetration were measured within the enamel and dentinal tubule microstructures using the ImageJ IJ 1.46r software [Schneider et al., 2012]. All descriptive data, including the mean, range, and standard deviation of silver penetration, were obtained and analyzed using the IBM SPSS software (v24; IBM Corp., Armonk, NY, USA).

Results

The effects of 38% SDF on the enamel of the deciduous teeth are presented in Figure 1. Compared with carious lesions without SDF treatment (Fig. 1a), silver deposition was evident in demineralized enamel, and the intensity was related to the degree of enamel demineralization (Fig. 1b–e). The study revealed that silver precipitation

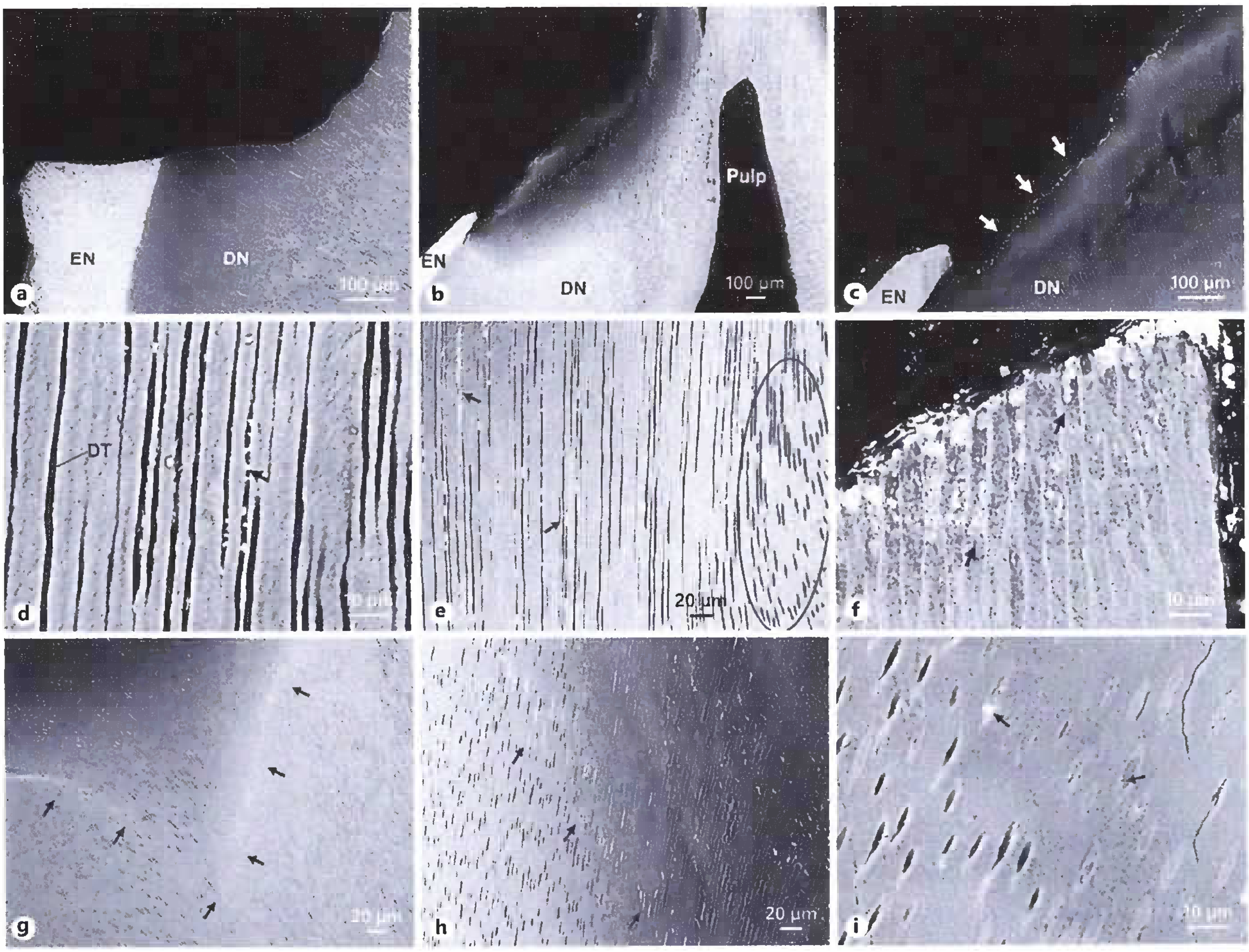


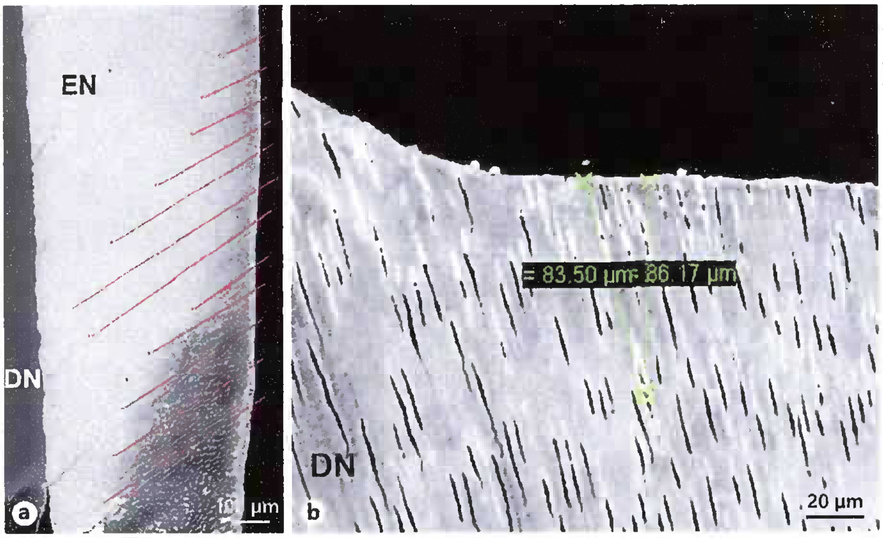
Fig. 2. Silver penetration into the dentin treated with 38% SDF. **a** Carious lesion without SDF treatment. Dentin demineralization was identified. **b** Carious lesion was treated with SDF. **c** Magnified view of the highly concentrated silver particles lined along the surface of a carious lesion. **d** Vertically sectioned view of silver penetrated into the dentinal tubules. **e** Silver precipitation was observed in the vertical dentinal tubules, but did not extend into the dentinal tubules that had changed direction (circled). **f** Magnified view of the silver deposited and penetrated into the dentinal tubules. **g** Dense and bright silver-enriched zones identified in the deepest carious lesions. **h** Magnified view of silver-enriched zones showing silver precipitation in the dentinal tubules toward the deepest demineralized lesions. **i** A portion of the dentinal tubules was empty, and the other portion was apparently sealed by silver particles and other minerals. Arrows indicate silver particles. DN, dentin; DT, dentinal tubule; EN, enamel; SDF, silver diamine fluoride.

extended through the enamel rods and rod sheaths (Fig. 1f, g). Apparently, silver penetrated through the pellicle into the demineralized enamel (Fig. 1h), but penetration into the sound enamel was not seen (Fig. 1i).

The effects of 38% SDF treatment on the dentin are presented in Figure 2. Upon comparing SDF-treated lesions to carious lesions without SDF treatment (Fig. 2a), silver particles were observed not only to line along the surface of the carious lesions (Fig. 2b, c), but also to penetrate into the dentinal tubules (Fig. 2d–f). Zones of dense and bright silver-enriched dentin were formed at the margins and bottoms of the lesions (Fig. 2g). Close investigation of the zones revealed that highly concentrated silver particles were precipitated in the innermost demineralized lesions (Fig. 2h), and the dentinal tubules were partially sealed by silver particles and other minerals (Fig. 2i).

As illustrated in Figure 3, the silver penetrations were measured in the enamel (Fig. 3a) and dentinal tubule

Fig. 3. Illustrations of the computer program-generated measurements of silver penetration into carious lesions. **a** Measurements of silver penetration in enamel. **b** Measurements of silver penetration in dentin. **c** Summary table of the measurement obtained from the thirteen SDF-treated carious lesions (multiple lesions in three teeth). * Number of measurements per lesion. DN, dentin; EN, enamel; SD, standard deviation; SDF, silver diamine fluoride.



Color version available online

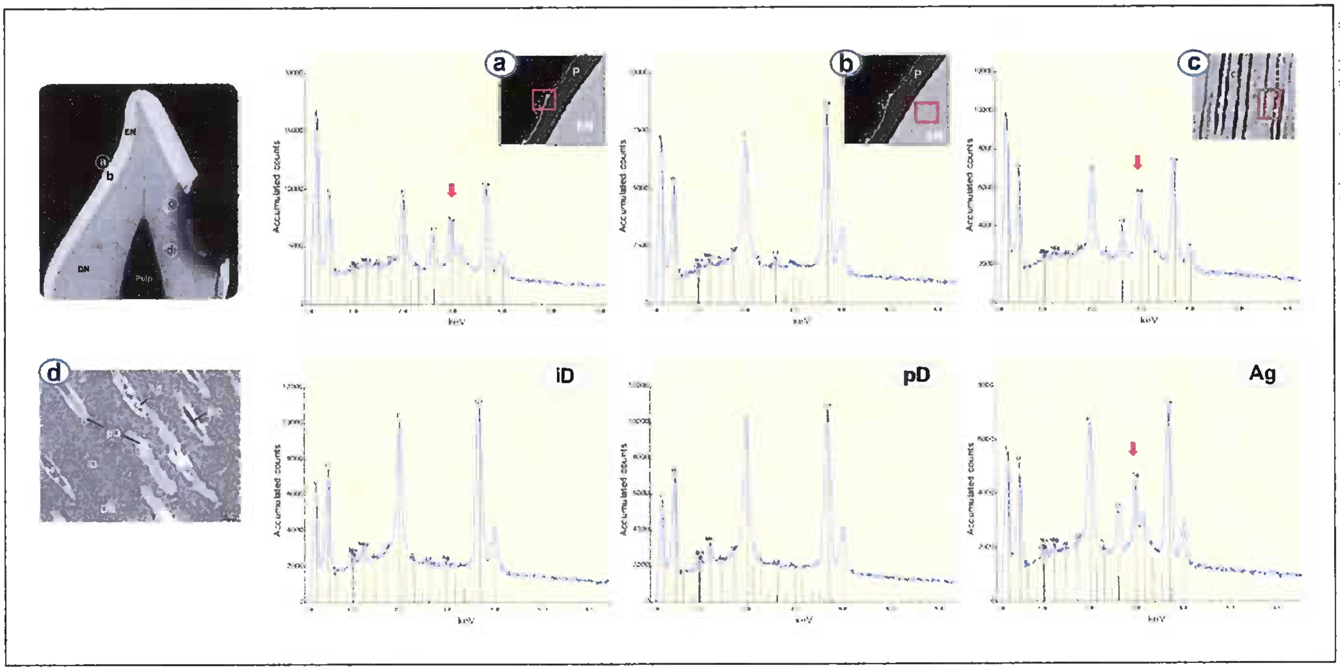
c Measurement of silver penetration on SDF-treated teeth

Carious lesion	*n**	Mean	SD	Range	Minimum	Maximum
1	30	514.51	202.18	393.90	129.75	823.64
2	26	681.38	349.70	1,286.10	70.56	1,356.66
3	11	1,464.49	287.51	1,027.71	770.29	1,798.00
4	27	532.17	164.49	637.29	122.06	759.35
5	49	1,069.93	364.57	1,623.03	16.75	1,639.79
6	72	1,268.84	430.40	2,458.44	31.77	2,490.22
7	40	646.30	307.89	909.56	362.48	1,272.04
8	61	1,005.33	588.95	1,786.38	77.78	1,864.16
9	35	448.91	188.91	778.23	63.06	841.29
10	72	480.76	181.50	816.73	134.32	951.05
11	90	570.29	328.33	1,116.26	73.34	1,189.59
12	48	700.67	304.36	1,142.21	70.08	1,212.29
13	42	455.97	182.66	689.57	66.34	755.91
Sum	603	744.65	448.69	2,473.46	16.75	2,490.22

(Fig. 3b) anatomical microstructures. A total of 603 measurements (mean 46 ± 22.25) were obtained from thirteen carious lesions of the ten teeth treated with SDF using the ImageJ IJ 1.46r software [Schneider et al., 2012]. The average depth of the silver penetration was 744.65 ± 448.69 μm (range 16.75–2,490.22 μm) (Fig. 3c).

EDX analyses confirmed that the particles that lined the pellicle layer were silver (Fig. 4a) which did not penetrate into sound enamel (Fig. 4b). The silver penetrated into the dentinal tubules (Fig. 4c) rather than into the intertubular dentin (Fig. 4d). The EDX spectrum analysis further revealed that carbon, oxygen, phosphorus, silver, calcium, and chlorine were the main elements detected in the carious lesions treated with 38% SDF (Fig. 4). Additionally, sodium, magnesium, aluminum, silicon, zinc, and sulfur were the minor elements detected within the SDF precipitation "zone."

The micro-CT analysis showed no silver deposition on the sound enamel surface (Fig. 5a). As soon as the demineralization occurred, silver penetration was observed (Fig. 5b). The study also showed that silver had the potential to go through the incipient carious lesions (Fig. 5c) and form a zone of dense silver precipitation surrounding the carious lesions (Fig. 5b–f). In cavitated caries, the silver-enriched zones were diffused as the lesion enlarged (Fig. 5d–f). Finally, in the deep lesions, the silver precipitation was observed in the pulp chamber (Fig. 5f).



Color version available online

Fig. 4. Illustration of the qBSE-SEM and EDX spectra of carious lesions treated with SDF. **a** Silver deposition could be seen on the surface of the pellicle overlaying sound enamel treated with SDF. **b** Silver penetration was not evident on the sound enamel. **c** Silver particles were detected in the dentinal tubules. **d** Silver particles were not detected in the intertubular dentin or peritubular dentin, but were detected in the dentinal tubules. Ag, silver particles; DN, dentin; DT, dentinal tubule; EDX, energy-dispersive X-ray spectroscopy; EN, enamel; P, pellicle; qBSE-SEM, quantitative backscattered electron scanning electron microscopy; SDF, silver diamine fluoride; pD, peritubular dentin; iD, intertubular dentin.

Discussion

Fully matured dental enamel is a highly mineralized tissue containing 96% minerals, 4% organic matter, and water by weight [Nanci, 2013b]. The fundamental structural units of enamel are tightly ordered rods and interrods. The rods are arranged roughly perpendicular from the dentinoenamel junction towards the tooth surface [Nanci, 2013b]. The arrangement and curvature of the rods are clinically significant because they modulate the enamel resistance to the mechanical forces during tooth functioning, the mineralization pattern, and the speed of caries progression. In this study, we observed that the structural characteristics of enamel could facilitate the spread of silver penetration from enamel to dentin. The silver particles penetrated from the surface vertically following the direction of the rods into the demineralized enamel and dentin. The degree of silver penetration was associated with the magnitude of enamel demineralization. Importantly, the study revealed that silver precipitation extended through the boundary between rods and interrods, known as the rod sheaths (or periprismatic sheaths), which are composed of multiple protein accumulates [Nanci, 2013b]. A body of evidence suggests that the presence of the rod sheaths and cariogenic bacterial invasion in the microstructures are responsible for the initial loss of enamel minerals at the ultrastructural and nanoscale levels, leading to caries formation [Fejerskov, 2015].

This study showed that the rod sheaths, specifically in partially demineralized enamel with enlarged gaps between the rods and interrods, likely provided the path for silver penetration. Theoretically, silver particles could reach the demineralized ultrastructural sites and therefore cease the demineralization. Clinically, the effectiveness of SDF application on an incipient carious lesion could be influenced by the configuration of rods and interrods, enamel thickness, as well as caries site and severity. The study also found that a limited amount of silver particles could be detected on the sound enamel surface (Fig. 1i, 5a), which could explain why SDF applications on caries-free tooth surfaces do not produce black staining. These findings suggested that the use of SDF as a potential caries-preventive agent warrants further study.

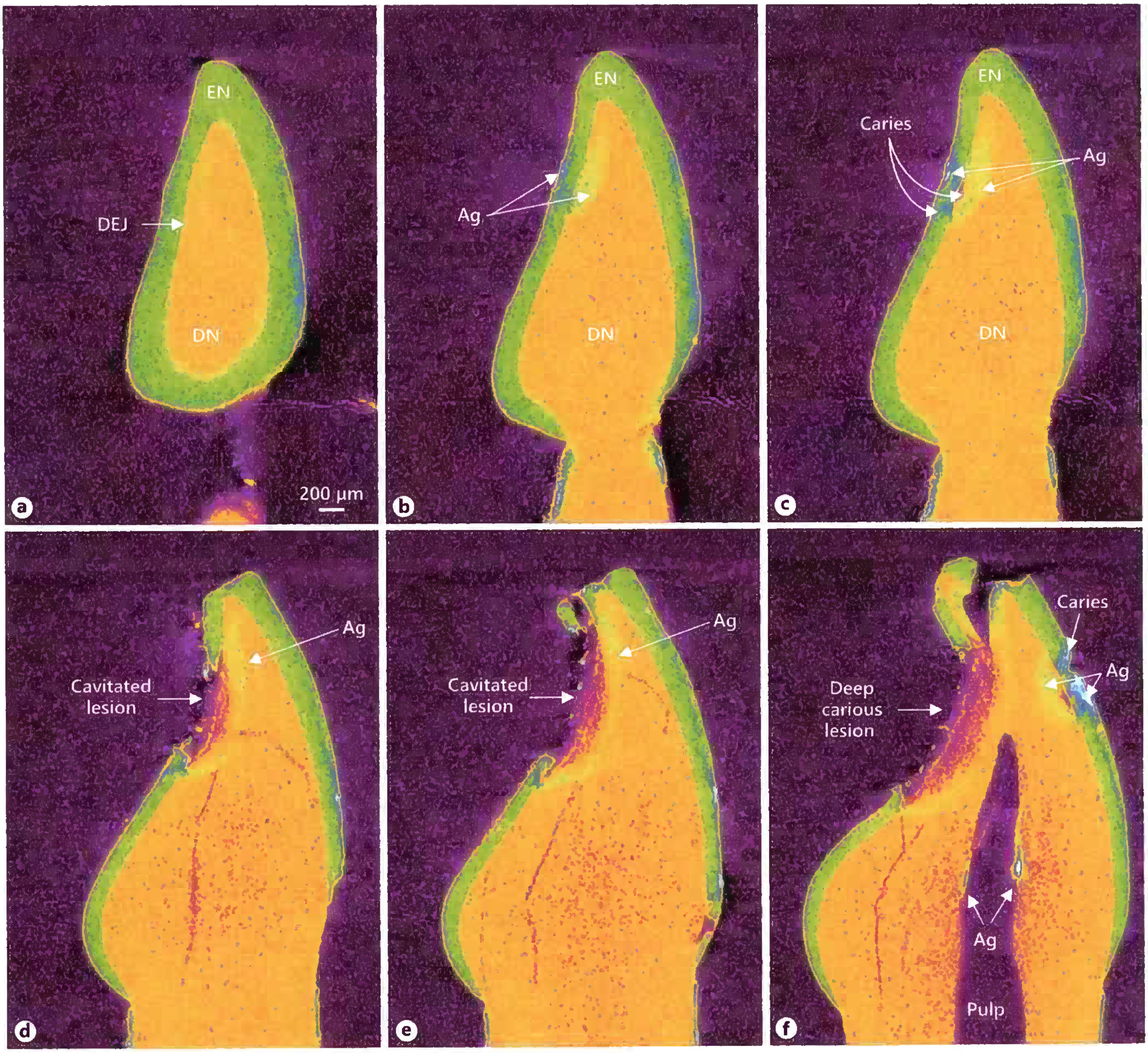


Fig. 5. Micro-computed tomography images of the effect of SDF on carious lesions. The extent of silver penetration was associated with the degrees of enamel and dentin demineralization. **a** No silver precipitation was observed on the sound enamel surface. **b** Silver penetration was observed on the demineralized enamel that was clinically intact and passed through the dentinoenamel junction into the dentin. **c** Silver precipitation occurred on the incipient lesions of the enamel and the dentin. **d, e** Cavitated lesions were surrounded by highly concentrated silver precipitations. **f** In the deep carious lesions, silver precipitations were observed inside the pulp chambers. Ag, silver particles; DEJ, dentinoenamel junction; DN, dentin; EN, enamel; SDF, silver diamine fluoride.

The matured dentin, organized into peritubular and intertubular dentin, is a major component of the tooth structure, which consists of minerals (70%), organic matrix (20%), and water (10%) which functions to support enamel and resist fracture [Nanci, 2013a]. The main characteristic feature of dentin is that the closely packed dentinal tubules extend through the entire thickness of the dentin, and the odontoblast processes run within the dentinal tubules. Accurate measurement of silver penetration in carious lesions is challenging, as the enamel rods or dentinal tubules are arranged differently in different sections of the tooth. To ensure accuracy, four strategies were used. First, we defined the affected areas in which silver particles were detected. Second, we drew lines approximately every 5 μm from the surface of the tooth or the carious lesion to the end point of silver particles deposited within the targeted areas. Third, the ImageJ IJ 1.46r software [Schneider et al., 2012] was used to generate the measurements. Finally, an average of those measurements was produced for each examined lesion.

In our study, the silver particle could be detected as deep as 2,490 μm (with an average depth of 744 μm) in the dentinal structures of the deciduous teeth. This depth is greater than a previously reported depth of 25–200 μm based on microhardness measurements of SDF-treated carious lesions [Chu and Lo, 2008] or a depth of 20–100 μm of silver penetration into SDF-treated dentin [Shimooka, 1972; Yamaga et al., 1972; Willershausen et al., 2015]. The variations can be explained by differences in the tooth specimen and the testing methods used. Here, we used extracted deciduous teeth with caries, while most of the previous studies used sound enamel and dentin blocks or permanent teeth. Since the structures and mineral contents of the enamel and dentin are different between the deciduous and permanent teeth [De Menezes Oliveira et al., 2010], a deeper silver penetration was expected in the deciduous teeth. However, as much as a 10-fold difference in the depth of silver penetration was unanticipated. Hence, the application of a highly concentrated SDF solution on the deciduous teeth should be used with caution.

The study used micro-CT to generate three-dimensional images of SDF-treated tooth specimens and to assess the extent of silver penetration. The results showed silver-enriched "zones" or temporarily formed "shields" surrounding the lesions. It is not clear how the silver particles were concentrated at the end of demineralized sites and what caused the absence of silver precipitation that exceeded the boundary. Here, we propose the following explanations for these observations. (1) The silver-enriched zone formation could be due to alterations in the microenvironment (e.g., the changes in the equilibrium parameter of the mineral molecular structure and an increased abundance of free minerals) in dentinal tubules as a result of carious development. (2) The "sudden" stop in silver precipitation could be due to changes in the direction of the dentinal tubules at the junction between the primary dentin (near the enamel) and secondary dentin (near the pulp chamber and less regular). Some evidence suggests that the dentinal tubules of secondary dentin fill with more calcified materials and the overall permeability of the dentin is reduced, thereby protecting the pulp [Nanci, 2013a]. (3) The dentinal tubules could be filled with cariogenic microorganisms or blocked by denatured dentin collagen matrix and metabolic byproducts resulting from a carious process that prevents silver penetration. (4) A partial remineralization could occur from the silver particles or other minerals that mechanically seal the demineralized dentinal tubules. In addition, we observed that with SDF application on a deep carious lesion, the silver penetration could extend into the pulp chamber. The potential pulpal injury associated with SDF treatment is not fully understood and, therefore, needs to be further investigated.

Dental caries is caused by alterations of mineral substances in the acidic oral environment, resulting in destruction of the tooth structure [Fejerskov and Larsen, 2015]. The primary mineral constituting both enamel and dentin is hydroxyapatite, which is a crystalline calcium phosphate $[Ca_{10}(PO_4)_6(OH)_2]$. A range of hypothetical chemical reactions have been proposed [Yamaga et al., 1972; Rosenblatt et al., 2009] between the hydroxyapatite, silver ions (254,709 ppm), and fluoride ions (44,860 ppm) in the commonly used 38% SDF solution. A study showed that deep carious lesions treated with SDF increased calcium, phosphate, and fluoride ions in caries-affected dentin [Sinha et al., 2011]. Yamaga et al. [1972] attributed the increased hardness of SDF-treated carious dentin to the deposition of silver phosphate. Mei et al. [2018] suggested that silver particles contributed to the increased hardening of the treated tooth surfaces.

In addition to determining the characteristics of SDF penetration, the study also aimed to investigate the inorganic composition of carious lesions treated with 38% SDF. The combined EDX and qBSE-SEM analysis revealed that carbon, oxygen, phosphorus, silver, calcium, and chlorine were the main elements detected in the carious lesions treated with SDF. Additionally, sodium, magnesium, aluminum, silicon, zinc, and sulfur were the minor elements detected within the SDF precipitation zone. The important elements consistently detected in the carious lesions were calcium, phosphorus, and silver. Previous studies have suggested that the main mechanism of SDF-induced caries arrest was the formation of silver phosphate (Ag_3PO_4), calcium fluoride (CaF_2), and fluorapatite $[Ca_{10}(PO_4)_6F_2]$ on treated lesions [Yamaga et al., 1972]. Based on their association with biological apatite formation, chemical stability, and solubility, fluoride has been classified as a key cariostatic element [LeGeros, 1991]; fluorohydroxyapatite formation in SDF-treated caries lesion might induce the lesion remineralization [Mei et al., 2018]. This study could not confirm the fluoride composition in the SDF-treated lesions due to the limitations of the methods used. The mechanistic role of high fluoride concentration (44,860 ppm fluoride ions) in SDF-treated carious lesion needs to be further investigated.

The study by Yamaga et al. [1972] also concluded that the black staining on SDF-treated tooth surfaces was due to the formation of Ag_3PO_4. In a recent review, Mei et al. [2018] suggested that silver compounds are the reason for the black staining of SDF-treated caries lesions. In fact, the presence of oxygen, phosphorus, and sulfur as well as increased chlorine in the SDF-treated carious lesions suggested that these elements could incorporate silver and form not only Ag_3PO_4 but also silver oxide (Ag_2O) and silver sulfide (Ag_2S). Due to the solubility product constants of these compounds at 25 °C (Ag_2O, 1.9×10^{-8}; Ag_3PO_4, 2.8×10^{-18}; Ag_2S, 8.0×10^{-51}) [Harris, 2015; Rumble, 2018], it is possible that in the presence of alkali hydroxide and hydrogen sulfide in the microenvironment, Ag_2S, Ag_3PO_4, and Ag_2O compounds that turn silver ions (Ag^+) to metallic silver nanoparticles (Ag^0) after light exposure contribute to the black staining resulting from SDF application on the surface of carious lesions.

Increasing evidence has suggested that certain oral bacteria and chronic inflammation (e.g., tonsillitis or periodontitis) are associated with high concentrations of hydrogen sulfide in the oral cavity [Salako and Philip, 2011; Takeshita et al., 2012; Ren et al., 2016; Choi et al., 2018]. Furthermore, Ag_2S is a dense black solid that is insoluble in all solvents, and its solubility product constant is much lower than that of Ag_2O, Ag_3PO_4, and other silver compounds [Harris, 2015; Rumble, 2018]. Here, we propose that future investigations should particularly focus on understanding the physiochemical reaction of silver compounds with carious lesion and antimicrobial effects of silver nanoparticles against cariogenic biofilm that will enable scientists to elucidate the cariostatic mechanism of SDF, eliminate the black-stain drawback of SDF, and design better chemotherapeutic agents for caries management.

One limitation of our study is that the surface preparation procedure might influence penetration by a capillary action resulting from the air-dry procedure. In a clinical setting, the recommended protocol for tooth preparation for SDF treatment is to isolate the decayed tooth, clean the teeth with a microbrush applicator, remove food debris or visible plaque with gauze, and dry the tooth with either triple syringe or cotton rolls. In this study, the extracted teeth were placed on a microcentrifuge tube rack. Since no physical interference or debris/caries removal was involved, the 4-h air-dry procedure was selected to accommodate procedural differences in surface preparation before application of the SDF solution. As SDF is a water-soluble solution with a specific gravity of 1.35 (Advantage Arrest), the SDF and the water in enamel and dentin were miscible, and the molecular differences between water and SDF were significant, therefore the osmotic difference between the two was likely to pull the SDF through the hard tissue porosity as fast as it would have traveled by capillary action. Therefore, the 4-h air dry should not alter the SDF penetration. These findings need to be further validated by more clinical studies.

In summary, the penetration and precipitation of silver were evident in the carious lesions of deciduous teeth after treatment with 38% SDF solution. The study revealed that the silver precipitation occurred through the pellicle complex, along with the rod sheaths, into the demineralized enamel rods and the dentinal tubules. Furthermore, the precipitation formed silver-enriched diffusion barriers surrounding the carious lesions at depths up to 2,490 μm and reaching the dental pulp tissue. The air-dry procedure might not be ideal for the surface preparation of the teeth for SDF treatment. Despite this limitation, our findings demonstrated that the extent of silver penetration and the scope of distribution were positively related to the degree of enamel and dentin demineralization. EDX-SEM analysis suggested that Ag_2O, Ag_2S, and/or Ag_3PO_4 are the potential key contributors for the black-stain resulting from SDF application on the surface of carious lesions. These findings provide new evidence for elucidating the mechanistic mode of action by which SDF interacts with the tooth. Furthermore, questions regarding the SDF antimicrobial mechanism associated with caries arrest or failure need to be answered.

Acknowledgments

This project was supported by a New York University Provost's Mega-Grants Initiative Award (Y. Li), China Scholarship Council Grant No. 201506015041 (Y. Liu), an AADR Student Research Fellowships (O.M. Nguyen), and the New York University College of Dentistry Faculty and Student Research Funds. Our thanks go to Dr. Amr Moursi, Dr. Courtney Chinn, and the staff members of the Department of Pediatric Dentistry for assisting the sample collection, as well as to Dr. John L. Ricci, Dr. Min Zhou, Julia Katris, and Gina Yildirim for technical assistance.

Statement of Ethics

The study was conducted at the New York University College of Dentistry. Approval for collecting the extracted teeth due to caries was obtained from the Institutional Review Boards of the New York University School of Medicine.

Disclosure Statement

All authors declare no potential conflicts of interest with respect to the authorship and/or publication of this work.

Author Contributions

Y. Li, W.J. Psoter, and T.G. Bromage conceived and designed the experiment and drafted the manuscript; Y. Liu and B. Hu performed the experiment; O.M. Nguyen and S. Rabieh assisted in the data analysis; M.A. Walters and F.C. Kumararaja critically reviewed the manuscript. All authors participated in the editing and final preparation of the manuscript.

References

Boyde A. Methodology of calcified tissue specimen preparation for scanning electron microscopy. In: Dickson GR, editor. Methods of Calcified Tissue Preparation. Amsterdam: Elsevier; 1984. p. 251–307.

Boyde A, Davy KW, Jones SJ. Standards for mineral quantitation of human bone by analysis of backscattered electron images. Scanning. 1995;17 Suppl. V:6–7.

Choi KY, Lee BS, Kim JH, Kim JJ, Jang Y, Choi JW, et al. Assessment of volatile sulfur compounds in adult and pediatric chronic tonsillitis patients receiving tonsillectomy. Clin Exp Otorhinolaryngol. 2018 Sep;11(3):210–5.

Chu CH, Lo EC. Microhardness of dentine in primary teeth after topical fluoride applications. J Dent. 2008 Jun;36(6):387–91.

Chu CH, Lo EC, Lin HC. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese preschool children. J Dent Res. 2002 Nov;81(11):767–70.

De Menezes Oliveira MA, Torres CP, Gomes-Silva JM, Chinelatti MA, De Menezes FC, Palma-Dibb RG, et al. Microstructure and mineral composition of dental enamel of permanent and deciduous teeth. Microsc Res Tech. 2010 May;73(5):572–7.

Fejerskov O. Pathology of dental caries. In: Fejerskov O, Nyvad B, Kidd EA, editors. Dental caries The disease and its clinical management. Oxford: John Wiley & Sons, Ltd.; 2015. p. 49–82.

Fejerskov O, Larsen MJ. Demineralization and remineralization: the key to understanding clinical manifestations of dental caries. In: Fejerskov O, Nyvad B, Kidd EA, editors. Dental caries The disease and its clinical management. Oxford: John Wiley & Sons, Ltd.; 2015. p. 155–70.

Gao SS, Zhang S, Mei ML, Lo EC, Chu CH. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment – a systematic review. BMC Oral Health. 2016 Feb;16(1):12–21.

Harris DC. Quantitative chemical analysis. 9th ed. New York (NY): W.H. Freeman and Co.; 2015.

Horst JA, Ellenikiotis H, Milgrom PL. UCSF protocol for caries arrest using silver diamine fluoride: rationale, indications and consent. J Calif Dent Assoc. 2016 Jan;44(1):16–28.

LeGeros RZ. Calcium phosphates in oral biology and medicine. Basel: Karger; 1991.

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. J Dent Res. 2005 Aug;84(8):721–4.

Lo EC, Chu CH, Lin HC. A community-based caries control program for pre-school children using topical fluorides: 18-month results. J Dent Res. 2001 Dec;80(12):2071–4.

Mei ML, Ito L, Zhang CF, Lo EC, Chu CH. Effect of laser irradiation on the fluoride uptake of silver diamine fluoride treated dentine. Lasers Med Sci. 2015 Apr;30(3):985–91.

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

Mei ML, Nudelman F, Marzec B, Walker JM, Lo EC, Walls AW, et al. Formation of Fluorohydroxyapatite with Silver Diamine Fluoride. J Dent Res. 2017 Sep;96(10):1122–8.

Nanci A. Dentin-pulp complex. In: Ten Cate's oral histology: development, structure, and function. St. Louis: Mosby; 2013a. p. 165–204.

Nanci A. Enamel. In: Ten Cate's oral histology: development, structure, and function. St. Louis: Mosby; 2013b. p. 122–64.

Peng JJ, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: a review. J Dent. 2012 Jul;40(7):531–41.

Ren W, Xun Z, Wang Z, Zhang Q, Liu X, Zheng H, et al. Tongue coating and the salivary microbial communities vary in children with halitosis. Sci Rep. 2016 Apr;6(1):24481.

Rosenblatt A, Stamford TC, Niederman R. Silver diamine fluoride: a caries "silver-fluoride bullet." J Dent Res. 2009 Feb;88(2):116–25.

Rossi G, Squassi A, Mandalunis P, Kaplan A. Effect of silver diamine fluoride (SDF) on the dentin-pulp complex: ex vivo histological analysis on human primary teeth and rat molars. Acta Odontol Latinoam. 2017 Apr;30(1):5–12.

Rumble JR. Solubility product constants of inorganic salts. In: CRC handbook of chemistry and physics, ed 99 (Internet Version 2018). Boca Raton, FL: CRC Press/Taylor & Francis; 2018. Section 5 p. 1–8.

Salako NO, Philip L. Comparison of the use of the Halimeter and the Oral Chroma™ in the assessment of the ability of common cultivable oral anaerobic bacteria to produce malodorous volatile sulfur compounds from cysteine and methionine. Med Princ Pract. 2011;20(1):75–9.

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012 Jul;9(7):671–5.

Shimooka S. On the penetration of silver nitrate and ammoniacal silver fluoride into microstructure of the sound dentin. Shigaku. 1972 Feb;59(6):534–66.

Sinha N, Gupta A, Logani A, Shah N. Remineralizing efficacy of silver diamine fluoride and glass ionomer type VII for their proposed use as indirect pulp capping materials – Part II (A clinical study). J Conserv Dent. 2011 Jul;14(3):233–6.

Suzuki T, Nishida M, Sobue S, Moriwaki Y. Effects of diammine silver fluoride on tooth enamel. J Osaka Univ Dent Sch. 1974 Sep;14:61–72.

Takeshita T, Suzuki N, Nakano Y, Yasui M, Yoneda M, Shimazaki Y, et al. Discrimination of the oral microbiota associated with high hydrogen sulfide and methyl mercaptan production. Sci Rep. 2012;2(1):215–22.

Willershausen I, Schulte D, Azaripour A, Weyer V, Briseño B, Willershausen B. Penetration potential of a silver diamine fluoride solution on dentin surfaces. An ex vivo Study. Clin Lab. 2015;61(11):1695–701.

Yamaga R, Nishino M, Yoshida S, Yokomizo I. Diammine silver fluoride and its clinical application. J Osaka Univ Dent Sch. 1972 Sep;12:1–20.

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 Jul;88(7):644–7.

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 Nov;40(11):962–7.