

ORIGINAL RESEARCH

Effects of LED curing light on silver diamine fluoride penetration into dentin

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Abstract

This *ex-vivo* study investigated the effect of a light-emitting diode (LED) curing light on the depth of penetration of Silver Diamine Fluoride (SDF) into carious lesions. Twenty-four primary teeth with untreated caries lesions were allocated into groups and treated within 5 min after extraction: (1) n = 6 treated for 1 min with one drop of SDF followed by 10 sec rinse with tap water, (2) n = 6 treated for 10 sec with one drop of SDF and exposed to LED light for 20 sec (30 sec total SDF exposure) followed by 10 sec rinse with tap water, (3) n = 6 treated for 10 sec with one drop of SDF followed by a 10 sec rinse with tap water, (4) n = 3 untreated, and (5) n = 3 untreated but exposed to LED light for 20 sec. Samples were prepared, embedded, sectioned and silver penetration was measured using backscattered electron imaging in the scanning electron microscope and energy-dispersive X-ray spectroscopy analysis. Results were expressed as the average relative depth of penetration (%) = Ag depth/lesion depth × 100 from 5 sites in each lesion. Group means were compared using mixed model analysis. Mean ± standard deviation (SD) penetration was: 86.4 ± 20.7% in Group 1, 94.3 ± 13.7% in Group 2, and 26.7 ± 13.9% in Group 3. Groups 1 and 2 were statistically similar and different from Group 3 ($p < 0.001$). Groups 4 and 5 had no silver present. Use of LED light for 20 sec after 10 sec SDF application appears to facilitate silver penetration, similar to a 1 min SDF application. Clinical studies are needed to define the role of silver penetration in sustained caries arrest.

Keywords

Silver diamine fluoride; Deciduous teeth; Caries arrest; LED curing light; Caries management

1. Introduction

Silver Diamine Fluoride (SDF), is a liquid solution which was approved by the Food and Drug Administration (FDA) in 2014 [1] as a device for the treatment of dentin sensitivity in adults over 21 years of age, but is now widely used for the arrest of dentinal caries lesions. Each of the components of the formulation have a specific function, namely, silver ions act as an antibacterial agent, fluoride promotes remineralization and ammonia provides stability to the solution [2]. The clinical application of SDF is simple and painless since it does not require the removal of carious tissue or local anesthetic injections [3]. In the United States, SDF is marketed as a 38% solution (Elevate Oral Care Company, West Palm Beach, FL, USA).

Several clinical trials have reported caries arrest rates of approximately 80% (ranging from 40 to 90%), for dentinal caries lesions in primary teeth, showing that SDF is a clinically effective therapy [4]. An American Academy of Pediatric Dentistry (AAPD) guideline, published in 2017, recommends its use as an alternative tool to traditional caries management in children, adolescents and people with special health-care needs

[1] and the World Health Organization (WHO) has recently included it in their list of essential medicines [5].

After the application of SDF, the arrested caries lesion turns black as a result of silver precipitation on the demineralized/carious dental tissues (enamel and dentin). Studies have found a highly remineralized zone rich in calcium and phosphate in the underlying layers which protect the dentin collagen in the arrested cavitated dentinal lesion [6]. Staining of demineralized tissues turns out to be permanent and is a sign of caries arrest.

Randomized controlled trials (RCTs) have shown that SDF caries arrest rates are higher on anterior teeth (90–94%) and smooth surfaces of the teeth than on posterior teeth (50–55%) and occlusal surfaces [7, 8]. This may be due to a variety of reasons ranging from size of the lesions, to ease of cleaning, to individual microbial profiles [9] and even exposure to natural light [3].

The manufacturer's instructions for 38% SDF do not establish the optimal exposure time to be absorbed into the dentinal tissues to achieve caries arrest (Elevate Oral Care product info). Studies that pool data from RCTs are inconclusive about

ideal exposure time, as application times in individual studies range from 10 sec to 3 min and comparisons on arrest rates are difficult between studies due to differences in application protocols and calibration of the arrest assessment [3]. It has been hypothesized that at least 1 min is required to achieve caries arrest [10]. A recent trial in children aged 1 to 3 years with high caries experience utilized an application time of approximately 10 sec due to the limited patient cooperation. Reported arrest rates were 36% after one year follow-up [11], which is surprisingly lower than other trials.

Light-emitting diode (LED) curing lights have become the standard for curing restorative materials in dentistry. These LED lights produce a narrow spectrum of predominantly blue light in the 400 to 500 nm range (with a peak about 460 nm), which is able to cure most light-cured materials efficiently and with minimal heat. Such LED lights are present in most dental offices.

It has been reported that exposure to a curing light accelerates the darkening of demineralized and carious tooth surfaces treated with SDF [3]. If the light aids silver penetration into affected tissues and improves outcomes of caries arrest, it would be a valuable technique to use in very young children and special-needs patients, when 1 min exposure time is not possible due to limited cooperation.

A recent study [12] explored the effects of a dental curing light on the penetration depth of SDF, dentin hardness and silver and fluoride ion precipitation into cavitated carious lesions of extracted anterior teeth using scanning electron microscopy. The authors reported that applying a dental curing light induces more silver precipitation in infected dentin, increases its hardness and reduces SDF penetration into sound dentin. Investigators used a 1 min SDF application, with and without light, followed by a 3 min dry time and repeated two weeks later. Although their results showed increased penetration when using a curing light after SDF application, their methods did not address the issue of the light aiding in the penetration of SDF applied for shorter time periods, especially on posterior teeth, where arrest rates are typically lower [7]. Another recent *in-vitro* study in primary molars [13] reported that SDF followed by LED light applied twice after a 2 weeks interval significantly improved the microhardness of dentin when compared to SDF alone. However, no application times or amount of SDF used were reported in their methodology.

The aim of this *ex-vivo* study was to determine the effects on the depth of penetration of SDF, when using a curing light after a 10 sec SDF application, compared to a 1 min SDF application without light exposure. Our null hypothesis is that exposure of LED curing light after SDF application will have no effect on the depth of penetration of SDF.

2. Materials and methods

This *ex-vivo* study did not require Institutional Review Board (IRB) oversight, consistent with NYU School of Medicine IRB policy and federal regulations governing human subject research. Primary teeth were collected from pediatric patients seen at New York University College of Dentistry Pediatric Dentistry Clinic whose planned treatment included extraction of a carious tooth. None of the specimens had any personal

identifiers. Teeth extracted during such planned treatment and with no identifiers do not require IRB approval for research purposes.

2.1 Tooth sample collection and preparation

Primary molars that were included in the study (24) had untreated caries lesions and were planned for extraction as they were judged to be clinically unrestorable. Immediately after extraction, visible debris and blood were cleaned with tap water and excess water was dried with a gauze pad. 1 drop of SDF 38% was placed on a dappen dish and applied to the open dentinal cavity (infected dentin surface) with a microbrush. All samples were treated within the first 5 min of extraction so as to minimize tissue drying and simulate a realistic clinical scenario.

Twenty-four extracted teeth were sequentially allocated into 5 groups:

1. Treated with 1 drop of SDF applied for 1 min followed by 10 sec rinse with tap water (6 teeth).
2. Treated with 1 drop of SDF applied for 10 sec followed by exposure to LED curing light for 20 sec, followed by 10 sec rinse with tap water (6 teeth). The total SDF exposure time was 30 sec.
3. Treated with 1 drop of SDF applied for 10 sec followed by 10 sec rinse with tap water (6 teeth).
4. Untreated control (3 teeth).
5. Untreated, but exposed to LED curing light for 20 sec (3 teeth).

SDF used on all treated teeth was 38% Advantage Arrest SDF (Elevate Oral Care, Palm Beach, FL, USA). The LED light used on samples in groups 2 and 5 was a Woodpecker Cordless LED-C (Guilin Woodpecker Medical Instrument Co., Guilin, China), with light intensity of 800–1000 mW/cm², and blue light of 421–480 nm wavelength, set on full mode at the working distance of approximately 1 cm that is recommended for clinical use. The 10 sec tap water rinse was done to simulate the salivary clearance that is seen as saliva production is stimulated by the metallic taste of the SDF as patients close their mouth after the application.

After the treatment, all samples were stored in a solution of 70% ethanol and kept at room temperature until all teeth were collected for evaluation. Once all samples were collected, they were inspected for visible signs of arrest, photographed and prepared for backscattered electron scanning.

2.2 Specimen preparation for electron microscopy

Samples were subject to dehydration using a graded series of ethanols, cleared with methyl salicylate, embedded in poly methyl methacrylate (PMMA) and polymerized by ultraviolet light. After complete penetration and polymerization of MMA, PMMA fills in all the spaces previously occupied by tissue fluids. Polymerized blocks were sawn through each tooth longitudinally with a Buehler Isomet 1000 (Buehler, Lake Bluff, IL, USA) diamond circular saw in a labio- or buccal-lingual orientation that provided the maximum extent of the enamel/dentin lesion. Each sawn surface was first ground with 600, 800 and 1200 grit carborundum papers, and then polished

with diamond compound to a 1 μm surface relief. Deionized (DI) water and ultrasonic cleaning, were used to clear the smear layer produced during grinding and polishing of the samples, and later analysis confirmed no surface contamination.

2.3 Backscattered electron imaging in the scanning electron microscope (BSE-SEM) and energy-dispersive X-ray spectroscopy (EDS) analysis

Dried and finely polished tooth sample surfaces were carbon coated with a Denton Desk V (Denton Vacuum, Moorestown, NJ, USA) to render them electrically conductive for SEM imaging and EDS analysis. All EDS data were collected using a Bruker Quantax 200 XFlash 6160 EDS detector (Bruker, Billerica, MA, USA) coupled with a Zeiss Gemini-300 FE-SEM (Carl Zeiss Microscopy, White Plains, NY, USA). All conditions for EDS were the same as for BSE-SEM. Images at field widths of 1 mm were obtained at high vacuum (HV) with a chamber pressure of 3.5×10^{-7} torr, an accelerating voltage of 8.0 kV, a 60 μm aperture with high current mode activated (rendering ~ 400 pA current), and a working distance of 8.5 mm to characterize the scope of silver precipitation and penetration (location, depth and range) in the dentin, and to examine enamel and dentinal tubule microstructural features in the tooth regions with caries. Block samples were numbered and assigned to a list kept by one of the investigators (GC).

2.4 Measurement of silver penetration

The total lesion depth was determined on all samples based on visible changes in dentin mineral density. This was done by 3 investigators until agreement was reached on the boundary of the lesions. Silver penetration depth was identified based on the changes in the enamel and dentin mineral density and silver deposition detected by BSE-SEM and EDS on 5 locations on each dentin lesion. For each sample, 5 locations that were bordered by sound dentin were identified and lesion depth and silver penetration depth were measured at each, along a line following the contour of the lesion. Element tracing was identified to confirm the presence of silver (Ag) in the dentin using EDS with ESPRIT version 2.2 software (Bruker Nano GmbH, Berlin, Germany). The depths of silver penetration were measured within the dentinal tubule microstructures using Adobe Photoshop V22.42 software (Adobe Inc., San Jose, CA, USA).

2.5 Statistical analysis

Lesion and silver penetration depths in the dentin were available from each of the 5 sites within the carious lesions of the 18 teeth treated with SDF. The proportion of the lesion depth occupied by silver was calculated as (Ag depth/lesion depth) and multiplied by 100 to be expressed as a percentage. If the distributions of these measures were skewed, they were described in terms of the median and inter-quartile range (IQR). When distributions were symmetrical, they were described in terms of the mean and standard deviation (SD) and compared between groups with a linear mixed model and *post-hoc* Sidak tests. Statistical significance is indicated by $p < 0.05$.

3. Results

The three groups that were treated with SDF 38% showed silver penetration in all samples. No silver was present in either of the control, untreated groups. In the treated groups, silver particles were observed along the surface of the caries lesions and in the body of the lesion penetrating the dentinal tubules (Fig. 1).

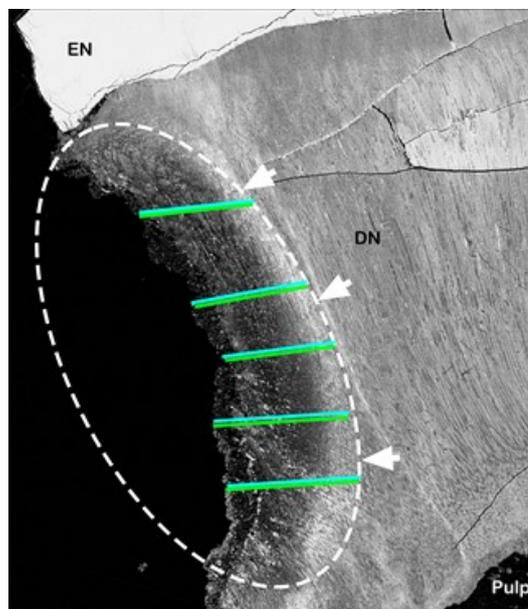


FIGURE 1. Image of BSE-SEM micrographs with visible evidence of differences in mineral density. A caries lesion after exposure to SDF (group 1) is enclosed by the dashed line, surrounded by sound dentin and away from the pulp tissue. A dense white hypermineralization area is noted at the deepest portion of the lesion (white arrows). Green lines follow the orientation of the lesion, and correspond to the depth of silver penetration, overlapping on blue lines where depth of the lesion was measured. EN is enamel and DN is dentin.

EDS analysis revealed that silver particles precipitated in the innermost demineralized lesion surface in groups 1 and 2, but not in group 3. Representative samples of each group are shown on Figs. 2,3,4.

Lesion depth varied widely among the samples, from 67 to 3396 μm , as would be expected from natural caries lesions, but the distributions were similar between groups ($p = 0.51$). The distributions of lesion depth and silver penetration were skewed due to outliers, particularly in Group 1. Median lesion depths averaged between approximately 500 and 700 μm , depending on group, and silver depths varied between approximately 150 and 600 μm (Table 1).

While there was considerable variability in lesion depths, silver penetration depths were consistently proportional. In this way, the distributions of relative depth of penetration were symmetrical and are treated parametrically. Mean (SD) relative penetration was quite high in groups 1 and 2, 86.4% (20.7) and 94.3% (13.7), respectively and considerably lower, 26.7% (13.9) in group 3 (Fig. 5). Analysis showed that groups 1 and 2 means were similar to one another and greater than

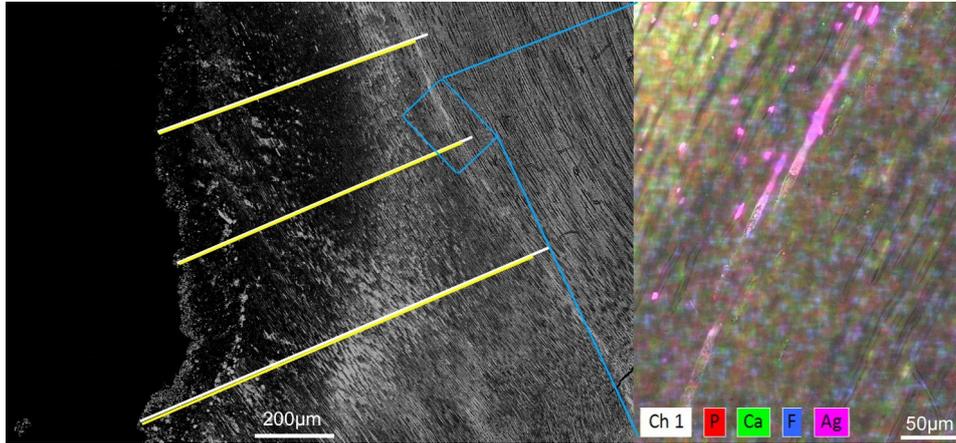


FIGURE 2. Left side is a magnified view of a representative sample of a Group 1 lesion showing 3 sites out of the 5 measurements taken. Yellow lines marking silver penetration, overlapping on white lines marking depth of the lesion. Blue rectangle denotes the area where EDS analysis was done. EDS analysis (right side) was used to confirm the minerals present in the dentin at the deepest area of silver penetration. Pink color denotes presence of silver (Ag), and yellow areas are an overlap of green and red, indicating areas with where Ca and P are prevalent.

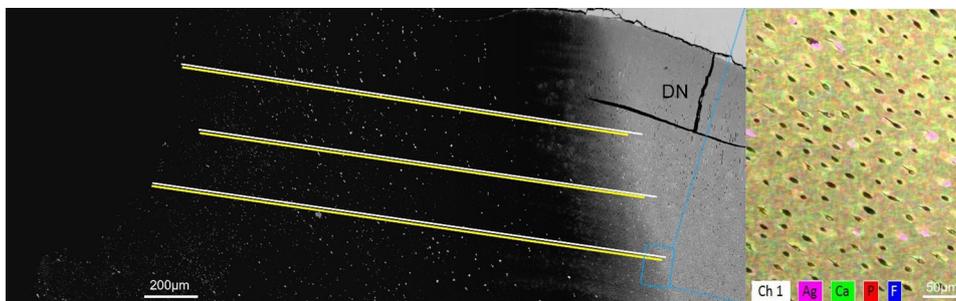


FIGURE 3. Left side is a magnified view of a representative sample of Group 2 lesion showing 3 sites out of the 5 measurements taken. Yellow lines marking silver penetration, overlapping on white lines marking depth of the lesion. Blue rectangle denotes the area where EDS analysis was done. EDS analysis (right side) was used to confirm the minerals present in the dentin at the deepest area of silver penetration. Pink color denotes presence of silver (Ag), and yellow areas are an overlap of green and red, indicating areas with where Ca and P are prevalent, DN is sound dentin.

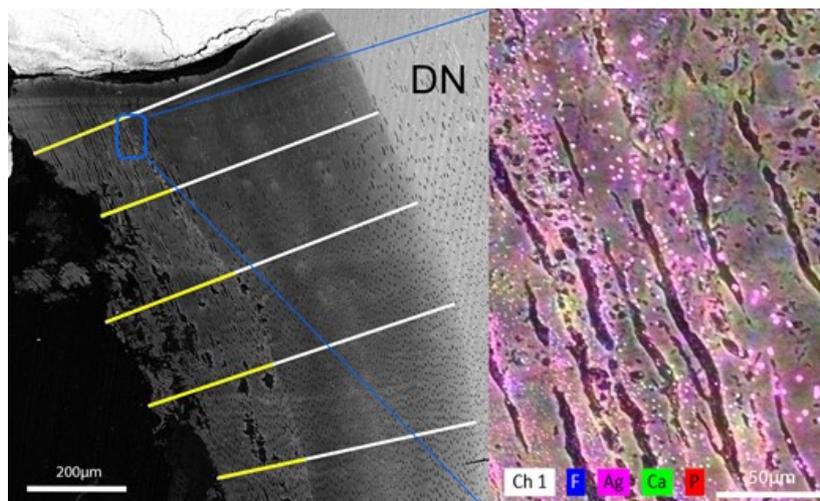


FIGURE 4. Left side is a magnified view of a representative sample of Group 3 lesion showing 3 sites out of the 5 measurements taken. Yellow lines marking silver penetration, overlapping on white lines marking depth of the lesion. Blue rectangle denotes the area where EDS analysis was done. EDS analysis (right side) was used to confirm the minerals present in the dentin at the deepest area of silver penetration. Pink color denotes presence of silver (Ag). DN is sound dentin.

TABLE 1. Median (IQR) of lesion depths and silver penetration depths in Groups 1, 2 and 3.

Group	Lesion depth (μm)	Silver depth (μm)
1 SDF 1 min	666.2 (314.5)	589.7 (545.8)
2 SDF 10 sec + LED 20 sec (total SDF 30 sec)	722.0 (1150.4)	624.3 (905.5)
3 SDF 10 sec	490.8 (306.7)	141.5 (138.7)

SDF: Silver Diamine Fluoride; LED: Light-emitting diode.

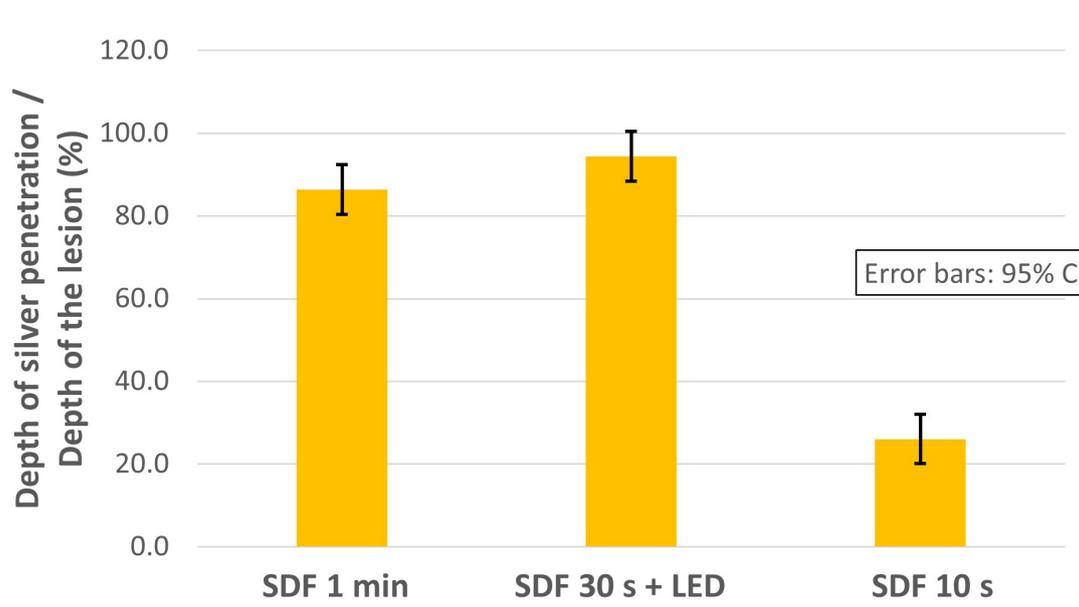


FIGURE 5. The mean relative depth of silver penetration (expressed as a percentage of silver penetration to the total depth of each lesion) was similar in the SDF 1 min and SDF 30 sec + light groups, and both showed greater depths of penetration than the SDF 10 sec group ($p < 0.001$). SDF: Silver Diamine Fluoride; LED: Light-emitting diode; CI: Confidence intervals.

group 3 (both $p < 0.001$). These results suggest that the 20 sec LED curing light exposure increased the efficiency of silver penetration into the dentinal tubules to the level seen following a 1 min exposure without light. No silver was present in the tubules of either of the control groups, which were not exposed to SDF.

4. Discussion

This study suggests that using a LED curing light for 20 sec after application of SDF for 10 sec (total SDF exposure time 30 sec) will increase silver penetration depth in the body of the lesion to match that of SDF application for 1 min. The mean % relative penetration values in these two groups were statistically similar but were more than three times higher than those for the 10 sec SDF alone group. This difference was statistically significant.

LED curing lights are an essential piece of equipment in every modern dental office, as they are used for curing most resin-based and many glass ionomer materials [14]. Utilizing this device as a step of the SDF application could increase the efficacy of the procedure, while keeping it cost-effective, time-saving and non-invasive. Other *in vitro* studies have reported that the use of lasers after SDF application, can increase the

fluoride uptake in the dentin [15] and increase its hardness [13]. However, the cost of the laser would add to the cost of the treatment, as lasers are not a staple of every dental practice.

The mechanism of action of SDF is still not fully understood. However, silver penetration could be a marker for how deep into the lesion or dentinal tissues the antibacterial and remineralizing properties of SDF would take effect [15]. Our study agrees with findings of Toopchi *et al.* [12], who conclude that applying a curing light during SDF treatment of caries lesions, induces more silver precipitation in infected dentin. The action of light at specific wavelengths may act to speed the reaction [12].

All samples from groups 1, 2 and 3 showed a highly mineralized surface area and silver penetration into dentinal tissues to different extents (Figs. 1,2,3,4). EDS analysis revealed that silver particles were precipitated in the innermost demineralized portion of the lesion in groups 1 and 2 but in group 3 it penetrated to less than one-third of the lesion depth.

In only one of the 24 samples, a complete blockage of the dentinal tubules in a small area close to the surface was observed, which could be because the sample at that depth was sliced precisely parallel to the direction of the dentinal tubules, as described by Seto *et al.* [17], who describe the “microwire” pattern from the analysis of one representative tooth (out of

5 primary and 2 permanent tooth samples studied). In the rest of our samples, complete tubular blockage was not seen, perhaps because of the single exposure to SDF, and the pattern of penetration was irregular in all 18 samples treated with SDF, with silver presence in some areas within the dentinal tissue. This could be due to the high amount of mineral loss in those areas, as noted by Sulyanto *et al.* [18], who showed 6% and 21% tubules occluded in carious teeth that had been treated with SDF *in vivo* and extracted after two minutes or three weeks, respectively.

Our study suggests that the penetration of silver into the dentinal lesions, is dependent on time of exposure of the SDF. It also seems that exposure to LED light enhances silver penetration. Although the mechanism of the blue light enhancement of silver uptake into dentinal caries lesions observed in this study is unknown, it may be useful to note that the diffusion of acids into enamel or dentin and the diffusion of calcium and phosphate out of these tissues is the basis for the formation of caries lesions [19]. The diffusion coefficient (D in cm^2/s) is a measure of how fast ions or molecules can move through a fluid, in this case water. Ions in water have D values of 10^{-5} to 10^{-6} cm^2/s . The reported D value for silver ions in water is 1.7×10^{-5} cm^2/s [20]. Thus, ions can diffuse through the water in dentin almost as fast as in water alone. In the present study the silver ions diffused to a median depth of 590 μm in 60 sec treatment (group 1). This corresponds to an apparent diffusion coefficient of about 1.4×10^{-5} cm^2/s indicating that the silver ions diffused into the carious lesions at about the same rate as they would diffuse in water. The driving force for diffusion is the very high concentration of silver ions at the surface. As soon as the surface is cleared, the driving force stops. In the case of group 3 where the diffusion time was 10 sec, the above assumptions lead to a predicted penetration of about 30%. The observed value was 26%. In the case of group 2 with a 10 sec SDF treatment and a 20 sec LED treatment (a total SDF exposure of 30 sec) simple diffusion predicts about 50% penetration if there is no effect of the light. Penetration for group 2 was 94% indicating that the blue light treatment approximately doubled the effective rate of diffusion of the silver ions into the lesion demonstrating a clear effect of the LED light over and above exposure time to SDF.

The mechanism of action of this observed enhancement by the curing LED light is unknown. Interaction between blue light and silver ions/silver nanoparticles has been shown to enhance antibacterial activity of silver [21] possibly due to the high absorption of blue light. The absorption of blue light by the silver ions and suspended nanoparticles in the present study may have increased their kinetic energy thereby enhancing the diffusion into the lesion. It is also possible that there is a photothermal effect due to the heat generated by the LED light exposure either in the water phase or due to absorption by the silver ions or nanoparticles. Further studies that are specifically designed to address the mechanism will be needed to determine the reasons for the observed phenomenon.

Even though our study protocol attempted to treat the teeth immediately after extraction and to mimic a live scenario, it has the inherent limitations of *ex-vivo* studies. A one minute application was used as the standard recommended application time, and a ten second application was used as reported in an

RCT that resulted in low arrest rates. More application times including a 30 second application time should be studied. The results are very clear and show that the treatment with blue LED curing light was effective in enhancing silver penetration into the lesions. However, clinical studies are needed to verify the relationship between silver penetration in the tissue and sustained arrest of demineralization and caries progress.

5. Conclusions

1. LED curing light (blue light) for 20 sec after 10 sec of SDF application (a total SDF application of 30 sec) seems to facilitate silver penetration, making it comparable to the current standard protocol of a 1 min SDF application with no light.
2. This easy modification to the protocol after application could be an important aid in SDF use, as it would be helpful in the clinical management of young children and patients with special health care needs who cannot tolerate longer exposure times. It could also improve the efficacy in occlusal surfaces on posterior teeth, where arrest rates are typically lower.
3. Clinical studies are needed to determine the correlation between silver penetration and sustained arrest of dentinal caries lesions as well as laboratory studies to determine the mechanism by which LED light promotes penetration of silver into dentinal tubules and tissue.

ABBREVIATIONS

SDF, silver diamine fluoride; LED, light-emitting diode; BSE, backscattered electron; SEM, scanning electron microscopy; EDS, energy-dispersive X-ray spectroscopy; PMMA, poly methyl methacrylate; IQRs, interquartile ranges; FDA, food and drug administration.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

AUTHOR CONTRIBUTIONS

YOC and SR—designed the research study. SR, GC and BH—performed the experimental analysis. MNJ—performed the statistical analysis. MNJ and TGB—provided help and advice on various steps of this study. YOC, SR, MNJ, GC and BH—analyzed the data. YOC, SR and GC—wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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