Enhanced effectiveness of silver diamine fluoride application with light curing on natural dentin carious lesions: an in-vitro study

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Research Article

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Abstract

Background: Silver diamine fluoride (SDF) is widely accepted as a dentinal caries arresting agent because of its effectiveness, easy handling, and short working time. When dealing with uncooperative patients, a reduced working time is desired. As SDF has been known to interact with light, the effects of light curing (LC) on SDF have not been investigated. This study aimed to compare mean mineral density difference and surface morphology between 10-and 60-second(s) SDF-applied dentin carious lesions and similarly SDF-treated teeth with additional 20s LC.

Methods: Forty primary molar blocks with natural dentin carious lesions were measured for baseline lesion depth and mineral density at baseline using Image-Pro Plus software. Samples were distributed into 4 groups; 38% SDF applied for 1) 10s (10SDF), 2) 60s (60SDF), 3) 10s+LC (10SDF+LC), and 4) 60s+LC (60SDF+LC), then underwent a 7-day bacterial pH-cycling. Dentin carious lesions’ mean mineral density difference was re-evaluated by digital subtraction radiographic analysis. Surface morphology and elemental profile were assessed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. The mean mineral density difference of the dentin lesions was analyzed using two-way ANOVA, generalized linear models analysis.

Results: Light curing was the only factor affecting the mean mineral density difference ($p=0.007$). Mean mineral density differences in 10SDF+LC and 60SDF+LC groups were significantly higher than those without light curing ($p=0.041$ and 0.041, respectively). The 60SDF+LC group showed a significantly higher mean mineral density difference than the 10SDF group ($p=0.010$), while the 10SDF+LC group was not different from the 60SDF group ($p=1.00$). Scanning electron microscopy showed denser mineral content layers, which likely were silver and chloride, in 10SDF+LC and 60SDF+LC groups than 10SDF and 60SDF groups, respectively.

Conclusion: Shortened application time with light curing enhanced SDF remineralization similarly to a conventional method. This could be valuable for managing dentin caries in young or uncooperative children and special-needs patients by reducing the SDF application time by 50% of the American Academy of Pediatric Dentistry recommendation while maintaining an adequate SDF remineralization effectiveness.

Background

Silver diamine fluoride (SDF) has become a popular caries-control method in arresting dentinal caries because it is easy to handle and costs less than other restorative materials [1, 2]. This colorless silver compound-containing solution is photosensitive, with 253,900 ppm of silver ions and 44,800 ppm of fluoride ions [3] and a pH of 9–10 [4]. With its unique property of arresting dentin caries without requiring caries excavation [5], SDF is considered an appropriate treatment for young or uncooperative children and special needs patients. Furthermore, aerosol-generating procedures have been avoided to reduce the risk
of airborne infection. This makes SDF a safer and more attractive choice in managing cavitated dentin lesions.

SDF arrests dental caries due to its bactericidal effects, interrupts demineralization, promotes remineralization in demineralized enamel or dentin, and inhibits dentin collagen degradation [4]. When SDF is exposed to light, the lesion turns dark [2] and the surface hardness increases [1]. Lesion depth also decreased after SDF application, which might have been due to calcium and phosphate aggregation on the lesion's surface [6]. However, no studies have evaluated the surface and remineralization effects of SDF treatment.

Light might play a role in the caries arrest by SDF because it is light-sensitive. Crystal and Niederman [2] hypothesized that SDF-treated dental caries in primary anterior teeth was arrested more than in posterior teeth because they could be cleaned easier and were exposed to more light. They also hypothesized that light increases the remineralization efficacy of SDF. Although two studies [7, 8] have investigated the effect of light curing on SDF-applied lesions by comparing the silver ion penetration in artificial caries lesions with that in lesions applied with SDF and light-cured, their results differed. Moreover, these studies did not address the mechanism by which silver precipitation results in remineralization after SDF application with light curing. The American Academy of Pediatric Dentistry (AAPD) recommends that SDF be applied for 1 min per tooth [9], which may be too long for young, uncooperative children. Light curing the applied SDF may accelerate silver precipitation, resulting in more rapid caries arrest and decreased application time.

Digital subtraction radiography (DSR) is a method to detect the progression of dental caries, including remineralization and demineralization, by superimposing digital radiographs from before and after the treatment; unchanged structures are subtracted, revealing only changes in mineralization. The advantages of this method are that it can detect carious lesion remineralization with increased sensitivity and reproducibility compared with conventional radiographs, and the whole tooth can be analyzed without being sectioned [10–12].

Currently, there is no report demonstrating the effect of light curing (LC) applied SDF on remineralization. Therefore, the aim of this in vitro study was to evaluate the remineralization effects of SDF application on natural dentin carious lesions in primary molars of different application times with additional LC compared with its application without LC, as analyzed by DSR, energy dispersive X-ray spectroscopy (EDS), and scanning electron microscopy (SEM). The null hypothesis was that the remineralization effects were not different between groups regardless of the additional light curing or SDF application time.

Methods

Sample size calculation and tooth selection
This study protocol was approved by the Ethics Committee (HREC-DCU 2020-008) and Institutional Biosafety Committee (DENT CU-IBC 007/2020).

Currently, no study has been done on the efficacy of LC on remineralization of dentin carious lesions after SDF application. Therefore, the sample size determination was carried out based on the authors’ pilot study before this one (5 samples/groups, a total of 4 groups), giving a partial eta squared of 0.304. Using the G*power program, a two-way ANOVA statistical test determined that at least 6 samples/group would be required in this study ($\alpha = 0.05$, $\beta = 0.20$). Due to the small sample size, the sample size was increased to 10 specimens/group to compensate for potential specimen preparation and data analysis errors. Therefore, 40 carious primary molars were used in this study.

Furthermore, we studied the SEM-EDS from the 40 specimens. The sample size calculation for elemental profiling (EDS) was performed based on an ex vivo study by Mei et al. [6], determining 16 carious primary molars; hence, 4 teeth per group were required. Surface morphology (SEM) was studied on 50% of the EDS samples.

The inclusion criterion for both analyzes was primary molars with either occlusal or occluso-proximal natural caries from 4- to 6-year-old children. The exclusion criteria were teeth with restorations, developmental defects, arrested caries, craze lines, or radiographic dental caries involving more than the middle 1/3 of the dentin.

**Specimen preparation**

Forty primary molars with carious dentin (10 samples/group), extracted according to the patient’s treatment plans, were collected from the dental department at Nongjik hospital in Pattani, Thailand, and stored in 0.9% sodium chloride at 4ºC until used. Subsequently, the teeth were cut along the buccolingual axis using a slow-speed cutter (Isomet 1000; Buehler Ltd., Lake Bluff, Illinois, USA). The samples were rinsed with deionized water in an ultrasonic bath and dried. Clear nail polish was applied on the specimens except for the dentin lesion. Finally, the specimens were fixed in 1x1x0.9 cm (WxLxH), self-curing acrylic resin blocks, and polished with 600-, 800-, and 1,000-grit silicon carbide papers (Fig. 1).

**Radiographic assessment**

Using an X-ray alignment system, baseline digital radiographs were taken to generate reproducible projection geometry (Fig. 2). A phosphor digital imaging plate No. 0 (CS 7600; Carestream Health, Rochester, New York, USA) in a protective sleeve was stabilized in a customized film holder. Each dentin block was put over the imaging plate with the buccal aspect of the tooth facing toward the X-ray tube. A 15-mm thick, rectangular, plexiglass plate was placed over the plexiglass supporter, acting as a soft tissue equivalent [13]. A customized device for locking the collimator was used to position the X-ray collimator perpendicular to the tooth and receptor [12]. The radiographs were taken using an intraoral X-
ray system (Kodak 2200; Carestream Health, Rochester, New York, USA) with settings of 70 kVp, 7 mA, and 0.119 s exposure time. The same machine and settings were used throughout the experiment by the same investigator (J.K.).

Permuted block randomization

After the digital radiographs were taken, they were saved as Tagged Image File Format with a resolution of 792 dots per inch. Image-Pro Plus software version 7.0 (Media Cybernatics; Rockville, Maryland, USA) was used to evaluate the baseline lesion depth (LD) and mineral density (MD) of the samples from groups divided. The specimens were excluded if the occlusal or occluso-proximal caries involved more than the middle 1/3 of the dentin. LD was determined by dividing the distance from the dentinoenamel junction (DEJ) to the deepest part of dentin lesions by the total depth of the dentin as measured parallelly from the DEJ to dentin-pulp junction. MD was measured by identifying two areas of interest (AOIs) at the same lesion depth on each specimen; the deepest part of the dentin lesion and the adjacent sound dentin as a control. The sizes of the measurement windows were set at 15x15 pixels for the outer 1/3 dentin lesion and normal dentin, and 30x30 pixels for the middle 1/3 dentin lesion to cover a larger area (Fig. 3). The MD measurement was described as mean density/intensity value. The baseline MD of each specimen (MD_{baseline}) was determined by the difference between the MD at the deepest part of the lesion (MD_{lesion}) and the MD at the adjacent normal dentin (MD_{control}).

The specimens were sterilized with hydrogen peroxide gas and divided into 4 groups according to their baseline LD and MD using permuted block randomization to ensure equal distribution of different LDs and MDs in all groups:

Group 1: SDF applied for 10 s (10SDF)
Group 2: SDF applied for 60 s (60SDF)
Group 3: SDF applied for 10 s + LED light curing 20 s (10SDF+LC)
Group 4: SDF applied for 60 s + LED light curing 20 s (60SDF+LC)

The specimens were immersed in artificial saliva (KCl, MgCl₂, CaCl₂, K₂HPO₄, KH₂PO₄, sodium carboxymethylcellulose, sorbitol, sodium benzoate, and deionized water) of pH ~ 7 for 1 h before SDF application to create the pellicle on the carious surface mimicking oral condition. Each application was performed in a dark, sterile laminar flow cabinet, using a microbrush soaked with premeasured 5 µL of 38% SDF (Saforide; Toyo Seiyaku Kasei Co., Ltd., Osaka, Japan). An LED dental curing light with an output intensity of 520 mW/cm² and a wavelength range of 450–470 nm (Demi Plus; Kerr, Orange, California, USA) was used in the LC groups. The light intensity was calibrated before curing 20 specimens and the light source contacted the tooth surface when light curing.
**Bacterial pH-cycling**

Due to the antibacterial property of SDF, the demineralization solution was prepared with two cariogenic bacteria, *Streptococcus mutans* (*S. mutans*) ATCC 25175 and *Lactobacillus casei* (*L. casei*) IFO 3533 as previously described [14, 15]. They were cultured on tryptic soy agar plates at 37°C, 5% CO₂ for 24 h. One colony of each culture was transferred to tryptic soy broth containing 0.5% yeast extract and incubated at 37°C, 5% CO₂ for 16 h. The incubated broths of each bacteria were diluted in tryptic soy broth containing 0.5% yeast extract, 2% sucrose, and 1% glucose to achieve optical densities of 0.1 as measured by a spectrophotometer at 540 nm. The cultures were mixed at a 1:1 ratio (≈ 1.37 x 10⁸ CFU ml⁻¹ of *S. mutans* and ≈ 7.8 x 10⁷ CFU ml⁻¹ of *L. casei*) to make a dentin demineralizing solution with the average pH of 6.31 ± 0.1[16].

The specimens were immersed in the demineralizing solution for 4 h and in artificial saliva without fluoride for 20 h. The specimens were rinsed using deionized water before changing the solution. The specimens were sterilized with hydrogen peroxide gas after bacterial-pH cycling for 7 d [15] and reassessed for post-treatment MD using DSR. The study flowchart is presented in Fig. 4.

**Digital subtraction radiographic analysis**

The samples were radiographed post-bacterial pH cycling as described for the post-treatment radiographs. They were superimposed with their baseline radiographs using Image-Pro Plus software to create digital subtraction radiographs (Fig. 5). At baseline, the two recorded AOI windows on the dentin lesion and normal dentin were later used to measure the MD of the same area on the subtraction radiographs. To compare the MD between samples, the MD of the subtraction radiographs at the normal dentin were adjusted (adjusted MD<sub>S-control</sub>) to an intermediary pixel grey value of 128 [17], and the MD of the subtraction radiograph at the dentin lesion was calculated (adjusted MD<sub>S-lesion</sub>) using the rule of three. The MD<sub>subtraction</sub> was the difference between the adjusted MD of the lesion and the normal dentin. A positive MD<sub>subtraction</sub> value represented remineralization, whereas a negative value represented demineralization.

\[
\text{MD}_{\text{subtraction}} = \text{adjusted MD}_{\text{S-lesion}} - \text{adjusted MD}_{\text{S-control}}
\]

Before the subtraction analysis, J.K. was calibrated for measuring mineral density on the images by an oral and maxillofacial radiologist (S.P.) using a randomized 20% of all the subtraction radiographs to ensure reproducibility and reliability by intraclass correlation coefficient (ICC). Each investigator separately measured the MD<sub>subtraction</sub> of all the samples. When there was any difference in the measurement, the two investigators discussed and agreed on the measurement. The mean MD<sub>subtraction</sub> in each group was calculated, and this value was referred to as the mean mineral density difference (mMDD).
**Statistical analysis**

Statistical analysis was performed using SPSS software version 22.0 (IBM, Armonk, New York, USA). The Shapiro-Wilk test was used to assess the distribution of the data. One-way ANOVA was used to compare the baseline LD and MD between the groups. The mMDD between the groups determined by the subtraction method was analyzed using two-way ANOVA, generalized linear models (GLMs) with Bonferroni post hoc test. The significance level was set at $p < 0.05$.

**Surface morphology and elemental profiling**

The sterilized post-treatment blocks were taped to an aluminum stand to undergo elemental profiling with SEM-EDS after 7 d of bacterial pH-cycling. Four samples were randomly selected from each group for assessing the post-treatment elemental profile using an INCAEnergy Energy Dispersive X-ray Spectroscopy (EDS) system (Oxford Instruments, High Wycombe, UK) with a scanning electron microscopy (SEM, JSM-IT300, JEOL, Japan) at a 15 kV operating voltage. Two of the four samples were randomly selected to assess their surface morphology by SEM (Quanta 250; FEI Company, Netherlands). Hence, a total of eight specimens were kept dry in a desiccator cabinet before being evaluated. After the elemental profile of the surface lesion was assessed, the specimens were coated by gold sputtering and taped to the aluminum stand to evaluate the surface lesion morphology after treatment at magnifications of 1,000x, 10,000x, and 30,000x with 20kV.

**Results**

The intraclass correlation coefficient value for $\text{MD}_{\text{subtraction}}$ was 0.925. The baseline LD and MD ± standard deviation (SD) in the 4 groups are shown in Table 1. The baseline LD and MD between the groups were not significantly different as determined by one-way ANOVA ($p = 0.050$ and 0.081, respectively). The post-treatment mMDD ± SD in each group is presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Mean LD and MD at baseline and mMDD between groups post-treatment.</td>
</tr>
</tbody>
</table>
Group | \( \text{LD}_{\text{baseline}} \pm \text{SD} \) | \( \text{MD}_{\text{baseline}} \pm \text{SD} \) | \( \text{mMDD} \pm \text{SD} \)  
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>10SDF</td>
<td>0.38 ± 0.15</td>
<td>-39.21 ± 19.26</td>
<td>3.47 ± 5.34</td>
</tr>
<tr>
<td>60SDF</td>
<td>0.52 ± 0.13</td>
<td>-25.19 ± 18.23</td>
<td>6.36 ± 5.08</td>
</tr>
<tr>
<td>10SDF + LC</td>
<td>0.47 ± 0.11</td>
<td>-41.14 ± 21.55</td>
<td>7.90 ± 3.51</td>
</tr>
<tr>
<td>60SDF + LC</td>
<td>0.50 ± 0.08</td>
<td>-49.84 ± 23.45</td>
<td>10.47 ± 6.63</td>
</tr>
</tbody>
</table>

LD lesion depth; MD mineral density; mMDD: mean mineral density difference; SD standard deviation

Two-way ANOVA, GLMs was used to evaluate the effects of two factors on the mMDD of the carious dentin lesions, LC and SDF application time. The results indicated no interaction between LC and application time on mMDD \((p = 0.920)\) (Fig. 6). When each factor was evaluated separately, LC tended to influence the mMDD \((p = 0.065)\); however, the application time did not \((p = 0.249)\).

Because there was no significant interaction effect between LC and application time on the mMDD, the interaction between the two factors was eliminated, and the data were re-analyzed (Table 2). This analysis indicated that LC significantly affected mMDD \((p = 0.007)\), however, application time did not \((p = 0.084)\). Post-hoc analyzes were conducted using Bonferroni correction (Table 3). The mMDD in the LC groups was significantly higher than those without LC. The 10SDF+LC group demonstrated a significantly higher mMDD than the 10SDF group \((p = 0.041)\). Furthermore, the 60SDF+LC group presented a significantly higher mMDD than the 10SDF and the 60SDF groups \((p = 0.010\) and 0.041, respectively). Comparing application times, the mMDD in the 10SDF and 60SDF groups were not significantly different \((p = 0.502)\). In addition, the mMDD in the 10SDF+LC group and 60SDF group were similar \((p = 1.00)\).

**Table 2**

The GLMs results, with mMDD as the dependent variable showing the differences in mMDD between factors.

<table>
<thead>
<tr>
<th>Factors</th>
<th>df</th>
<th>Diff mMDD (SE)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>No light</td>
<td>1</td>
<td>-4.27(1.58)</td>
</tr>
<tr>
<td>Light</td>
<td>1</td>
<td>-2.73(1.58)</td>
<td>0.084</td>
</tr>
</tbody>
</table>

GLM Generalized Linear Models; mMDD: mean mineral density difference; SE standard error; * \( p < 0.05 \) by two-way ANOVA, GLMs

**Table 3**
The GLMs results, with mMDD as the dependent variable, show the differences among multiple factors.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Diff mMDD (SE)</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDF 10 s x No light</td>
<td>-2.73 (1.58)</td>
<td>1</td>
<td>0.502</td>
</tr>
<tr>
<td>SDF 10 s x Light</td>
<td>-4.27 (1.58)</td>
<td>1</td>
<td>0.041*</td>
</tr>
<tr>
<td>SDF 60 s x Light</td>
<td>-7.0 (2.23)</td>
<td>1</td>
<td>0.010*</td>
</tr>
<tr>
<td>SDF 60 s x No light</td>
<td>2.73 (1.58)</td>
<td>1</td>
<td>0.502</td>
</tr>
<tr>
<td>SDF 10 s x Light</td>
<td>-1.54 (2.23)</td>
<td>1</td>
<td>1.000</td>
</tr>
<tr>
<td>SDF 60 s x Light</td>
<td>-4.27 (1.58)</td>
<td>1</td>
<td>0.041*</td>
</tr>
<tr>
<td>SDF 10 s x Light</td>
<td>4.27 (1.58)</td>
<td>1</td>
<td>0.041*</td>
</tr>
<tr>
<td>SDF 60 s x Light</td>
<td>1.54 (2.23)</td>
<td>1</td>
<td>1.000</td>
</tr>
<tr>
<td>SDF 60 s x Light</td>
<td>-2.73 (1.58)</td>
<td>1</td>
<td>0.502</td>
</tr>
<tr>
<td>SDF 60 s x Light</td>
<td>7.0 (2.23)</td>
<td>1</td>
<td>0.010*</td>
</tr>
<tr>
<td>SDF 10 s x Light</td>
<td>4.27 (1.58)</td>
<td>1</td>
<td>0.041*</td>
</tr>
<tr>
<td>SDF 10 s x Light</td>
<td>2.73 (1.58)</td>
<td>1</td>
<td>0.502</td>
</tr>
</tbody>
</table>

GLM Generalized Linear Models; mMDD: mean mineral density difference; SE standard error; * p < 0.05, Bonferroni test

The EDS analysis presented higher silver and chloride peaks in the 10SDF+LC and 60SDF+LC groups than those without LC. The surface morphology of the 10SDF group had a few spherical grains sparsely aggregated on the lesion's surface (Fig. 7). Densely packed spherical grains mixed with square-shaped particles tightly adhered to the intertubular dentin were seen in the 60SDF group. Compared to LC, the 10SDF+LC group demonstrated more spherical grains and square-shaped particles than the 10SDF group. A similar pattern was observed comparing the 60SDF+LC and 60SDF groups.

**Discussion**

This study evaluated the remineralization effects of SDF with different application times and additional LC on natural dentin carious lesions in primary molars compared with its application without LC, as analyzed by DSR, EDS, and SEM. The results demonstrated that LC was the only factor related to lesion remineralization and enhanced precipitation of silver ions after SDF application. These results indicated that light improves the remineralization efficacy of SDF.

Digital subtraction radiography was used to analyze the mineral change after treatment. To ensure the reproducibility and quality of the radiographs, the x-ray alignment system and same exposure factors were designed to generate reproducible projection geometry and the same radiographic density. The grey
value of the normal dentin was adjusted to the intermediary pixel grey values of an 8-bit radiograph which is 128 [17]. An additional device such as an aluminum step wedge was not used in this study.

The light source in the present study was an LED light curing device with an output intensity of 520 mW/cm², which is the highest intensity used in our clinical setting, and a wavelength range of 450–470 nm that triggers silver ion precipitation into metallic silver [18]. As silver ions in SDF partly combined with specific amino acids in collagen in the exposed dentin and were reduced to metallic silver, the demineralized dentin turns dark [19]. When the silver particles are exposed to light, the shape of the particles tends to change from spherical to plate-like triangular, square, or hexagonal [20]. Concordantly, the SEM images of the SDF-applied with LC groups illustrated more square-shaped particles on the dentin surface lesions compared with the non-LC groups. This strongly suggests that the particles observed were silver, consistent with the high quantities of silver observed from the elemental profile.

Furthermore, chlorine in this study was higher than that of phosphorus. Therefore, the silver compound formed after the application of SDF could likely be silver chloride rather than silver phosphate. The morphology of silver chloride particles, i.e., budding cubic particles, [21] was obviously present in the SDF60 + LC group. Due to the fact that silver chloride has a lower solubility than silver phosphate (8.9x10⁻⁵ and 6.5x10⁻⁴ g/100 ml, respectively) [22]. Silver phosphate dissolves when immersed in artificial saliva, releasing free silver ions to enhance antibacterial activity [23] and react with the chloride ions in artificial saliva to form silver chloride [22], which could play a major role in hardening the lesion surface [24]. Several studies also detected silver chloride after SDF application [3, 24, 25].

Seto et al. [26] concluded that the metallic silver obliterates the porous surface of the carious lesions and reinforces the structure of the remaining organic matrix, increasing the lesion hardness. Our results demonstrated that the deepest part of almost all of the carious lesions in each group gained mineral density or remineralized. However, this study could not identify the specific tooth structure with the remineralization because the evaluations were performed using DSR, which is two-dimensional. A clinical or histopathological examination is needed to investigate the characteristics of dental caries [27]. However, a previous study [8] investigated SDF penetration in dentin carious lesions with and without a 40 s light curing after SDF application. The study classified three different layers of dentin carious lesions using a scanning electron microscope and a stereomicroscope; infected dentin, affected dentin, and sound dentin. Their results demonstrated that SDF penetrated 60 ± 10 µm into the sound dentin in the light-cured group (test group) while penetrating 130 ± 50 µm in the group without light-curing (control). The zone with the most silver precipitation was infected dentin, which was 2.6-fold greater in the test group compared with the control group. The affected dentin caries zone presented no difference in silver precipitation in both groups. The authors concluded that SDF treatment with light curing induced more silver ion precipitation in infected dentin, causing less SDF penetration into sound dentin. Infected dentin is rich in bacteria and is where demineralization occurs. This agrees with Mei et al. [6] who reported mineral deposition 150 µm from the surface of SDF-applied dentin carious lesions of primary incisors without light curing.
The application time of SDF has been widely studied. Despite a 1-min recommendation by the AAPD [9] and the 3–4 min recommendation by Saforide’s manufacturer, application times ranging from 10 s to 3 min were used in the clinical study review by Crystal and Niederman [2]. Although Duangthip et al.[28] applied SDF for only 10 s, they demonstrated a better caries arrest rate than using 5% NaF at the same frequency. Our results revealed that the mMDD in the 10SDF and the 60SDF groups were not significantly different, suggesting that application time did not affect remineralization. This finding agrees with the results from a clinical study that found no relationship between the length of application time and the effectiveness of SDF treatment [29]. Notably, the AAPD suggests that when a reduced application time is used, careful monitoring is needed to evaluate caries arrest and consider the need for re-application [9].

The strength of this study is the use of natural carious primary molars with active lesions, the bacterial pH-cycling model, comprising cariogenic bacteria and artificial saliva that simulated the environment in the oral cavity. Unlike when using chemical pH-cycling, SDF can exhibit its bactericidal effect in the bacterial pH-cycling model [30]. The demineralizing solution had an average pH of 6.31 ± 0.1, which is lower than the critical pH of dentin (pH = 6.5) [16].

The limitation of this study is that it was performed in vitro. Although our method mimics the oral environment to some extent, the results may not be extrapolated to the clinical situation. Besides, EDS analysis could be carried out only at the surface of the lesions as specimen preparation required nail varnish coatings to prevent demineralization in unwanted areas during bacterial pH cycling. The elemental profile of the lesions beneath the surface was then unable to be assessed. These findings have practical implications for managing dentin caries in young or uncooperative children and special-needs patients to reduce chair time. However, clinical studies with long-term follow-ups are suggested to confirm these results before clinical use to improve clinical outcomes by reducing SDF application time through using additional LC.

**Conclusion**

Light curing enhances the remineralization efficacy of SDF, as shown by DSR, EDS, and SEM analyses. The remineralization due to applying SDF on the lesions for 10 s with 20 s LC (total time of 30 s/cavity) was not significantly different from those treated using a 60 s application time with or without LC. The results could be valuable for managing dentin caries in young or uncooperative children and special-needs patients by reducing the SDF application time by 50% of the AAPD recommendation while maintaining an adequate SDF remineralization effectiveness.

**Abbreviations**

DSR: Digital Subtraction Radiography

EDS: Energy Dispersive X-ray Spectroscopy

GLMs: Generalized Linear Models
LC: Light Curing
LD: Lesion Depth
MD: Mineral density
mMDD: mean Mineral Density Difference
SDF: Silver Diamine Fluoride
SEM: Scanning Electron Microscopy

Declarations

Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee (HREC-DCU 2020-008) and Institutional Biosafety Committee (DENT CU-IBC 007/2020) of the Faculty of Dentistry, Chulalongkorn University. Written informed consent was obtained from all guardian participants.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors’ contributions
CT, SP, PT, and OT conceived the original idea and designed the experiments. JK performed the experiments, analyzed, interpreted the data, wrote the original draft, reviewed, and edited the manuscript. SP calibrated digital subtraction of JK and edited the manuscript. KP wrote the original draft, reviewed, and edited the manuscript. KJ helped in designing and interpreting the statistical analysis. All authors read and approved the final manuscript.

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References


31.


Figures
Figure 1
Specimen preparation
Figure 2

X-ray alignment system. a longitudinal section; b top view; c x-ray collimator in a customized locking device. 1: intraoral imaging plate; 2: primary molar block; 3: plexiglass supporter; 4: plexiglass; 5: a customized device for locking collimator.
Lesion depth measurement

Figure 3

Lesion depth (LD) and mineral density (MD) measurement. **a** sagittal view diagram; **b** sagittal view radiograph; green line – the distance from dentinoenamel junction (DEJ) to the deepest part of dentin lesions, pink line – the DEJ to the dentin-pulp junction. **c** sagittal view diagram; **d** sagittal view radiograph; green box – the area of interest (AOI) of dentin lesion, pink box – AOI of normal dentin.
Figure 4

Flow chart of the study
Figure 5

Two-dimensional radiographs of before and after treatment, and digital subtraction radiographs; white arrows indicate the remineralized area.
Figure 6

The interaction between two factors, light and application time, on mMDD.
Figure 7

SEM images and elemental profile of the dentin carious lesion's surface of each group.