IN-VITRO REMINERALIZING EFFECT OF SILVER DIAMINE FLUORIDE ON DENTIN CARIES IN PRIMARY TEETH

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ABSTRACT
INTRODUCTION: Minimal intervention approaches are now utilized to remineralize carious lesion and maintain a functional tooth. Remineralizing agents as silver diamine fluoride (SDF) was found to be able to arrest caries lesions by effectively remineralizing the demineralized tooth structure.

OBJECTIVE: Evaluate and compare the remineralizing effect of 38% SDF and 5% sodium fluoride (NaF) varnish on dentin caries-like lesions of primary teeth.

MATERIALS AND METHODS: Forty sound primary teeth were selected. The enamel layer of each tooth on the labial surface was removed exposing the dentin, which was then covered with nail varnish exposing a 4x4 dentin window that underwent demineralization. Each tooth was sectioned through the window labiolingually into mesial and distal specimens. One half was treated with test material (test group), while the other was left untreated (control group). Specimens were assigned to 2 groups according to treatment agent; group I: 20 specimens were treated using 38% SDF; group II: 20 specimens were treated using 5% NaF. Evaluation was conducted using Energy dispersive X-ray spectrometer (EDX) and Polarized light microscope. Independent t test was used to compare between groups while within group comparisons were done using Repeated Measures ANOVA followed by post hoc test with Bonferroni correction while paired t test was used to compare lesion depth within group. Significance level was set at P value 0.05.

RESULTS: Both groups showed a statistically significant percentage increase in calcium (P=0.002) and calcium phosphate ratio (P<0.0001). This increase was in favor of SDF. Statistically significant percent reduction in lesion depth was found in the SDF group in comparison to the NaF group (83.18 (7.76) and 45.73 (12.76) respectively, P<0.0001).

CONCLUSION: 38% SDF solution and 5% NaF varnish showed remineralizing effect on artificial carious dentin. However, SDF demonstrated superior remineralizing potential than NaF in terms of mineral content as well as lesion depth.

KEY WORDS: Silver diamine fluoride (SDF), Remineralization, Sodium fluoride varnish, Primary teeth.

INTRODUCTION
Dental caries remains one of the most common chronic diseases worldwide (1). It is a dynamic process; its progression depends on the remineralization and remineralization balance whether the lesion will continue into cavitation or it will be reversed. (2)

The treatment of dental caries has been redirected to the arrest of disease progression rather than focusing on restorative approaches alone (3). Minimal intervention dentistry (MID) is a concept that shifted the traditional surgical approach to a medical model. It breaks the ‘repeat restoration cycle’ to ensure the maintenance of teeth functionality for life. When the disease process is interrupted early the pain is reduced, as well as the cosmetic and financial burdens of treatment (4).

Fluorides have been widely recommended for almost a hundred years for the prevention of caries. Ten Cate and Buzalaf (2019) (5) explained the fluoride activity on the demineralization and remineralization processes. Fluorides are able to rebuild partially dissolved crystals, in the presence of calcium and phosphate, to form fluorhydroxyapatite crystals when the acid attack is neutralized and pH rises above the critical level. These crystals are more resistant to future acid attacks. Calcium fluoride is also precipitated that releases fluoride ions during acid attacks, those ions adsorb to the surface of the crystals inhibiting their remineralization. (6)

Topical application of 5% sodium fluoride (NaF) varnish increases the fluoride intake by dental tissue due to its high concentration and the increase in the contact time between fluoride and tooth structure. It is considered safe due to the thin layer and small amounts actually used (7). Despite their proven efficacy and cost effectiveness, the necessity of patient cooperation as well as the multiple application annually require alternative agents and preparations that provide further benefits (8).

Silver diamine fluoride (SDF) is another form of professionally applied fluoride. It contains the highest...
fluoride concentration available for dental use, with 44,800 ppm fluoride (9). It is the first material to combine the remineralizing capacity of sodium fluoride with the antibacterial effect of silver nitrate (10). The reason behind the increased popularity of SDF is for its safety and effective control and arrest of caries (11). The first SDF product was approved in the USA by the Food and Drug Administration (FDA) in 2014. Silver diamine fluoride 38% is an alkaline liquid that consists of 24.4-28.8% silver, 5.0-5.9% fluoride and ammonia (12).

The clinical success of SDF has gained the attention of clinicians and researchers to further study and understand the mechanism of SDF in the remineralization of carious dentine (13). Silver ions in the SDF are responsible for the antimicrobial action, moreover, the silver reacts with hydroxyapatite crystals resulting in the precipitation of silver phosphate that forms an insoluble layer blocking the dentinal tubules and rendering the outer dentin layer resistant to further mineral loss (5). The remineralizing capacity of SDF is promoted by the fluoride ions found in the solution. In the presence of calcium and phosphate, fluoride forms the more acid resistant fluorapatite crystal as well as the precipitation of calcium fluoride; this in turn also increases the mineral density and hardness (14). Silver diamine fluoride has also shown to protect the collagenous matrix that acts as a scaffold for the new crystal deposition, by inhibiting, matrix metalloproteinases and forms a silver-protein conjugation increasing the degradation resistance (15).

The main drawback of SDF is the esthetic results due to the black staining of demineralized carious lesions (16). However, several studies found that parents were satisfied with the outcome and the acceptability to the treatment increased with the increase in the required behavior management of the child (17,18,19).

Several in vivo studies on SDF have shown its effectiveness in arresting dentin caries (18-21) however to understand further the action of SDF on caries, in vitro studies have been conducted to evaluate the remineralizing capacity of SDF on artificial carious lesions (22-24). Some of these studies were conducted on permanent dentin while others on primary enamel. Therefore, the aim of this study was to evaluate and compare the remineralizing effect of 38% SDF and 5% NaF in dentin caries-like lesions in primary teeth. The null hypothesis of the study was that there is no difference in the remineralizing capacity in terms of mineral deposition and lesion depth reduction following the application of 38% SDF and 5% NaF on artificial dentin caries.

**METHODS**

An experimental *in vitro* study was conducted in the labs of Department of Pediatric Dentistry and Dental Public Health and Department of Oral Biology, Faculty of Dentistry, and Department of Geology, Faculty of Science, Alexandria University. To evaluate the remineralizing effect of SDF on dentin of primary teeth. Approval for the study conduction was granted by the Research Ethics Committee of Faculty of Dentistry (IORG0008839).

Sample size was estimated based on the assumption alpha error = 5% and beta error = 20%. With minimal required sample size of 10 specimens per group (number of groups=2) (total sample size=20 specimens) for the EDX evaluation, for the polarized light study the minimal required sample size of 10 specimens per group (number of groups=2) (total sample size=20 specimens) (25,26).

Forty maxillary extracted or exfoliated anterior primary teeth free from any cracks, developmental defects or caries examined using a magnifying lens, were included in the study. They were collected from the outpatient clinics of the pediatric department, Faculty of Dentistry, Alexandria University. Selected teeth were cleaned with fluoride free pumice and brush and stored in deionized water at 4°C until required for use. Each tooth was embedded in acrylic resin blocks with the labial surface facing upwards. The enamel layer on the labial surface was removed using a model trimmer under running water, and smoothened with silicon carbide paper (27). A 4x4 window of dentin was created by placing a 4x4 mm self-adhesive labels followed by coating with nail varnish then the label was removed (28).

Dentin caries-like lesion was achieved by pH cycling to simulate the natural caries process in the oral environment, teeth were placed in 10 ml of demineralizing solution (2.2mM calcium chloride, 2.2mM potassium dihydrogen phosphate, 0.05M acetic acid and 1M potassium hydroxide to adjust pH to 4.8) for 8 hours, followed by 10 ml of remineralizing solution (1.5mM calcium chloride, 0.9mM sodium dihydrogen phosphate, 0.15M potassium chloride pH=7) for 16 hours for 14 days (29). Teeth were then sectioned labiolingually in a longitudinal direction through the center of the window giving 2 halves, each half was considered a specimen, where one half was treated while the other half was left untreated to serve as its control.

Random allocation of the 40 teeth (80 specimens) was conducted using computer generated list (http://www.random.org/) (31) into two groups; group I where the test half was treated with 38% SDF solution (Advantage Arrest) (Elevate Oral Care, LLC, West Palm Beach, Fl). Group II where the test half was treated with 5% NaF varnish (Profluoride varnish) (VOCO GmbH, Cuxhaven, Germany).

All specimens were rinsed with deionized water and stored in artificial saliva (200g Methyl-p-hydroxy methyl cellulose, 10.00g Sodium carboxy methyl cellulose, 0.625 g potassium chloride, 0.059g Magnesium chloride hydrate, 0.166 g Calcium chloride hydrate, 0.804g Dipotassium Hydrogen phosphate, 0.326 g monopotassium phosphate, one liter distilled, and 0.05 M water Sodium hydroxide will be to obtain a pH 7.0) for 24 hours. All specimens underwent pH cycling to simulate oral environment, for 10 days, they were immersed for 6 hours in demineralizing solution (pH=4.8) followed by 18 hours in remineralizing solution (pH=7).

Energy Dispersive X-ray spectrometer (EDX)[JEOL, Tokyo Japan] was used for the quantitative evaluation of the mineral content. The analysis provides us with the micro analysis of the specimen composition, each constituent atomic % was determined by measuring its characteristic re-emission using the scanning electron microscope. Calcium (Ca) and phosphorus (P) distribution...
was determined by peaks on the software’s graph, with corresponding readings. Ca/P ratios were calculated (24). Olympus polarized light microscope CX31 (America Inc) was used for qualitative histologic examination, as well as quantitative evaluation of the lesion depth. Ground sections of dentin were examined, where they show different birefringence. Rays refracted by minerals found in the samples with two planes of transmission (birefringence) where the positive birefringence refers to the slower rays while the negative birefringence refers to bigger amplitude and faster rays, demineralized dentin showed lesion with positive birefringence while remineralized dentin showed negative birefringence. The mean lesion depth was measured with three lines one in the center and one on each side of the lesion in micrometers (31).

**Statistical analysis**

Normality was checked using descriptive statistics, plots, and normality tests. Minerals content, Lesion depth and percent reduction were found to be normally distributed so, means and standard deviations (SD) were calculated. Microanalysis of minerals content was compared between groups using independent t test while within group comparisons were done using Repeated Measures ANOVA followed by post hoc test with Bonferroni correction. Paired t test was used to compare lesion depth between test and control within each group. While independent t test was applied in comparison between SDF and NaF. Percent change was calculated according to the following formula; 

\[
\text{Percent change} = \left(\frac{\text{Test values} - \text{Control values}}{\text{Control values}}\right) \times 100
\]

Percent change in minerals content was compared using Mann Whitney U test and independent t test was used for percent change in lesion depth.

Data were analyzed using SPSS for Windows version 25.0. Significance level was set at P value 0.05.
RESULTS
Within group comparison, results of the present study show that after remineralization the mean Ca content increased significantly in both SDF and NaF groups (P<0.0001, P=0.0001 respectively). The P content also showed significant increase (P=0.0001, P=0.0001 respectively), as well as the Ca/P ratio (P<0.0001, P=0.006 respectively). By comparing the two study groups, the percentage increase in SDF was significantly higher when compared to NaF in Ca (P=0.002) and in Ca/P ratio (P<0.0001), however there was no statistically significant difference in percentage change in phosphate (P=0.695) (Table 1).

Regarding the lesion depth, a statistically significant reduction in mean lesion depth was found after remineralization in both SDF and NaF groups (P<0.0001, P<0.0001 respectively) with percentage reduction (83.18%, 45.73% respectively). By comparing the two study groups, the percentage reduction in lesion depth was significantly higher in the SDF group in comparison to the NaF group (P<0.0001) (Table 2).

Qualitative evaluation
Examination of the SDF group revealed an evident remineralizing potential where the majority of study samples showed reduction in lesion depth with strong negative birefringence, while a few specimens revealed partial remineralization with a narrow zone of positive birefringence was still detected. The fluoride group upon examination showed a noticeable amount of remineralization, however, less than that shown for the SDF group. Most of the specimens showed remineralization with a few dentinal tubules still showing signs of demineralization, while others showed only partial remineralization with demineralized zones.

Table (1): Calcium and phosphate content in dentin. And Ca/P ratio at demineralization, remineralization and percentage change in Silver diamine fluoride and Sodium fluoride groups.

Table (2): Lesion depth at demineralization, after remineralization and percentage reduction in Silver diamine fluoride and Sodium fluoride groups.

**DISCUSSION**
The present study evaluated and compared the remineralizing potential of SDF and NaF varnish in terms of mineral content and lesion depth of dentin caries-like lesion. It fills the gap in knowledge about the difference in action of both SDF and NaF on dentin of primary teeth by comparing their remineralizing capacity. Results of this study rejected the null hypothesis in which 38% SDF was found to be more effective in dentin remineralization than 5% NaF varnish. Although there was a significant increase in the Ca, P and Ca/P ratio in both groups after remineralization, the percentage increase in SDF group was higher than NaF group with significant difference. Moreover, there was a notable reduction in lesion depth in both groups with significantly higher percentage of reduction in SDF group than NaF group. The promising results of this study have implication for the arrest and management of dentin caries in primary teeth.

In the current study the significant increase in Ca, P, Ca/P ratio after remineralization in the SDF group was in agreement with previous studies (32, 33). The high remineralization potential may be due to the higher fluoride content of SDF at 38%, where it promotes the formation of calcium fluoride, which releases calcium and fluoride ions when dissolved in a salivary environment (33). This process is responsible for the formation of fluorapatite by the replacement of the hydroxyl ion. Another feature responsible for its remineralizing capacity is the alkaline pH. This high alkalinity promotes ion exchange by creating the ideal conditions for the reposition of ions and the formation of fluorapatite (9). Remineralization may also be induced by the protection of exposed collagen, SDF stabilizes the collagen matrix by inhibiting host-derived proteases or matrix metalloproteinases. This exposed and stabilized collagen act as a scaffold for the mineral deposition and crystal formation (34). Silver phosphate and silver chloride are formed as byproducts of the reaction between SDF with hydroxyapatite in dentin. They have low solubility and precipitate on the dentin surface providing a protective layer and prevent the loss ions, this action may contribute...
to the increased remineralization potential and caries arresting property of SDF (9).

The current study shows consistency with data obtained from ex vivo studies that demonstrated favorable remineralization of dentin caries when treated with SDF where increased microhardness of arrested dentin was found (24, 35), Furthermore a dense layer of a high remineralized zone in the outer region with density higher than sound dentin associated with increase in calcium and phosphate was observed in the arrested dentin carious region (24). Moreover, Trieu et al. compared the capability of SDF and NaF in arresting dentin caries. They found that SDF was statistically more effective than NaF showing almost double effectiveness in caries arrestment and management of primary teeth (36) The reason behind the efficiency of SDF to arrest caries may be due to the synergistic effect between the silver and fluoride ions to enhance the mineral formation with the consequent increase in microhardness, and the alkaline property of the SDF alters the acidic microenvironment around the lesion. (13). However, neutral sodium fluoride varnish depends mainly on a single acidic microenvironment around the lesion. (13). However, neutral sodium fluoride varnish depends mainly on a single mechanism which is the mineral content restoration, rendering the hard tissue more resistant to future pH drops (37).

The increase in mineral content of the test groups was associated with lesion depth reduction, where in both treated groups lesion depth was significantly reduced in comparison to their control counterparts. This provides information regarding the microstructural changes that occurred during remineralization. Additionally, it is a reflection on the remineralization capacity. The two study groups showed remineralized dentin with strong negative birefringes and regularly arranged dentinal tubules, in comparison to their control group, that showed broad bands of demineralization extending into the thickness of dentin with strong positive birefringes. This goes in line with previous studies that assessed the lesion depth using Micro-CT (23,24,33).

By comparing the two study groups, the reduction of lesion depth in SDF group was more significant than NaF group. This goes in line with previous studies (22,32) Gao et al., (38) conducted a systemic review on the effect of professionally applied fluoride treatment on caries remineralization and arrest, they concluded that SDF was more efficient in arresting dentine caries, this is confirmed by the increase in mineral content and reduction of lesion depth found in our study. They also found that sodium fluoride varnish was more effective in the remineralization of early enamel caries.

One of this study’s limitation is the difficulty in duplicating the natural oral environment and its complexity. Our demineralization and remineralization phases provided a controlled environment that in turn focused on the assessment of the tested agents without the influence of salivary flow, pH fluctuation and the action of cariogenic bacteria that may affect the outcome of the rested agents. Within the limitation of the present study, it was shown that SDF has better remineralizing capacity on artificially demineralized dentin than NaF with increased reduction of lesion depth.

**CONCLUSION**

Based on the results of the current study it can be concluded that both 38% SDF and 5% NaF varnish can be used for remineralization of dentin caries-like lesions. However, SDF showed significantly more remineralization potential with higher mineral deposition and greater lesion depth reduction when compared to NaF varnish.

**CONFLICT OF INTEREST**

No conflicts of interest were declared by the authors.

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**REFERENCES**


Remineralizing agents on demineralized dentin

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