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Longitudinal *In Vitro* Effects of Silver Diamine Fluoride on Early Enamel Caries Lesions

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Clinical Relevance

Fluoride varnish is likely a better choice than silver diamine fluoride (SDF) for treatment of early, incipient, noncavitated, white-spot enamel lesions.

SUMMARY

This laboratory study evaluated the longitudinal surface microhardness changes in early, incipient, noncavitated, white-spot, enamel caries lesions treated with silver diamine fluoride (SDF). Five intervention groups (SDF, $AgNO_3$, KF, 5% sodium fluoride varnish (FV), deionized water (DI)) × two-time intervals after intervention (immediate & delayed pH-cycling) resulted in 10 groups (n=18). Silver nitrate ($AgNO_3$) and potassium fluoride (KF) groups served as controls to assist in evaluating if remineralization effects were due to the silver or fluoride component in SDF. Early, incipient, noncavitated, white-spot, enamel caries lesions were created in bovine enamel, the extent of demineralization was determined using Vickers surface microhardness (VHN_{lesion}). Intervention treatments were applied. Half the specimens from each group underwent immediate 5-day pH-cycling, and half were stored in an incubator with artificial saliva for 2 weeks before undergoing 5-day pH-cycling. After pH-cycling, lesion hardness was evaluated using VHN_{post}. Specimens were then exposed to a second demineralization challenge, and lesion softening was evaluated (VHN_{secdem}). Hardness variables were calculated: $\Delta VHN = VHN_{post} - VHN_{lesion}$; $\Delta VHN_{secdem} = VHN_{secdem} - VHN_{post}$. Data were analyzed using two-way ANOVA (α=0.05). Immediately cycled, SDF had significantly (p<0.0001) greater remineralization than DI, $AgNO_3$, and FV. All delayed cycling groups had significantly greater remineralization than FV (p<0.0001). Significantly greater remineralization was noted in delayed $AgNO_3$

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($p \leq 0.0001$), DI ($p=0.0003$), and FV ($p=0.0006$) compared to immediately cycled. After the second demineralization challenge, FV had significantly less surface softening than $AgNO_3$ ($p=0.0002$), DI ($p=0.0003$), KF ($p=0.0225$), and SDF ($p=0.0388$) intervention groups. No significant difference was found between the pH-cycle timings ($p=0.2710$). Based the present findings, FV may be better suited than SDF to treat early, incipient, noncavitated, white-spot, enamel caries lesions.

INTRODUCTION

Silver diamine fluoride (SDF) has been used for over 80 years in Japan, Argentina, Australia, Brazil, and China,[1,2] as a topical medicine at concentrations of up to 38% for the treatment of tooth hypersensitivity and to arrest caries lesions.[2] In 2014, the use of 38% SDF in the United States was approved by the Food and Drug Administration (FDA) for treating adults with dentin hypersensitivity.[2,3,4,5] In 2018, the American Dental Association (ADA) approved use of SDF as an interim caries-arresting medicament.[2] A recent review indicated that 38% SDF could be used to treat asymptomatic cavitated coronal caries as well as cavitated and noncavitated root caries.[3] While both SDF and fluoride varnish (FV) have been approved by the FDA for treatment of tooth sensitivity, only FV is currently used off-label for primary prevention of dental caries lesions.[2] The 2018 clinical practice guidelines published by the ADA recommended biannual applications of 38% SDF as the treatment of choice to arrest cavitated lesions on coronal surfaces of both primary and permanent teeth.[3] However, the ADA publication also stated that there currently is not enough research to recommend using SDF on noncavitated proximal lesions.[3]

In vitro studies have shown that SDF can penetrate up to 25 microns into enamel and up to 200 microns into dentin, which leads to two to three times more fluoride retention compared to applications of sodium fluoride (NaF) or stannous fluoride (SnF_2).[2,6,7] This greater retention of fluoride suggests SDF will have a greater efficacy in preventing and arresting caries lesions than NaF or SnF_2.[7] It was also reported in a systematic review and meta-analysis that SDF was 89% more effective than other fluoride treatments in arresting and controlling caries.[4] Similarly, *in vitro* studies found that upon a second demineralization challenge, demineralized dentin treated with SDF was better able to resist further demineralization compared to nondemineralized dentin.[8] This increased resistance to further demineralization was attributed to both the high fluoride levels (44,800 ppm) as well as the high levels of silver that were deposited on the demineralized surfaces after application of SDF.[4,8]

A review article suggested that SDF was more effective than FV and could be a possible caries-prevention intervention, but further research was needed.[7] Additionally, the University of California San Francisco (UCSF) published protocol for caries arrest using SDF noted that annual application of SDF produced better prevention of caries lesions in both children and elderly compared to applying FV four times a year.[2] In one of the few clinical studies that examined SDF efficacy in preventing caries in the permanent dentition, it was found that SDF was 65% effective in preventing caries in permanent first molars.[9] Application of SDF only takes one minute[2] and has shown to reduce the progression of caries by 89%.[4] However, research is lacking on the effects of SDF on early enamel caries lesions and its longitudinal effects.[10,11] Likewise, the acid resistance of remineralized enamel lesions that have been previously treated with SDF has not yet been investigated. Therefore, the purpose of our research was to investigate the longitudinal surface microhardness changes in early, incipient, noncavitated white-spot, enamel caries lesions treated with SDF. This *in vitro* study aimed to test the hypotheses that: 1) SDF treatment will result in increased surface microhardness of early, incipient, noncavitated, white-spot lesions in enamel compared to all other tested interventions; and 2) specimen storage for 2-weeks in artificial saliva will result in greater surface rehardening in lesions treated with SDF compared to all other interventions.

METHODS AND MATERIALS

Specimen Preparation

Enamel specimens, 4 × 4 mm, were obtained from bovine teeth using a low-speed saw (IsoMet, Buehler, Lake Bluff, IL, USA). During specimen preparation, all specimens were stored in deionized water (DI) containing 0.1% thymol. A polishing unit (Struers Rotopol 31/Rotoforce 4, Struers Inc, Cleveland, OH, USA) was used to grind and polish specimens to create flat, smooth, and uniform enamel and dentin surfaces for microhardness testing. The enamel surface of the specimens was ground smooth with 1200-, 2400-, and then 4000-grit silicon carbide paper, followed by a 1-μm diamond polishing suspension on a polishing cloth. After polishing, specimens were rinsed and sonicated in DI for 3 minutes as a final cleaning step. Under 20× magnification, specimens were inspected for cracks, hypomineralization (white spots) areas, and any other flaws present in the enamel surface, which would result in their exclusion from use in this study. All surfaces of

specimens, except the enamel surface to receive testing, were coated with acid-resistant, colored nail varnish (Sally Hansen Advanced Hard As Nails Nail Polish, Red, New York, NY, USA). Prepared specimens were stored at 100% relative humidity at 4°C until further use. A total of 219 specimens were prepared.

Sound Enamel Surface Microhardness

Using a Vickers diamond indenter with a 200-g load for 10 seconds, four baseline indentations (2100 HT; Wilson Instruments, Norwood, MA, USA) were placed 150 μm to the right of the center of each sound enamel specimen (approximately 150 μm apart from each other). The average sound Vickers hardness values (VHN_{sound}) were recorded for each specimen. Specimens with a VHN_{sound} between 300 and 400 were included in the study.

Artificial Caries Lesion Creation

Artificial caries lesions were created in the specimens by a 36-hour immersion in a solution of 0.1 M lactic acid, 0.2% Carbopol 907, 3.0 mM $CaCl_2 \times 2H_2O$, 6.0 mM KH_2PO_4, 63.0 mM KCl, and 3.1 mM NaN_3, with the pH adjusted to 5.0 using KOH. Upon removal from the chemical lesion creation solution, specimens were rinsed with DI.

Postlesion Creation Surface Microhardness

Postlesion creation indentations (VHN_{lesion}) were placed approximately 150 μm to the right of the VHN_{sound} indentations, as described when obtaining sound microhardness values. Specimens were included if their mean VHN_{lesion} value fell within the range of the mean VHN_{lesion} value of all specimens +/− 2 standard deviations of VHN_{lesion} of all specimens and had a standard deviation less than 12.5. The percentage surface microhardness change ($\%SMHC_{lesion}$) was calculated as follows: $\%SMHC_{lesion} = (VHN_{lesion} - VHN_{sound})/VHN_{sound} \times 100\%$. The 180 accepted specimens were stratified into 10 groups (two pH cycle timings × five intervention groups; n=18 per group) to ensure no statistically significant differences in mean VHN_{lesion} between the groups.

Application of Interventions

The five intervention groups with their active ingredients are displayed in Table 1. FV was applied according to the manufacturer's instructions. Specimens were air dried for 1 minute after application and then rinsed with DI. The applied FV was not artificially removed from the specimens. For all other interventions, the UCSF SDF application protocol was followed.[2,12] A microbrush was used to apply the intervention for 10 seconds to a dried enamel surface. After application, specimens were air dried for 1 minute and then rinsed with DI.[2]

pH Cycling Phase

The chosen pH cycling model was based on a model that is described elsewhere.[13,14] Immediately following application of the interventions, half of the specimens for each group (n=18) underwent immediate pH cycling for 5 days. The remaining specimens were stored for 2 weeks to be subjected later to the same pH cycling procedure.

The daily pH cycling schedule (Table 2) included two 1-minute fluoride exposures separated by four alternating cycles of 30-minute remineralization in artificial saliva [1.5 mM calcium dichloride ($CaCl_2$) × 2 H_2O (water); 0.9 mM KH_2PO_4 (potassium phosphate); 130.0 mM KCl

Table 1: *Intervention Group Products*

Intervention	Study Purpose	Manufacturer	Fluoride Source and Concentration	Silver Concentration	Noteworthy Ingredients
Silver Diamine Fluoride (SDF)		Elevate Oral Care	38% SDF; 44,800 ppm	253,900 ppm	—
Prevident 5% NaF Varnish (FV)	Clinical Reference Standard	Colgate	5% NaF; 22,600 ppm	—	Xylitol
Potassium Fluoride (KF)	Fluoride Control	Sigma-Aldrich	44,800 ppm	—	—
Silver Nitrate ($AgNO_3$)	Silver Control	Sigma-Aldrich	—	253,900 ppm	—
Deionized Water (DI)	Negative Control	—	—	—	—

Table 2: *Daily pH Cycling Regimen*

Duration	Specimen Treatment
1 minute	Fluoride Toothpaste Exposure
30 minute	Remineralization
60 minute	Demineralization
30 minute	Remineralization
60 minute	Demineralization
30 minute	Remineralization
60 minute	Demineralization
30 minute	Remineralization
60 minute	Demineralization
1 minute	Fluoride Toothpaste Exposure
(Overnight)	Remineralization

(potassium chloride); 20.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); 3.1 mM NaN_3 (sodium azide), adjusted to pH 7.0 with KOH (potassium hydroxide)],[12] followed by 60-minute demineralization in the lesion creation solution. The specimens were stored in artificial saliva overnight.

For the fluoride exposures, a fluoride toothpaste slurry was prepared by mixing toothpaste (Crest Cavity Protection, 0.243% sodium fluoride; Proctor and Gamble, Mason, OH, USA) with artificial saliva in a 1:2 ratio in a beaker with a magnetic stirrer.[15] Fresh slurry was prepared immediately prior to each 1-minute fluoride exposure.

The specimens that underwent the 2-week delayed pH cycling were stored in an incubator at 37°C in artificial saliva at approximately 100% relative humidity. During the 2 weeks of storage, specimens were taken out of the incubator daily, rinsed with DI, blotted dry, and a new 50-μL drop of artificial saliva was pipetted on the top of specimens. After 2 weeks of storage, the delayed specimens followed the 5-day pH cycling described above.

Post-pH Cycling Surface Microhardness

Post-pH cycling indentations (VHN_{post}) were placed approximately 150 μm to the left of the VHN_{sound} indentations, as described when obtaining sound microhardness values. The extent of surface rehardening was calculated as follows: $\Delta VHN = VHN_{post} - VHN_{lesion}$. The $\%SMHC_{post}$ was calculated as follows: $\%SMHC_{post} = (VHN_{post} - VHN_{lesion})/VHN_{lesion} \times 100\%$.

Secondary Demineralization

After post-pH cycling microhardness evaluations, all specimens were immersed for 24 hours in the chemical lesion creation solution, as described above. Subsequently, specimens were rinsed with DI.

Postsecondary Demineralization Surface Microhardness

Postsecondary demineralization indentations (VHN_{secdem}) were placed approximately 150 μm to the left of the VHN_{post} indentations, as described when obtaining sound microhardness values. The extent of surface softening was calculated as follows: $\Delta VHN_{secdem} = VHN_{secdem} - VHN_{post}$. The %SMHC was calculated as follows: $\%SMHC_{secdem} = (VHN_{secdem} - VHN_{post})/VHN_{post} \times 100\%$. Positive ΔVHN and %SMHC values indicated lesion rehardening, while negative values indicated further demineralization.

Surface Images

Surface images of all the specimens were acquired after completion of the final hardness measurements. For this, one representative specimen from each of the 10 groups was placed under an optical microscope (D3100, Nikon, Tokyo, Japan) equipped with a digital camera (Infinity 1, Lumenera, Ottawa, ON, Canada). One image of each specimen was acquired under standardized conditions and saved.

Radiographs

Four specimens from each of the 10 groups, which had representative ΔVHN_{secdem} data for their group were selected for radiographic analysis. The 40 specimens were mounted on plastic rods and sectioned with a hard tissue microtome (Silverstone-Taylor Hard Tissue Microtome, Series 1000 Deluxe; Silverstone-Taylor, SciFab, Lafayette, CO, USA). One approximately 100-μm section was obtained from each specimen. The sections were X-rayed at 45 kV and 45 mA at a fixed distance for 12 seconds.

Statistical Analysis

Outcomes of the primary variables, ΔVHN and ΔVHN_{secdem}, were analyzed using two-way ANOVA, with factors for interventions ($AgNO_3$, DI, FV, KF, and SDF) and pH cycling modes (two-week delay and immediately), as well as interactions between the factors to identify the significant effects of intervention and pH cycling. All pair-wise comparisons from ANOVA analysis were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5%. Summary statistics were calculated for the exploratory objectives $\%SMHC_{lesion}$, $\%SMHC_{post}$, and $\%SMHC_{secdem}$. Analysis was performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Sample Size Calculations

With a sample size of 18 specimens per treatment-storage combination, the study had 80% power to detect a ΔVHN post-pH cycling difference of 14.6 between any two groups, assuming two-sided tests, each conducted at a 5% significance level and ΔVHN standard deviation 15.

RESULTS

VHN_{sound} (mean ± standard deviation) varied between groups from 363 ± 18 to 378 ± 13 (Table 3). VHN_{lesion} was virtually identical between the groups (all 81±8). Lesion creation resulted in an approximate 78% reduction in mean hardness in all the groups ($SMHC_{lesion}$). The interaction between interventions and pH cycling modes was significant for ΔVHN ($p<0.0001$) but not for ΔVHN_{secdem} ($p=0.8636$). The ΔVHN_{secdem} data were affected by the type of intervention ($p=0.0012$) only and not by pH cycling modes ($p=0.2710$). The ΔVHN data are shown in Figure 1, whereas the ΔVHN_{secdem} data can be found in Figure 2. All other hardness data can be found in Table 3.

Rehardening

Rehardening (ΔVHN; mean ± standard deviation; Figure 1) values for specimens that were immediately pH-cycled were significantly ($p<0.0001$) higher in the SDF (62±14) and KF (64±18) intervention groups than all other intervention groups, which ranged between 9±10 (FV) and 43±18 ($AgNO_3$). There was no statistically significant difference between KF and SDF groups ($p=0.6947$) and between $AgNO_3$ and DI (40±17) treated specimens ($p=0.5132$), respectively. However, both $AgNO_3$ and DI intervention groups had significantly ($p<0.0001$) greater rehardening than the FV intervention specimens.

In the 2-week delayed pH-cycled specimens, SDF (60±14), KF (62±14), $AgNO_3$ (69±15), and DI (57±13) groups had significantly ($p<0.0001$) greater rehardening values than the FV (26±11) group (Figure 1). While the $AgNO_3$, DI, and FV intervention groups all experienced significant increases in rehardening values after the 2-week delay, only the $AgNO_3$ intervention group had statistically ($p=0.0127$) greater rehardening values than the DI intervention group. However, no statistically significant difference was found between the rehardening values of $AgNO_3$ and KF ($p=0.1255$), $AgNO_3$ and SDF ($p=0.0556$), DI and KF ($p=0.3285$), DI and SDF ($p=0.5542$), or KF and SDF ($p=0.6991$) intervention groups.

When comparing rehardening (ΔVHN) values of each intervention group to the different pH-cycles, a substantial and significant increase in rehardening was noted amongst the $AgNO_3$ ($p<0.0001$), DI ($p=0.0003$), and FV ($p=0.0006$) intervention groups after the 2-week delay compared to their respective immediately cycled rehardening values (Figure 1). The opposite effect, though not statistically significant, was seen in both the KF ($p=0.6301$) and SDF ($p=0.6343$) intervention groups, with greater rehardening values recorded in immediately pH-cycled specimens compared to the 2-week delayed pH-cycled specimens (Figure 1).

The $\%SMHC_{post}$ data (Table 3) mirrored the ΔVHN data (Figure 1) in that it showed the same rank order of rehardening for all the interventions and both the models.

Table 3: *Vickers Hardness Data and Calculated Variables for All Interventions (Mean [SD])*

Intervention	Mode	VHN_{sound}	VHN_{lesion}	$\%SMHC_{lesion}$	VHN_{post}	$\%SMHC_{post}$	VHN_{secdem}[a]	$\%SMHC_{secdem}$[a]
SDF	Immediate	374 (14)	81 (8)	−78 (2)	143 (17)	77 (18)	134 (15)	−6 (10)
FV		364 (15)	81 (8)	−78 (2)	90 (15)	11 (12)	91 (24)	3 (35)
KF		371 (26)	81 (8)	−78 (2)	145 (23)	79 (19)	133 (20)	−8 (9)
$AgNO_3$		363 (18)	81 (8)	−78 (3)	124 (16)	54 (14)	111 (12)	−11 (6)
DI		370 (17)	81 (8)	−78 (2)	121 (20)	50 (21)	106 (13)	−12 (7)
SDF	Delayed	364 (14)	81 (8)	−78 (2)	141 (16)	75 (18)	130 (17)	−7 (14)
FV		372 (14)	81 (8)	−78 (2)	107 (14)	32 (14)	102 (28)	−4 (23)
KF		378 (13)	81 (8)	−79 (2)	143 (18)	77 (17)	132 (20)	−7 (8)
$AgNO_3$		366 (18)	81 (8)	−78 (3)	150 (16)	87 (23)	130 (18)	−13 (8)
DI		369 (15)	81 (8)	−78 (2)	138 (17)	71 (16)	122 (19)	−12 (9)

Abbreviations: VHN, Vickers hardness number of sound enamel (VHN_{sound}), after lesion creation (VHN_{lesion}) or after completion of the pH cycling phase (VHN_{post}); %SMHC, percent surface microhardness change after lesion creation ($\%SMHC_{lesion}$), after completion of the pH cycling phase ($\%SMHC_{post}$) or after secondary demineralization ($\%SMHC_{secdem}$); SDF, silver diamine fluoride; FV, fluoride varnish; KF, potassium fluoride; $AgNO_3$, silver nitrate; DI, deionized water.

[a]*Individual group means are shown for information only; see results and Figure 2 for intervention means for $\%SMHC_{secdem}$.*

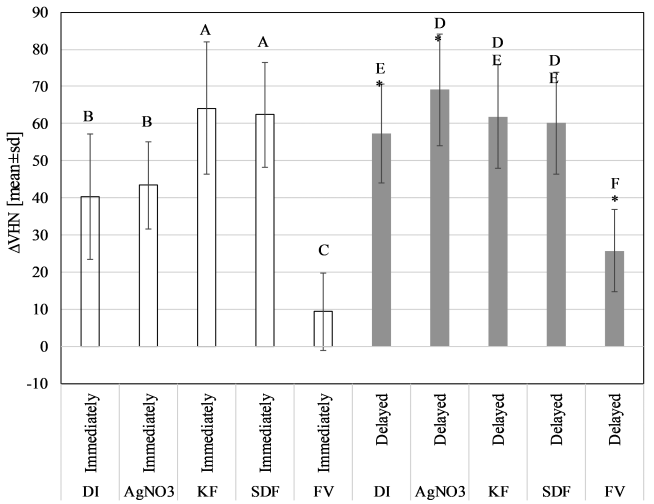

Figure 1. *ΔVHN Data Summary Chart. Mean change in surface microhardness (ΔVHN = VHN_{post} - VHN_{lesion}). Significant differences between intervention groups of the same pH cycle timing are represented by different letters. Asterisks indicate a significant difference compared to immediately cycled counterparts. Abbreviations: ΔVHN, mean change in Vickers hardness number; VHN_{post}, Vickers hardness number after completion of the pH cycling phase; VHN_{lesion}, Vickers hardness number after lesion creation; DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

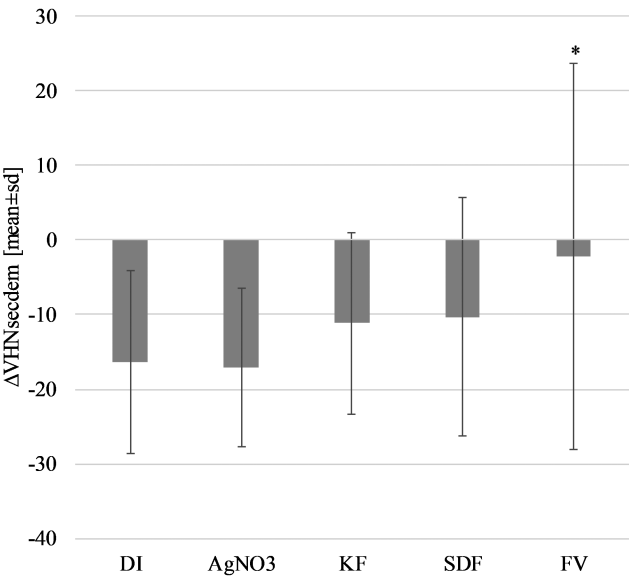

Figure 2. *ΔVHNsecdem Data Summary Chart. Mean change in surface microhardness (ΔVHNsecdem = VHNsecdem - VHNpost). Asterisk indicates FV intervention significantly different than all other interventions. Abbreviations: ΔVHN_{secdem}, mean change in Vickers hardness number after secondary demineralization; VHN_{post}, Vickers hardness number after completion of the pH cycling phase; DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

Secondary Demineralization

Specimens in the FV intervention group (ΔVHN_{secdem}; mean ± standard deviation: −2±26) had significantly less surface softening than specimens in the $AgNO_3$ (−17±11; p=0.0002), DI (−16±12; p=0.0003), KF (−11±12; p=0.0225), and SDF (−10±16; p=0.0388) intervention groups (Figure 2). However, no statistically significant difference was found between the surface softening values of DI and $AgNO_3$ (p=0.8562), DI and KF (p=0.1785), DI and SDF (p=0.1178), $AgNO_3$ and KF (p=0.1273), $AgNO_3$ and SDF (p=0.0813), or KF and SDF (p=0.8254) intervention groups.

The $\%SMHC_{secdem}$ data mirrored the ΔVHN_{secdem} data (Figure 2) in that it showed the same rank order of softening for all the interventions and both the models (FV: −1±29; $AgNO_3$: −12±7; DI: −12±8; KF: −7±8; and SDF: −7±12).

Surface Images

Figure 3 shows surface images of a representative specimen from each intervention group and model. Specimens in the FV, KF, and DI groups displayed a more or less natural tooth color, irrespective of the model. However, dark staining can be seen in both SDF and $AgNO_3$ groups. Comparing between models, it appears that specimens in the SDF groups were less dark in the delayed than in the immediate model, whereas the opposite was the case in the $AgNO_3$ groups.

Radiographs

Figure 4 shows a cross-sectional radiographic image of a representative specimens from each group that was obtained after secondary demineralization. An early subsurface caries lesion of slightly varying severity can be observed in all the specimens.

DISCUSSION

Caries management has shifted from surgical treatment to a more preventive approach, focusing on detecting and arresting caries lesions at early stages.[16,17] This early detection and prevention trend has led to further research into the different applications of fluoride, such as SDF, and resistance to subsequent acid attacks after treatment with these atraumatic, noninvasive, and prevention modalities. However, research lacks on the effects of SDF on early enamel caries lesions and the acid resistance of remineralized enamel lesions previously treated with SDF.[10,11]

The purpose of immediate pH cycling was an attempt to mimic the effects of a patient with poor oral hygiene that undergoes a second demineralization immediately

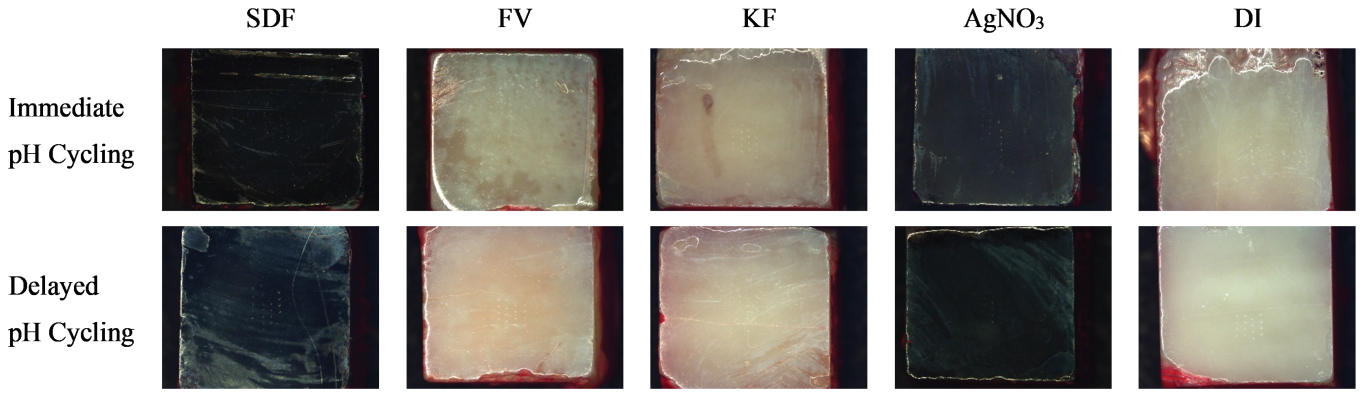

Figure 3. *Surface images of one representative specimen from each intervention group and model obtained after secondary demineralization. Abbreviations: DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

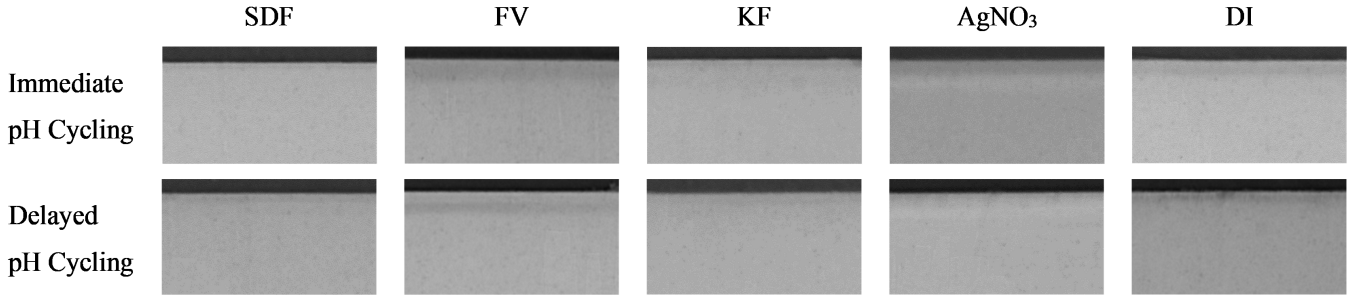

Figure 4. *Radiographic images (500 µm width) of a cross-section of one representative specimen from each intervention group and model obtained after secondary demineralization. Abbreviations: DI, deionized water; $AgNO_3$, silver nitrate; KF, potassium fluoride; SDF, silver diamine fluoride; FV, fluoride varnish.*

after application of SDF. The purpose of the 2-week delayed storage in artificial saliva before being pH cycled for 5 days was to mimic a patient with good oral hygiene that reverts to poor oral hygiene habits and is then exposed to a second demineralization 2 weeks after application of SDF.

Vickers SMH was chosen over the "gold standard" TMR to analyze the changes in surface hardness, as Vickers is better equipped to analyze shallow lesions.[12, 18-20] Utilization of V-SMH also allowed us to analyze changes in specimen hardness throughout different stages of our study, as TMR is inherently destructive.1[2, 21] We did, however, include one radiographic image of a representative specimen from each group (Figure 4) for better visualization. By analyzing surface rehardening and softening with V-SMH, it provided a more clinically similar evaluation of our lesions, as practitioners often diagnose caries lesion arrest by the hardness of the lesion surface along with its appearance.[22] Furthermore, percentage changes in surface hardness were calculated at all stages of the experiment (Table 3) to allow for better interpretation of the present findings and their possible extrapolation to the clinical situation.

Based on our results, our first hypothesis that SDF treatment would result in increased surface microhardness of early, incipient, noncavitated, white-spot lesions in enamel compared to all other tested interventions, can be partially accepted, as SDF and KF treatments resulted in similar extents of surface microhardness recovery. Both SDF and KF intervention groups had the same amount of fluoride (44,800 ppm fluoride), which can explain these results and also highlight that silver ions in SDF do not appear to interfere in the remineralization process. The second hypothesis that specimen storage for 2 weeks in artificial saliva would result in greater surface rehardening in lesions treated with SDF compared to all other interventions, however, was rejected. This is speculated to be due to the low viscosity of SDF, which appeared to provide a more immediate impact on surface rehardening compared to the more viscous FV that remained on the enamel surface longer and resulted in greater remineralization of the delayed FV group compared to its immediate counterpart, unlike what the SDF group experienced.

Results for $\Delta VHN_{postlesion}$ data representing the extent of surface rehardening were mostly as anticipated.

Both the SDF and KF intervention groups exhibited the greatest fluoride concentration, which also resulted in the greatest amount of surface rehardening. Similar findings were reported in other studies.[6,23,24] While the $AgNO_3$ group did not contain fluoride, the low solubility of silver ions has been found to play a role in increasing caries lesion hardness.[25] We, therefore, anticipated the $AgNO_3$ group to exhibit some rehardening, but the effect of the silver ions may have been diluted by the twice daily fluoride exposures, thus limiting the full effects of the silver ions.

The post-pH cycling data (Figure 1) was surprising in that all the intervention groups, including DI, had statistically significant greater surface rehardening than FV. This could be due to the fact that the FV remained on the specimens prior to undergoing pH cycling, which is something that has not been done before. The purpose in removing FV prior to further testing is to represent the FV film being mechanically removed in the oral cavity as would be experienced with toothbrushing and mastication.[6,15,25,26,28] However, the present study aimed to mimic FV being retained in hard-to-reach areas, such as interproximal or occlusal areas, as well as buccal surfaces of partially erupted third molars. The FV film may have provided protection as a mechanical barrier limiting the full effects of the pH cycling regimen, thus affecting the FV hardness data. It is also important to note that the FV film appeared to have naturally worn off the specimens during the pH cycling phase, which enabled V-SMH testing. While the FV treated specimens did not experience remineralization from fluoride, calcium and phosphate ions diffusing through the varnish coating, this could occur in some FV as different varnishes have different permeability characteristics. This may be an area worth exploring in future studies.

The high variability we encountered in both the FV groups after secondary demineralization is likely due to the continuous wearing off of the FV throughout the study. While it did not affect hardness measurements directly, it may have done indirectly, as a thin film of resin may have remained in certain areas on the specimens' surface, thus protecting some areas from an acid attack where still present and offering no protection for other specimens where it already wore off. This scenario also likely occurs *in vivo*, and future research may consider studying differences in wear resistance between different FVs.

When evaluating the ΔVHN data between the different pH-cycle timings, it is apparent that the $AgNO_3$, DI, and FV intervention groups had significantly greater surface rehardening after the 2-week delay compared to immediately cycled specimens (Figure 1). The results for the $AgNO_3$ group could be explained by the possibility that silver ions require more time to interact with enamel and enhance remineralization, as speculated in a similar study.[6] Interestingly, both immediately cycled KF and SDF groups experienced significantly greater surface rehardening than immediately cycled $AgNO_3$; but after the 2-week delay, the $AgNO_3$ group had the numerically greatest extent of surface rehardening of all the groups. It can be speculated that fluoride in KF and SDF provides a more immediate impact on surface rehardening, while silver ions in $AgNO_3$ assist with sustaining surface hardness over time. We were also intrigued to find that the 2-week delayed SDF did not exhibit greater surface rehardening than each of its individual components—silver and fluoride, which were represented by the $AgNO_3$ and KF intervention groups.

Additionally, the low viscosity of the SDF, $AgNO_3$, and KF interventions may have provided an advantage over the FV group. It has been noted in other studies that the viscosity of products may play a role in fluoride absorption to enamel surfaces.[27] The lower the viscosity, the greater the amount of surface rehardening may be experienced, such as what we saw with our SDF, $AgNO_3$, and KF groups, due to the liquid being able to fully contact the enamel surface and perhaps also penetrate deeper into the caries lesion, and thus resulting in better fluoride ion absorption.[28] On the contrary, the more viscous a product is, such as our FV treatment, the more it may result in a slower diffusion of fluoride ions into the enamel surface as well as a prolonged contact time.[28]

The secondary demineralization data, ΔVHN_{secdem} data (Figure 2), revealed no significant differences between the different pH-cycle timings. However, all intervention groups exhibited significantly more surface softening compared to the FV intervention group. This was surprising, especially since SDF contained almost twice the amount of fluoride as FV. However, these findings are in agreement with previous studies, which found SDF provided a short-term increase in surface microhardness of demineralized enamel but was not as effective as FV in reducing enamel surface demineralization.[27,28] It was also found that while fluoride in SDF reduced surface softening in enamel, it was not able to prevent subsurface mineral loss to the same extent as FV.[27] This difference between SDF and FV was speculated to be due to SDF forming and releasing a smaller amount of fluoride on the enamel surface than FV.[27] Another possible reason for this finding was that silver in SDF may have competed with fluoride depositing on the enamel surface, thus making SDF less effective in preventing further surface softening on enamel compared to FV.[27]

Interestingly, there was no significant difference in the extent of surface softening after secondary demineralization between DI, $AgNO_3$, KF, or SDF (Figure 2). This finding correlates to what other research concluded, that silver ions found in $AgNO_3$ and SDF produce minimal effects on the prevention of enamel demineralization, explaining why $AgNO_3$ and DI had no significant difference in amount of surface softening.[29] In another study, it was speculated that SDF was better at preventing demineralization in dentin than in enamel due to the silver in SDF expressing a greater affinity to bind to proteins found in dentin, which are absent from enamel.[27] Research has also found SDF to not be as effective as FV in preventing demineralization of enamel upon exposure to a demineralization challenge, which correlates with our ΔVHN_{secdem} findings.[27] However, we expected no significant differences to exist between $AgNO_3$, KF, or SDF, given the synergistic effects of the silver and fluoride components in SDF. Additionally, we expected KF and SDF to have significantly less surface softening than DI, due to the high concentration of fluoride, which has shown to inhibit enamel demineralization.[6,24,25,30]

While we did not quantify enamel discoloration as a result of an SDF treatment presently, it is apparent that a single application of SDF to an early enamel caries lesion will result in dark staining that also appears to be resistant to repeated acid challenges (Figure 3). A similar observation was made in the $AgNO_3$-treated specimens, however, not in the other groups, suggesting that the staining is due to silver rather than fluoride ions.

Several limitations need to be considered in the interpretation of the present findings. A chemically induced (artificial) enamel caries lesions was employed. While universally accepted in *in vitro* and *in situ* caries research, these lesions are only a surrogate for *in vivo* lesions. In the oral cavity, caries lesions form over considerably longer periods of time, including periods of remineralization, exposure to fluoride, and salivary proteins. This results in lesions that may respond in a different manner than those studied presently.

The implementation of a 5-day pH cycle rather than a longer 20-day cycle may be another limitation. While a shorter pH cycle is less likely to completely reharden lesions, it allows for easier analysis of the different interventions post-pH cycling outcomes and for a faster secondary demineralization to occur.[12] However, a shorter cycle may inadequately represent the natural de- and remineralization processes in the oral cavity, curtailing our results. Additionally, had we altered our daily pH cycle regimen to have less time in the remineralization solution, more closely mimicking a high caries risk patient whose oral cavity is in constant demineralization, our findings may have been much different with little surface rehardening experienced, making it difficult to observe both the re- and demineralization effects of our study. While our study did not evaluate the dark staining associated with SDF, it would be interesting to investigate whether there is a correlation between the color change in specimens and the extent of surface rehardening.

Despite the limitations encountered in our study, we found SDF to be an effective intervention to reharden the surface of early, incipient, noncavitated, white-spot lesions in enamel. However, SDF was not as effective as FV in preventing surface softening in these lesions after a secondary demineralization challenge. In an effort to better understand the effects of SDF on early enamel lesions, we believe it would be beneficial for future research to incorporate cariogenic biofilm models and a wider range of FV products.

CONCLUSION

Based on our findings, FV may be better suitable than SDF to treat early, incipient, noncavitated, white-spot, enamel caries lesions. Further research is needed on SDF, and its prevention of enamel surface softening upon exposure to demineralization challenges before SDF can be recommended over FV in the treatment of early, incipient, noncavitated, white-spot, enamel caries lesions.

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Conflict of Interest

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