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ORIGINAL RESEARCH

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Comparative evaluation of enamel microhardness between silver diamine fluoride and fluoride varnish on artificially induced caries in primary teeth: an *in vitro* study

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Abstract

Background: Despite being entirely preventable, dental caries remains highly prevalent in children, often beginning as early enamel demineralization (white spot lesions). Fluoride-based products are the mainstay for prevention and remineralization, with 5% Sodium Fluoride Varnish (FV) and 38% Silver Diamine Fluoride (SDF) being the most commonly used. However, evidence on SDF's remineralization potential in early primary tooth enamel caries is limited. **Methods**: This *in vitro* study evaluated the effectiveness of SDF and FV in remineralizing artificial enamel caries by measuring enamel microhardness (EMH). Sixty primary teeth were randomized into four groups (n = 15): sound enamel (healthy enamel), SDF, FV, and demineralized untreated control. Artificial caries was induced via an 8-day exposure to a pH 4.4 demineralizing solution. EMH was measured using a Vickers Microhardness tester. Data were analyzed via one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test ($p \leq 0.05$). **Results**: Results showed significant differences among groups ($p < 0.001$). FV produced the greatest increase in EMH, significantly outperforming SDF and control. SDF also improved EMH compared to untreated teeth, but was less effective than FV. **Conclusions**: Under these *in vitro* conditions, FV demonstrated superior remineralization capacity compared to SDF for early enamel lesions in primary teeth. However, these findings should be interpreted within the context of a chemically-induced lesion model without biofilm, and clinical decision-making should consider that FV and SDF have different indications and may serve complementary roles depending on lesion type, substrate, and clinical circumstances.

Keywords

Fluoride varnish; Silver diamine fluoride; Enamel microhardness; Remineralization; Caries prevention

1. Introduction

Despite the fact that dental caries is preventable, its prevalence is high, and it is the most common oral disease in children. This can be caused by various factors, including diet, water fluoridation, parental education, difficulty accessing dental care, and inadequate oral hygiene. Early stages of dental caries typically appear as a white spot lesion on the enamel surface, known as demineralization [1]. Dental caries at this stage are entirely reversible, and this process is known as remineralization. One of the most effective techniques for enhancing the remineralization of dental tissues is the regular application of fluoride products. In addition to its remineralization effect, fluoride can be used to prevent dental caries, as well as arrest caries activity and prevent its progression [2]. Recently, the treatment approach for remineralization and arresting dental caries has gained more acceptance due to its ease of application, minimal cost, and requiring a minimal level of cooperation from children [2, 3].

There are many fluoride-releasing products commercially available in the market. However, the most commonly used in dental practice are 5% Sodium Fluoride Varnish (FV) and 38% Silver diamine fluoride (38% SDF). The effectiveness of both agents is well-documented in the literature [4–6].

Furthermore, numerous published randomized clinical trials and systematic reviews have demonstrated that the topical application of FV and 38% SDF is significantly effective in preventing and arresting the demineralization of the enamel surface. In addition, topical application of FV is effective in preventing dental caries and is primarily recommended for

children with a high caries risk [7, 8]. The active ingredient of FV is sodium fluoride, and its concentration is usually 22,600 ppm. It was originally developed to prolong the contact time between fluoride and dental enamel, acting as a slow-releasing fluoride reservoir when the oral pH drops, as it adheres to the tooth structure for extended periods [9, 10]. FV is effective in preventing dental caries and remineralizing non-cavitated lesions in primary teeth [11]. Also, its use has been effective in controlling white spot lesions, dentin hypersensitivity, and root caries in permanent teeth [12, 13].

Recently, the use of SDF is gaining popularity among pediatric dentists [14]. Although it is commonly used off-label for caries prevention and arrest, Silver Diamine Fluoride (SDF) was initially approved by the United States Food and Drug Administration (FDA) in 2014 for the treatment of dentinal hypersensitivity [15]. However, due to its harmless nature, ease of use, and cost-effectiveness, the topical application of 38% SDF is considered safe and even more effective than fluoride varnish in arresting the progression of dental caries in primary teeth, particularly for cavitated dentin lesions [2, 16]. Additionally, there are well-established guidelines by the American Academy of Pediatric Dentistry (AAPD) to support the application of 38% SDF to arrest caries in primary teeth [17]. Although two standard concentrations are available, 12% and 38% SDF, multiple applications of 38% SDF are more effective in arresting caries than 12% [17, 18].

Despite the increased use of 38% SDF as a caries-arresting medicament, there is a lack of studies evaluating its remineralization effect in the early stages of enamel caries in primary teeth [19]. Although high-concentration fluoride agents have been widely investigated, most evidence regarding SDF emphasizes caries arrest in dentin and clinical settings involving cavitated lesions [14, 17, 19, 20]. By contrast, comparative data focusing specifically on early enamel lesions in primary teeth under standardized *in vitro* conditions remain limited. In this context, a controlled pH-cycling model combined with Vickers microhardness allows a direct, standardized comparison of the enamel remineralization potential of SDF and FV in primary teeth. Therefore, the present study aims to address this focused gap by evaluating these agents under a uniform artificial-lesion model.

Accordingly, the purpose of this study is to evaluate the effectiveness of Silver Diamine Fluoride (Advantage Arrest®, USA) and fluoride varnish (Clinpro™ White Varnish, 3M ESPE, USA) in remineralizing artificial enamel caries on primary teeth by assessing the level of Enamel Microhardness (EMH). The null hypothesis is that there would be no difference in EMH among treatment groups.

2. Materials and methods

2.1 Research design

An *in vitro* study was conducted in the Physical Laboratory of the College of Dentistry, King Saud University (KSU), Riyadh, Saudi Arabia. The study adhered to the approved research protocol and ethical guidelines of the King Saud University Institutional Review Board (IRB) under reference number E-24-8755.

2.2 Sample size

Sample size was estimated based on an effect size (f) of 0.60 derived from Mashhour *et al*. [21]. Sample size was estimated based on the results of a previous study [21] with $\alpha = 0.05$ and power $(1 - \beta) = 0.85$. The analysis indicated that a minimum of 60 sound primary molars (n = 15 per group) was required. An independent-group design was selected rather than a paired approach to avoid potential measurement carry-over effects between pre- and post-treatment surfaces on the same tooth.

2.3 Study size standardization and exclusion criteria

Previously extracted sound primary molars, due to orthodontic reasons, were obtained from the pediatric dental clinics of King Saud University, Riyadh, Saudi Arabia. The teeth collection process was conducted in full accordance with the rules and regulations of human subjects in the pediatric dentistry department of King Saud University, Riyadh, Saudi Arabia.

Teeth were collected in a de-identified manner and cannot be linked in any way to the patient from whom they came. All collected teeth were initially wiped with a 2 × 2 gauze pad to remove any residual soft tissue immediately after extraction. After extraction, teeth were cleaned of soft-tissue remnants, disinfected in 0.1% thymol solution for 24 h, stored in saline at 4 °C until use, and the container was kept in a secure area within the pediatric department. Teeth were examined by two independent, calibrated examiners, both visually and under a digital microscope (Hirox HRX-01, Hirox Co., Ltd., Tokyo, Japan), according to the International Caries Detection and Assessment System (ICDAS) [20, 22]. Exclusion criteria included any tooth with enamel demineralization, cracks, developmental anomalies, or visible or detectable caries.

Both examiners were calibrated prior to sample assessment, achieving excellent agreement. The roots of all teeth were sectioned below the cementoenamel junction to standardize crown orientation. Each buccal surface was ground flat using sequential 600-, 800-, and 1200-grit silicon carbide papers under water cooling to produce a uniform testing area for Vickers indentation. After surface flattening and polishing, specimens were thoroughly rinsed with deionized water and gently ultrasonicated for 3–5 min to remove residual debris and minimize smear-layer interference before the experimental procedures.

2.4 Randomization

A total of (N = 60) teeth were randomly divided into four groups of 15 teeth, each following the “sample” function of R 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Randomization was performed on sound primary molars prior to artificial caries induction (for Groups B–D) to minimize baseline structural variability across experimental groups, ensuring that any inherent differences in enamel composition, thickness, or mineral density were evenly distributed before the demineralization challenge. This approach ensured that each tooth had an equal chance of being allocated to one of the four groups, thereby eliminating selection bias. The groups were: Group A: Sound enamel, Group B: SDF (Advantage

Arrest®, USA), Group C: FV (Clinpro™ White Varnish, 3M ESPE, USA), and Group D: Demineralized untreated control (Table 1). The operator performing surface treatments and the examiner performing microhardness measurements were blinded to the group allocation. Allocation concealment was maintained using sealed, coded containers.

2.5 Mounting and enamel preparation

Teeth were mounted to self-curing acrylic resin material (Ortho-Jet™ for Orthodontic Appliances) with an enamel surface exposed for the study protocol. A 4×4 mm^2 adhesive tape was placed on the center of the buccal surface of every tooth. The entire tooth was then coated with an acid-resistant nail varnish. Then, the adhesive tape was removed, and the exposed areas were prepared.

2.6 Artificial caries preparation and pH cycling

Artificial caries were induced via an 8-day pH cycling protocol adapted from Delbem *et al*. [22]. Importantly, pH cycling was performed only before treatment application to create standardized artificial lesions; no additional cycling was performed after treatment. Each daily cycle consisted of immersion in 10 mL of demineralizing solution (2.2 mM Calcium chloride ($CaCl_2$), 2.2 mM Potassium dihydrogen phosphate (KH_2PO_4), 0.05 M acetic acid, pH 4.4) for 4 h, followed by immersion in 10 mL of remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 150 mM Potassium chloride (KCl), 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.0) for 20 h at 37 °C. The demineralizing solution pH was adjusted to 4.4 using dropwise addition of 1 M Potassium hydroxide (KOH) (or Sodium hydroxide (NaOH)), and both solution pHs were verified daily using a calibrated pH meter.

Specimens were thoroughly rinsed with deionized water between solution changes. This cycling protocol was repeated for seven consecutive days using fresh solutions for each cycle. On day 8, specimens underwent a final 24-h immersion in remineralizing solution. Following pH cycling, all specimens were visually examined under a digital microscope to confirm ICDAS score 2 (distinct visual change in enamel visible when both wet and dry), indicating successful artificial caries induction.

Specimens were stored in artificial saliva (Fusayama/Meyer formulation; Sigma-Aldrich, St. Louis, MO, USA) for 24 h at 37 °C, according to the manufacturer's specifications.

2.7 Groups and treatment protocol

For Groups A–D, the experimental sequence was: surface preparation; artificial caries induction by pH cycling for Groups B–D; topical agent application (when applicable); 24 h storage in artificial saliva; and microhardness measurement. Specimens were randomized to Groups A–D prior to lesion induction. After artificial caries induction, the allocation codes were revealed, and topical agents were applied according to the previously randomized group assignment as follows:

- Group A: (sound enamel): The control group consisted of 15 sound primary molars that were left untreated and stored in artificial saliva for 24 h. There was no artificial caries preparation in this group.
- Group B: SDF (Advantage Arrest®, Elevate Oral Care, USA): Silver diamine fluoride was applied with a micro-brush (Kerr, Orange, Calif., USA) to the exposed enamel for 2 min. Then, the teeth were washed with 10 mL of deionized water for 30 s, dried with compressed air, and finally stored in artificial saliva for 24 h. After that, the teeth were rinsed with deionized water for 1 min before microhardness testing.
- Group C: FV (Clinpro™ White Varnish, 3M ESPE, USA): FV was applied to the exposed enamel using a micro-brush and allowed to air dry for 5 min before being placed in artificial saliva for 24 h. Then, the fluoride varnish was carefully removed from all specimens using a cotton swab dipped in acetone to avoid harming the enamel surface. The teeth were rinsed with deionized water for 1 min before microhardness testing.
- Group D: Control group (demineralized untreated control): Teeth were left untreated after artificial caries preparation and stored in artificial saliva for 24 h. They were then rinsed with deionized water for 1 min before microhardness testing.

2.8 Vickers microhardness test (VMHT)

Enamel microhardness was assessed once per specimen as a final outcome, immediately after the 24 h post-treatment storage in artificial saliva. No baseline (pre-treatment) microhardness measurements were performed due to the independent-group design. Enamel surface microhardness was measured using a Vickers Microhardness tester (Highwood DMH 7, Model HWMMT-X7, TTS Unlimited, Osaka, Japan) under a 50 g load for 15 s. Five indentations per specimen were made, each spaced at least 100 μm apart and 200 μm from the enamel edge. The mean of the five readings was recorded as the Vickers Hardness Number (VHN). The instrument was calibrated prior to each testing session using a certified reference block.

TABLE 1. Study groups and allocation of specimens (N = 60).

Groups	Counts	Total sample size
Group A: Sound enamel, no artificial caries induced.	N = 15	N = 60
Group B: SDF (Advantage Arrest®, USA).	N = 15	
Group C: FV (Clinpro™ White Varnish, 3M ESPE, USA).	N = 15	
Group D: Control group (Demineralized untreated control), artificial caries induced, no treatment applied.	N = 15	

SDF, Silver Diamine Fluoride; FV, Fluoride Varnish.

2.9 Statistical analysis

Data were analyzed using one-way ANOVA to compare mean surface microhardness (SMH) among the four groups, followed by Tukey's *post-hoc* test for pairwise comparisons. Normality was confirmed with the Shapiro-Wilk test and homogeneity of variances with Levene's test. The effect size was calculated to determine the magnitude of differences. A significance level of $p \leq 0.05$ was used for all tests. Statistical analysis was performed using IBM SPSS Statistics (Version 29, IBM Corp., Armonk, NY, USA). Descriptive and *post-hoc* statistics are presented in separate tables (Tables 2 and 3, respectively) to facilitate clarity: Table 2 summarizes central tendency and variability for each group, while Table 3 details pairwise comparisons with confidence intervals.

3. Results

Descriptive analysis showed that 60 primary teeth were allocated to four groups (n = 15 each). Sound enamel (Group A) presented the highest mean microhardness (Mean (M) = 468.85, Standard Deviation (SD) = 1.36), whereas the demineralized untreated control (Group D) showed the lowest values (M = 192.17, SD = 2.63). Among treated specimens, fluoride varnish (Group C) yielded higher microhardness than silver diamine fluoride (Group B) (M = 377.65, SD = 2.75 *vs*. M = 333.13, SD = 2.22). The relatively small standard deviations indicate limited dispersion of microhardness values within each group (Table 2).

A one-way ANOVA showed a statistically significant difference in EMH among the four groups (F = 44,316.31, $p < 0.001$). Tukey's HSD (Honestly Significant Difference) *post-hoc* test revealed that all pairwise comparisons were statistically significant ($p < 0.001$). FV exhibited significantly higher microhardness than SDF (mean difference = 44.52, 95% Confidence Interval: 42.10–46.94), and both treated groups showed significantly higher values than the untreated control (Table 3).

4. Discussion

This study confirms that the type of preventive treatment significantly affects EMH, with the highest EMH in the caries-free group. This underscores the importance of maintaining healthy primary teeth and preventive measures for oral health. While FV and SDF both effectively promote remineralization [7, 8], the clinical literature suggests that SDF is particularly effective in arresting caries progression in cavitated dentin lesions in primary teeth [2, 16, 23].

Teeth treated with FV (Clinpro™ White Varnish, 3M ESPE, USA) showed significantly higher enamel hardness compared to those treated with SDF (Advantage Arrest®, Elevate Oral Care, USA), which is consistent with previous *in vitro* findings [23, 24]. Although the fluoride ions in SDF are more soluble than those found in FV, the remineralization and retention of fluoride following the application of FV may be higher than those in SDF, potentially resulting in greater remineralization potential and, consequently, higher EMH under these experimental conditions [23, 25]. Unlike SDF, FV has a prolonged retention time on tooth surfaces, hence maximizing fluoride exposure. Additionally, the absence of silver particles in FV may prevent interference with remineralization processes [26–30].

From a material–tooth interface perspective, it is plausible that silver-containing reaction products formed after SDF application could alter surface chemistry in a way that may be less conducive to fluoride diffusion into the hydroxyapatite lattice compared with the calcium fluoride-like reservoirs typically associated with FV. A silver phosphate-rich surface layer may

TABLE 2. Descriptive statistics of enamel microhardness values across study groups.

Group	Count	Max	Min	Mean (VHN)	SD
A—Sound enamel	15	471.33	465.83	468.85	1.36
B—SDF (Advantage Arrest®, USA)	15	336.58	328.89	333.13	2.22
C—FV (Clinpro™ White Varnish, 3M ESPE, USA)	15	381.87	371.58	377.65	2.75
D—Demineralized untreated control	15	197.01	188.51	192.17	2.63

SDF, Silver Diamine Fluoride; FV, Fluoride Varnish; SD, standard deviation; VHN, Vickers Hardness Number; Max, Maximum; Min, Minimum.

TABLE 3. Tukey's *post hoc* comparison of enamel microhardness between groups.

Group 1	Group 2	Mean Diff	*p*-adj	Lower CI	Upper CI
A—Sound enamel	B—SDF	−135.72	<0.001	−138.14	−133.29
A—Sound enamel	C—FV	−91.2	<0.001	−93.62	−88.77
A—Sound enamel	D—Demineralized untreated control	−276.68	<0.001	−279.11	−274.26
B—SDF	C—FV	44.52	<0.001	42.1	46.94
B—SDF	D—Demineralized untreated control	−140.96	<0.001	−143.39	−138.53
C—FV	D—Demineralized untreated control	−185.48	<0.001	−187.91	−183.06

SDF, Silver Diamine Fluoride; FV, Fluoride Varnish; Mean Diff, mean difference; p-adj, adjusted p-value (Tukey); CI, confidence interval.

favor antimicrobial effects but may not provide an optimal physicochemical environment for enamel remineralization in a sterile model. This type of interface-focused interpretation follows the broader principle that understanding chemical and ultrastructural changes at the material–tissue boundary is critical to explain clinical behavior [31, 32]. However, it should be emphasized that this mechanistic interpretation remains hypothetical and requires confirmation through spectroscopic or ultrastructural analyses.

Despite FV demonstrating superior EMH recovery compared to SDF in this model, treatment with SDF resulted in increased EMH when compared to the untreated group, consistent with previous findings [24]. A critical consideration is that this *in vitro* model did not capture the antimicrobial contribution of SDF. A substantial portion of SDF's clinical caries-arrest effect is mediated by sustained antimicrobial activity against cariogenic bacteria within biofilms [29], which cannot be evaluated in a chemically-induced lesion model. Therefore, the relative performance of SDF observed here may underestimate its clinical effectiveness in scenarios where biofilm management is a primary concern.

These findings have important clinical implications for managing early childhood caries, though they must be interpreted within the context of this study's limitations. Specifically, these results apply to early, non-cavitated enamel lesions under standardized *in vitro* conditions without biofilm, and may not be directly extrapolated to cavitated dentin lesions or clinical caries arrest situations. FV may be considered for remineralizing incipient enamel lesions in cooperative patients where esthetics and multiple follow-up visits are feasible [7, 8, 11]. However, SDF remains valuable for cavitated lesions requiring immediate caries arrest, high-risk patients needing antimicrobial intervention, and situations with limited patient cooperation or restricted access to care [2, 16, 23].

Clinical decision-making is multifactorial and scenario-driven; the selection of these agents should reflect lesion stage (incipient enamel *vs*. cavitated dentin), substrate, esthetic considerations, caries risk, and the feasibility of follow-up [17]. Importantly, these agents should not be viewed as mutually exclusive but rather as potentially complementary. Sequential therapy combining SDF's antimicrobial properties with FV's remineralization capacity may offer synergistic benefits, though this approach requires clinical validation. Neither agent restored sound enamel properties in this study, underscoring that prevention remains superior to treatment and these agents must be integrated into comprehensive caries management strategies [1, 9].

This study has inherent limitations that should be considered when interpreting these findings. The *in vitro* design lacks biological components, including salivary pellicle, proteins, enzymes, and biofilm. Their absence is particularly relevant for SDF, as its clinical efficacy largely derives from antimicrobial activity not captured here [29]. The independent-groups design precluded baseline microhardness measurements, although it avoided surface damage from repeated indentations and enabled valid between-group comparisons. Our single-application protocol differs from clinical practice, where agents are reapplied over time [17, 18], and Vickers microhardness assesses surface properties without directly quantifying subsurface remineralization. Complementary techniques, such as micro-computed tomography (CT), would strengthen future investigations [23, 24]. In addition, although not evaluated in this model, clinical use of SDF requires attention to potential soft-tissue irritation if inadvertently applied to mucosa, as well as other safety considerations, reinforcing the need for careful isolation and controlled application. Furthermore, although this study focused on remineralization potential, the clinical application of SDF also requires consideration of esthetic outcomes (*e.g.*, black staining of treated tissues) and the importance of controlled application protocols [28].

Future research should prioritize clinical validation through randomized controlled trials comparing single and multiple applications over twelve to twenty-four months with standardized caries activity assessment [3, 5]. Enhanced *in vitro* and *in situ* models incorporating salivary pellicle formation, biofilm components, and aging protocols with thermocycling and mechanical stress would provide more clinically relevant data [27, 33]. Advanced characterization techniques, including transverse microradiography, confocal microscopy, and micro-CT imaging, should complement microhardness testing to assess subsurface remineralization patterns [23, 25]. Mechanistic studies investigating why FV demonstrates superior remineralization in enamel despite SDF's higher fluoride concentration are particularly needed [26, 28]. Additionally, combination therapy protocols, dose-response studies with different SDF concentrations [18], cost-effectiveness analyses, and patient-centered outcomes research would inform evidence-based clinical decision-making and optimize preventive care strategies for early childhood caries [14, 15].

5. Conclusions

This *in vitro* study demonstrates that both FV and SDF significantly enhance enamel microhardness compared to untreated artificial caries lesions, thereby rejecting the null hypothesis. FV demonstrated superior remineralization capacity (377.65 VHN) compared to SDF (333.13 VHN), with a mean difference of 44.52 VHN ($p < 0.001$) under these experimental conditions. These findings suggest that FV may be the preferred agent for remineralizing early, non-cavitated enamel lesions in primary teeth under conditions similar to this model. However, these results should be interpreted in the context of a chemically induced lesion model without biofilm, and they do not diminish SDF's established value for caries arrest in cavitated lesions or its antimicrobial properties, which were not captured by this study design. Clinical decision-making should account for the distinct primary indications of FV and SDF and their potential complementary roles, depending on lesion type, substrate, esthetic concerns, and clinical circumstances.

AVAILABILITY OF DATA AND MATERIALS

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

AUTHOR CONTRIBUTIONS

WEA and AL—conceptualization. WEA and MHA—methodology. RAA—formal analysis. WEA, MHA, RAA, AMA, MA and MKA—investigation. WEA and CFM—writing–original draft. CFM and AL—supervision. WEA—funding acquisition. All authors contributed to writing–review and editing. All authors have read and agreed to the published version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical clearance and consent to participate were obtained from the King Saud University Institutional Review Board (project number E-24-8755). As the study used previously extracted teeth and patient identities are anonymized, the requirement for informed consent was waived by the ethics committee.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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