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Evaluation of Remineralization and Antimicrobial Potential of Various Silver Diamine Fluoride Solutions: In Vitro Study

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EVALUATION OF REMINERALIZATION AND ANTIMICROBIAL POTENTIAL OF VARIOUS SILVER DIAMINE FLUORIDE SOLUTIONS: IN VITRO STUDY

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Abstract

Background: This in vitro study evaluated and compared the remineralizing and antimicrobial effects of three commercially available 38% silver diamine fluoride (SDF) solutions: e-SDF, NuNamel SDF, and Advantage Arrest.

Methods: Remineralization-related effects were evaluated on artificially demineralized human dentin using Vickers surface microhardness testing and scanning electron microscopy, with sound and demineralized dentin serving as controls. Antimicrobial activity against *Streptococcus mutans* ATCC 25175 and *Lacticaseibacillus casei* ATCC 4646 was assessed using the disk diffusion assay. Dual-species biofilms formed on dentin disks were treated with the SDF formulations and analyzed using confocal laser scanning microscopy. Chlorhexidine (0.2%) and sterile saline were used as positive and negative controls, respectively.

Results: All SDF-treated groups exhibited significantly higher microhardness values than demineralized dentin and significantly lower values than sound dentin ($p < 0.001$), with no significant differences among the SDF formulations. Scanning electron microscopy demonstrated SDF-associated surface deposits and partial dentinal tubule occlusion. All SDF formulations produced significantly larger inhibition zones than chlorhexidine against both bacterial species. Confocal laser scanning microscopy revealed significantly lower live biomass and live/dead biomass ratios in all SDF groups than in the saline control. e-SDF also produced significantly lower live biomass and live/dead biomass ratios than chlorhexidine, whereas no significant differences were detected among the SDF formulations.

Conclusions: The three 38% SDF formulations demonstrated comparable remineralizing potential and marked antimicrobial activity, supporting their use as minimally invasive agents for caries management. Further studies are needed to establish the clinical relevance of these findings.

Keywords: silver diamine fluoride; remineralization; antimicrobial activity; surface microhardness; biofilm; dental care; bacteria; prevention

1. Introduction

Dental caries is a biofilm-mediated and multifactorial disease resulting from complex interactions among the dental biofilm, fermentable dietary carbohydrates, dental hard tissues, saliva, and time [1-3]. The disease is characterized by repeated cycles of demineralization and remineralization occurring at the interface between the tooth surface and the surrounding biofilm [1, 4, 5]. Under physiological conditions, these processes remain in a dynamic balance. However, when acid production within the biofilm repeatedly exceeds saliva's protective and buffering capacity, the balance shifts toward net mineral loss, initiating and progressing carious lesions [6-9].

Dental plaque is a structurally and functionally organized microbial biofilm whose composition and metabolic activity are influenced by local environmental conditions [3, 10]. Frequent exposure to fermentable carbohydrates and sustained reductions in biofilm pH favor acidogenic and aciduric microorganisms, promoting the development of a cariogenic microbial community [3, 7, 10]. Among these microorganisms, *Streptococcus mutans* (*S. mutans)* contributes to caries development through its capacity for adhesion, extracellular polysaccharide production, acid generation, and survival under acidic conditions [11]. Lactobacilli, including *Lacticaseibacillus casei (L. casei)*, are particularly associated with the progression of established carious lesions because of their strong acidogenic and aciduric properties [12]. Nevertheless, dental caries is not caused by a single bacterial species but results from the collective activity of a complex microbial community interacting with host, dietary, and environmental factors [10, 13, 14].

Silver diamine fluoride (SDF) is a non-invasive topical agent used primarily to manage active dentin caries in children and patients for whom conventional restorative treatment may be challenging [15, 16]. Its caries-arresting effect is attributed to the complementary actions of silver and fluoride ions. Silver ions exert antimicrobial activity against cariogenic microorganisms and biofilms, whereas fluoride inhibits demineralization and promotes

remineralization of dental hard tissues [17-19]. SDF may also preserve the demineralized dentin matrix by inhibiting collagen-degrading enzymes, including matrix metalloproteinases and cysteine cathepsins [20, 21].

Several commercial products are marketed as 38% SDF solutions; however, differences in their formulations and silver and fluoride ion availability have been reported [22]. Whether these variations translate into differences in antimicrobial and remineralization-related performance remains unclear, as direct comparisons under standardized experimental conditions are scarce. Therefore, assessing dentin surface characteristics, antibacterial activity against planktonic bacteria, and cariogenic biofilm responses may provide a more comprehensive evaluation of their biological performance.

Accordingly, this *in vitro* study aimed to compare the remineralization-related and antimicrobial potential of three commercially available 38% SDF solutions: e-SDF (Kids-e Dental, India), NuNamel SDF (NuSmile, USA), and Advantage Arrest (Elevate Oral Care, USA). Remineralization-related effects were assessed using Vickers surface microhardness testing and scanning electron microscopy (SEM). Antimicrobial activity against *Streptococcus mutans* ATCC 25175 and *Lacticaseibacillus casei* ATCC 4646 was evaluated using the disk diffusion assay, and bacterial viability and biomass in a dual-species biofilm were assessed using confocal laser scanning microscopy (CLSM). The null hypothesis was that the three SDF solutions would not differ in their remineralization-related or antimicrobial effects.

2. Materials and Methods

2.1. Study Design and Ethical Approval

This *in vitro* study evaluated the remineralization-related and antibacterial effects of three commercially available 38% SDF solutions. The protocol was approved by the Yeditepe University Non-Interventional Clinical Research Ethics Committee (12 June 2026; approval no. 202605Y1142) and was conducted in accordance with the Declaration of Helsinki.

Human third molars extracted for routine clinical reasons, independently of this study, were collected and used as specimens. No teeth were extracted specifically for this study. Written informed consent was obtained from the patients for the use of the extracted teeth.

A priori power analysis was performed using G*Power software (version 3.1; Heinrich Heine University, Düsseldorf, Germany). The calculations were based on the findings of Shimizu et al. [23] for the remineralization experiment and Hamama et al. [24] for the antibacterial experiment. At $\alpha = 0.05$ and 80% power, the minimum required sample size was eight specimens per group for both experiments.

Specimen preparation and microhardness testing were performed at Yeditepe University; caries induction and disk diffusion at Istanbul University; CLSM at the Aziz Sancar Institute of Experimental Medicine; and SEM at Hacettepe University Advanced Technologies Research and Application Center.

2.2. Materials

Three commercially available 38% SDF solutions were evaluated: e-SDF (Kids-e Dental, India), NuNamel SDF (NuSmile, USA), and Advantage Arrest (Elevate Oral Care, USA). For the antibacterial experiments, 0.2% chlorhexidine gluconate (CHX; Klorhex®, Drogsan Pharmaceuticals, Ankara, Türkiye) and sterile saline were used as the positive and negative controls, respectively. Table 1 presents the compositions of the SDF solutions, obtained from the manufacturers' product specifications and safety data sheets.

Table 1 Manufacturer information and composition of the SDF solutions used in the study

Product	Manufacturer (Country)	Silver / Silver Fluoride	Fluoride / Ammonium Fluoride	Ammonia / Ammonium Hydroxide	Other components
e-SDF	Kids-e Dental, India	Silver: 26.4%	Fluoride: 5.36%	Ammonia: 7.32%	Deionized water (balance)
NuNamel SDF	NuSmile, USA	Silver fluoride: 20–50%	Ammonium fluoride: >5%	Ammonium hydroxide: >5%	Deionized water (≤75%)
Advantage Arrest	Elevate Oral Care, USA	Silver fluoride: 20–50%	Ammonium fluoride: >5%	Ammonium hydroxide: >5%	FD&C Blue No. 1 (<1%); Deionized water (≤75%)

Abbreviations: SDF, silver diamine fluoride; FD&C, Food, Drug, and Cosmetic.

2.3. Experimental Groups and Treatment Allocation

The specimens were allocated to five groups according to the experimental procedures used for the remineralization and antimicrobial evaluations. Tables 2 and 3 present the group allocation, treatments, manufacturers, countries of origin, and sample sizes.

Table 2. Experimental groups and treatment allocation for the remineralization assessment

Group	Description	Treatment	Manufacturer	Country of Origin	n
Group 1	Experimental Group 1	e-SDF (38% SDF)	Kids-e Dental	India	8
Group 2	Experimental Group 2	NuNamel SDF (38% SDF)	NuSmile	USA	8
Group 3	Experimental Group 3	Advantage Arrest (38% SDF)	Elevate Oral Care	USA	8
Group 4	Negative Control	Artificial caries-affected dentin (no treatment)	—	—	8
Group 5	Positive Control	Sound dentin (no artificial caries, no treatment)	—	—	8

Three experimental groups received 38% SDF solutions (e-SDF, NuNamel SDF, or Advantage Arrest). Untreated artificial caries-affected dentin served as the negative control, and untreated sound dentin served as the positive control. Each group comprised eight dentin specimens (n = 8).

Abbreviations: SDF, silver diamine fluoride; n, number of specimens per group; USA, United States of America.

Table 3. Experimental groups and treatment allocation for the antimicrobial assessment.

Group	Description	Treatment	Manufacturer	Country of Origin	n
Group 1	Experimental Group 1	e-SDF Kids-e Dental (38% SDF)	Kids-e Dental	India	8
Group 2	Experimental Group 2	NuNamel (38% SDF)	NuSmile	USA	8
Group 3	Experimental Group 3	Advantage Arrest (38% SDF)	Elevate Oral Care	USA	8
Group 4	Negative Control	Saline solution	—	—	8
Group 5	Positive Control	Klorhex® (0.2% chlorhexidine gluconate)	Drogsan İlaçları San. ve Tic. A.Ş.	Türkiye	8

Three experimental groups received 38% SDF solutions (e-SDF, NuNamel SDF, or Advantage Arrest). Saline served as the negative control, and 0.2% chlorhexidine gluconate served as the positive control. Each group comprised eight dentin specimens (n = 8).

Abbreviations: SDF, silver diamine fluoride; n, number of specimens per group; USA, United States of America.

2.4. Specimen Selection and Preparation

A total of 45 caries-free and fracture-free human permanent third molars extracted for routine clinical reasons, primarily periodontal or orthodontic indications, were included. Teeth presenting caries, restorations, developmental defects, cracks, fractures, or other structural abnormalities were excluded following examination under ×2.5 magnification using dental loupes (Keeler Ltd., Windsor, Berkshire, UK).

The teeth were cleaned and stored in distilled water containing 0.1% thymol at 4°C for no longer than 2 months. The roots were separated from the crowns using an IsoMet 1000 precision saw (Buehler, Lake Bluff, IL, USA) under continuous water cooling. Two dentin disks were obtained from each crown, yielding 90 specimens.

The specimens were embedded in self-curing acrylic resin (Imicryl Acrylic Repair Material, Konya, Türkiye). The exposed dentin surfaces were standardized to 8 mm in diameter and sequentially polished with 600-, 800-, and 1200-grit silicon carbide papers using a Phoenix Beta grinder-polisher (Buehler) at 600 rpm.

Of the 90 specimens, 40 were used for microhardness testing, 10 for SEM analysis (n = 2 per group), and 40 for CLSM analysis. The disk diffusion assay was performed using five independent replicates for each test solution and bacterial strain.

2.5. Evaluation of Remineralization-Related Effects

2.5.1. Artificial Dentin Caries Formation

Artificial dentin caries lesions were induced using a modified microbiological caries model [25]. Except for specimens assigned to the sound-dentin control group, sterilized specimens were individually immersed in 25 mL of cariogenic medium consisting of brain-heart infusion (BHI) broth supplemented with yeast extract, sucrose, and glucose. The medium was inoculated with a fresh culture of *S. mutans* ATCC 25175, and its pH was adjusted to 4.0. Specimens were incubated under 5–7% CO_2 conditions at 37 °C for 15 days; the medium was renewed every 48 h. Following caries induction, the specimens were sterilized to eliminate microorganisms and thoroughly rinsed with deionized water.

2.5.2. SDF Application

After gentle drying, the respective SDF solution was applied to the dentin surface using a sterile microbrush and left undisturbed for 60 s. One drop of SDF was used for approximately five specimens. Excess material was removed with a cotton pellet.

2.5.3. pH Cycling

Following SDF application, specimens underwent a modified 14-day pH-cycling protocol. Each 24-h cycle comprised 6 h in demineralizing solution, rinsing with distilled water, and 18 h in remineralizing solution. The demineralizing solution contained 50 mM acetic acid, 2.2 mM KH_2PO_4, 2.2 mM $Ca(NO_3)_2$, and 0.1 ppm NaF (pH 4.3-4.5). The remineralizing solution contained 20 mM HEPES, 130 mM KCl, 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, and 1 mM NaN_3 (pH 7.0, adjusted with KOH). Both solutions were replaced every 3 days.

2.5.4. Surface Microhardness Testing

Dentin surface microhardness was measured using a Micromet 5114 tester (Buehler, Lake Bluff, IL, USA) with a Vickers diamond indenter. A 100-g load was applied for 15 s, and five indentations were made at standardized, non-overlapping locations on each exposed dentin surface. The mean of these five measurements was recorded as the representative Vickers hardness number (VHN) for each specimen.

2.5.5. SEM Analysis of Dentin Surface Morphology

Two specimens from each remineralization group were selected for qualitative SEM analysis (n = 10). The specimens were examined to characterize dentin surface morphology and evaluate surface deposits and dentinal tubule patency or occlusion following treatment. Specimens were rinsed, dehydrated through a graded ethanol series of 25%, 50%, 70%, 96%, and 100% ethanol (30 min each; 100% twice), air-dried for 24 h, mounted on aluminum stubs, and gold-palladium sputter-coated for 45 s (Leica EM ACE200, Leica Microsystems, Germany). Dentin surfaces were examined using field-emission SEM (GAIA3 Triglav™, TESCAN, Brno, Czech Republic) at ×500, ×2500, and ×10,000 magnifications to evaluate morphology, surface deposits, and dentinal tubule patency or occlusion.

2.6. Microbiological Tests

2.6.1. Disk-Diffusion Assay

The antibacterial activities of the test solutions against *S. mutans* ATCC 25175 and *L. casei* ATCC 4646 were evaluated using the disk diffusion assay. Fresh suspensions of each bacterial strain were prepared separately in 2

mL of BHI broth and adjusted to a turbidity equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The bacterial concentrations were verified by tenfold serial dilution and culture on BHI agar.

Subsequently, 100 µL of each standardized bacterial suspension was uniformly spread onto BHI agar plates. After the inoculated surfaces had been allowed to dry for 5 min, sterile blank antibiotic discs (Bioanalyse) impregnated with the test solutions were placed on the agar. The plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone surrounding each disc in millimeters. Antibacterial activity was determined by measuring the diameter of the inhibition zone surrounding each disc in millimeters. Five independent replicates were performed for each bacterial strain and test solution (n = 5).

2.6.2. Evaluation of Antibacterial Activity Using Confocal Laser Scanning Microscopy

2.6.2.1. Bacterial Suspension Preparation

Fresh 24-h cultures of *S. mutans* ATCC 25175 and *L. casei* ATCC 4646 were prepared separately in BHI broth supplemented with 5% sucrose. Each bacterial suspension was adjusted to a 0.5 McFarland standard and subsequently diluted to approximately 1×106 CFU/mL. The two suspensions were combined at a 3:2 ratio to prepare the dual-species inoculum.

2.6.2.2. Artificial saliva preparation

Artificial saliva was prepared in the laboratory according to the formulation described by Pratten et al. [26], consisting of Lab-Lemco powder (1 g/L), yeast extract (2 g/L), proteose peptone (5 g/L), porcine gastric mucus (2.5 g/L), sodium chloride (0.35 g/L), calcium chloride (0.2 g/L) and potassium chloride (0.2 g/L). Following autoclaving, 1.25 mL of a 40% urea solution was added, and the pH was adjusted to 7.0. The solution was subsequently sterilized by filtration through a 0.22-µm membrane filter.

2.6.2.3. Biofilm Formation

Each dentin specimen was placed in an individual well of a sterile 24-well plate. For surface conditioning, 1 mL of artificial saliva was added to each well, and the specimens were incubated at 37 °C for 1 h. The artificial saliva was subsequently aspirated, and the specimens were gently rinsed with phosphate-buffered saline (PBS). Each specimen was then transferred to a new well containing 1.6 mL of BHI broth supplemented with 5% sucrose and 100 µL of the dual-species inoculum. The plates were incubated under anaerobic conditions at 37 °C for 24 h to allow biofilm formation.

2.6.2.4. Application of the Test Solutions

The specimens were rinsed with sterile saline to remove non-adherent bacteria. Subsequently, 25 µL of the respective SDF solution was applied for 60 s. The positive-control group received 5 µL of 0.2% CHX for 60 s, whereas the negative-control group received sterile saline. All specimens were then rinsed with 1 mL of sterile saline.

2.6.2.5. Live/Dead Staining and CLSM Analysis

Live/Dead bacterial viability stain (Invitrogen, Thermo Fisher Scientific, USA) was prepared using 0.4 µL each of components A and B per 1 mL of dimethyl sulfoxide. A 20-µL aliquot was applied to each specimen and incubated in darkness for 15 min; specimens were held at 4 °C for approximately 1 h before imaging. CLSM (Leica Microsystems, Heidelberg, Germany) images were acquired from three non-overlapping regions per specimen at ×10 using 488- and 532-nm excitation and identical acquisition settings. Live and dead cells were visualized in green and red, respectively. COMSTAT quantified dead biomass (CH1), live biomass (CH2), total biomass (CH1 + CH2), and the live/dead ratio (CH2/CH1); biomass was expressed as $\mu m^3/\mu m^2$.

2.6.2.6 SEM Analysis of Biofilm Morphology

Following the antibacterial treatments, two representative biofilm specimens from each group were randomly selected for qualitative SEM analysis. The specimens were placed in separate cassettes and dehydrated in a graded ethanol series of 25%, 50%, 70%, 96%, and 100% (twice), with 30 min immersion at each concentration. They were subsequently air-dried for 24 h. The dried specimens were mounted on aluminum stubs and sputter-coated with a gold–palladium alloy for 45 s using a sputter coater (Leica EM ACE200, Leica Microsystems, Germany). Biofilm morphology and surface distribution were examined using a field-emission scanning electron microscope (GAIA3 Triglav™, TESCAN, Brno, Czech Republic) at ×500, ×2500, and ×10,000 magnifications.

2.7. Statistical Analysis

Statistical analyses were performed using NCSS 2007 Statistical Software (NCSS LLC, Kaysville, UT, USA). Data were summarized using the mean, standard deviation, median, and interquartile range, as appropriate. Normality was evaluated using the Shapiro–Wilk test.

Normally distributed variables were compared using one-way analysis of variance followed by Bonferroni-adjusted pairwise comparisons. Non-normally distributed variables were analyzed using the Kruskal–Wallis test followed by Dunn's post hoc test. All tests were two-sided, and statistical significance was set at $p < 0.05$.

3. Results

3.1. Surface microhardness

Surface microhardness differed significantly among the five groups ($p < 0.001$; Table 4). Sound dentin had the highest microhardness (68.51 ± 3.23 VHN), and artificial caries-affected dentin had the lowest (43.77 ± 2.91 VHN). Mean values for e-SDF, NuNamel SDF, and Advantage Arrest were 57.82 ± 2.05, 57.31 ± 1.03, and 54.75 ± 0.81 VHN, respectively.

Bonferroni-adjusted comparisons showed that all three SDF-treated groups had significantly higher microhardness than artificial caries-affected dentin and significantly lower microhardness than sound dentin (all $p < 0.001$). No significant differences were detected among e-SDF, NuNamel SDF, and Advantage Arrest (e-SDF vs. NuNamel SDF, $p = 0.990$; e-SDF vs. Advantage Arrest, $p = 0.066$; NuNamel SDF vs. Advantage Arrest, $p = 0.172$).

Table 4 Surface microhardness values (mean ± standard deviation) and Bonferroni post hoc multiple-comparison results

Groups	Treatment	Mean ± SD (VHN)	p value	Post-hoc
Group 1	e-SDF	57.82 ± 2.05^{b}	p<0.001*	a > b > c
Group 2	NuNamel SDF	57.31 ± 1.03^{b}		
Group 3	Advantage Arrest	54.75 ± 0.81^{b}		
Group 4	Artificial caries	43.77 ± 2.91^{c}		
Group 5	Sound dentin	68.51 ± 3.23^{a}		

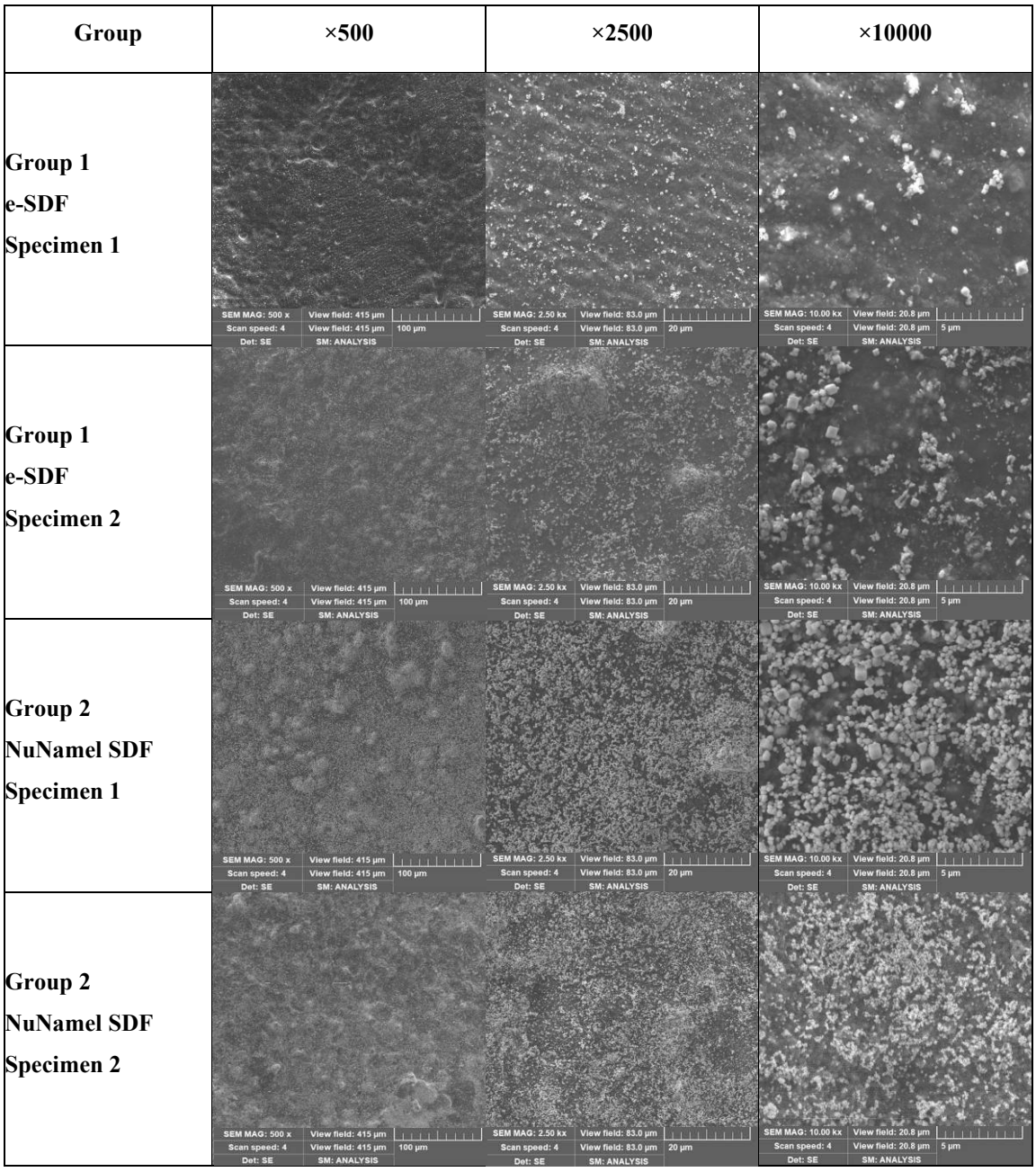
**p < 0.05 according to the one-way analysis of variance (ANOVA).*

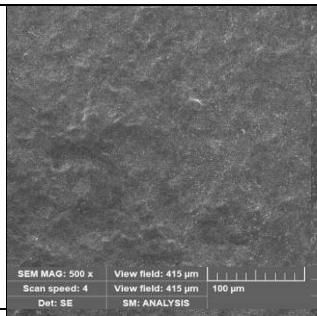
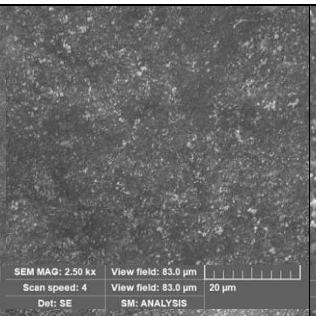
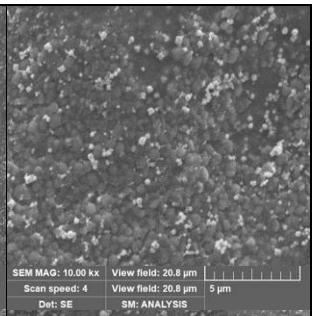
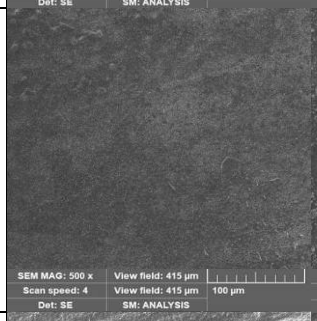
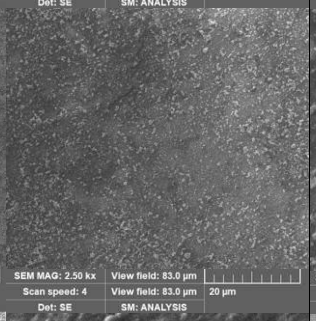
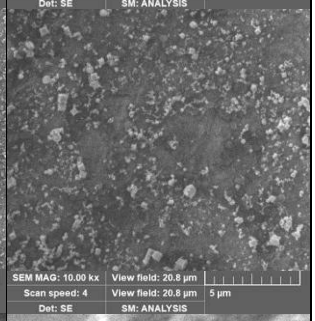
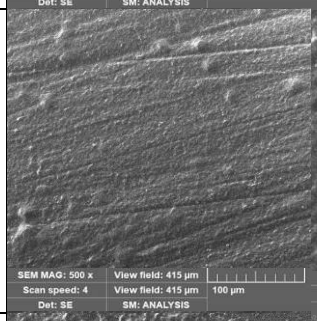
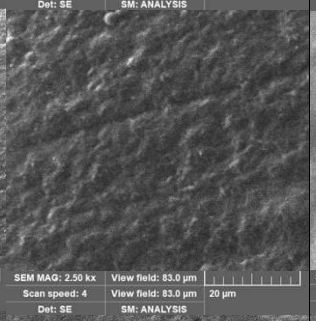
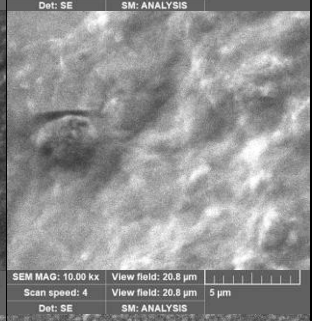
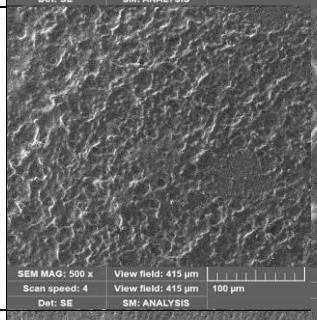
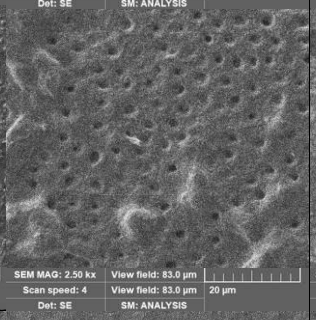
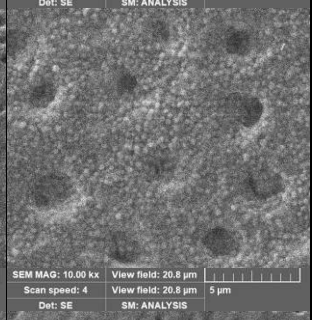
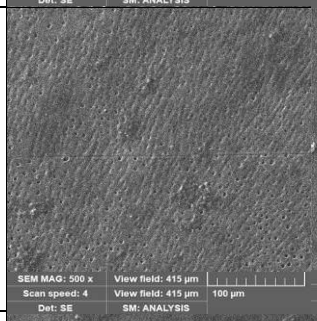
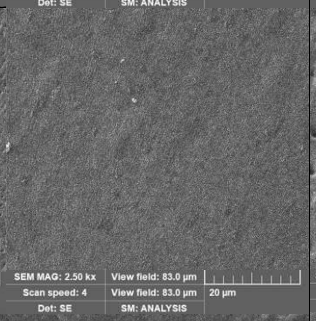
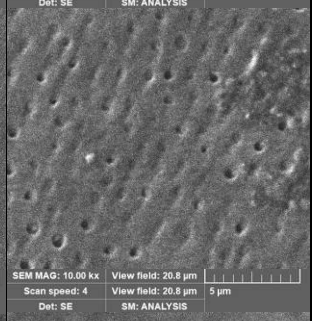
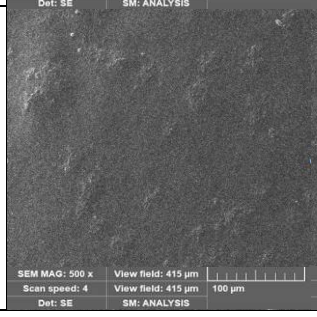
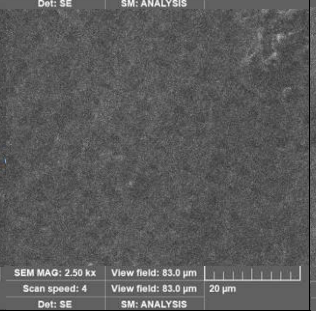
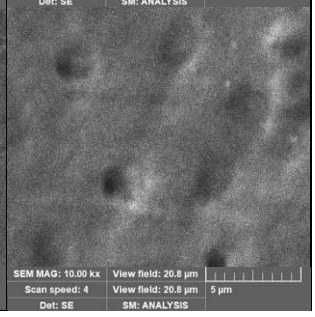
Different superscript lowercase letters in the same column indicate statistically significant differences between groups ($p < 0.05$) according to the Bonferroni post-hoc test; identical letters indicate no statistically significant difference. The ">" symbol indicates a statistically significantly higher surface microhardness value. Abbreviations: SDF, silver diamine fluoride; VHN, Vickers hardness number.

3.2. SEM evaluation of dentin surface morphology

SEM examination revealed distinct morphological differences among sound, artificial caries-affected, and SDF-treated dentin surfaces (Fig. 1). Sound dentin exhibited a relatively smooth and homogeneous surface with regularly distributed dentinal tubule openings. In contrast, artificial caries-affected dentin showed an irregular and porous surface, with numerous patent tubules and widened tubule orifices.

Fig. 1 Representative scanning electron microscopy images of dentin surface morphology



Group 3 Advantage Arrest Specimen 1			
Group 3 Advantage Arrest Specimen 2			
Group 4 Artificial Caries Specimen 1			
Group 4 Artificial Caries Specimen 2			
Group 5 Sound Dentin Specimen 1			
Group 5 Sound Dentin Specimen 2			

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Sound dentin exhibits a relatively smooth surface with regularly distributed dentinal tubule openings, whereas artificial caries-affected dentin shows an irregular, porous surface with patent tubules and widened tubule orifices. SDF-treated surfaces display granular and crystalline deposits: dense and relatively homogeneous coverage with NuNamel SDF, heterogeneous deposits with localized aggregates with e-SDF, and finer, less continuous deposits with partial tubule coverage with Advantage Arrest.

Abbreviations: SEM, scanning electron microscopy; SDF, silver diamine fluoride.

All three SDF-treated groups exhibited granular and crystalline surface deposits with varying distributions. NuNamel SDF produced dense and relatively homogeneous granular coverage that largely obscured the underlying dentin and dentinal tubule openings. The e-SDF specimens also showed substantial deposition; however, the particles were more heterogeneously distributed and formed localized aggregates. Advantage Arrest produced fine granular deposits with partial tubule coverage, although the deposited layer appeared less continuous than that observed with NuNamel SDF.

3.3. Disk-diffusion assay

Inhibition-zone diameters differed significantly among the tested solutions for both *S. mutans* and *L. casei*(both $p < 0.001$; Table 5).

Against S. mutans, no significant differences were detected among the three SDF formulations, whereas all SDF formulations produced significantly larger inhibition zones than 0.2% CHX (all $p \leq 0.002$). Against L. casei, e-SDF produced a significantly larger inhibition zone than Advantage Arrest ($p = 0.020$), while no other significant differences were observed among the SDF formulations. All three SDF formulations produced significantly larger inhibition zones than 0.2% CHX (all $p < 0.001$).

Table 5 Inhibition-zone against *S. mutans*ATCC 25175 and *L. casei*ATCC 4646 (mean ± standard deviation)

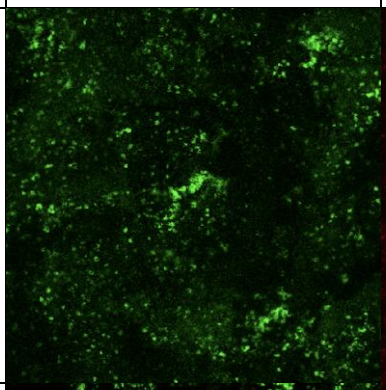
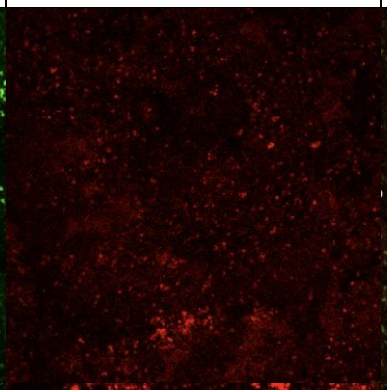
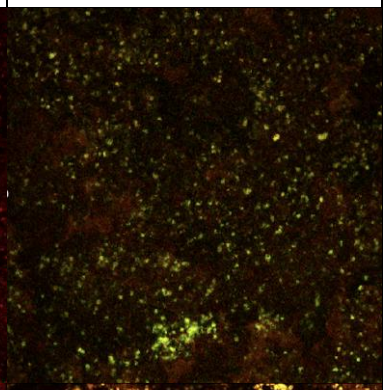
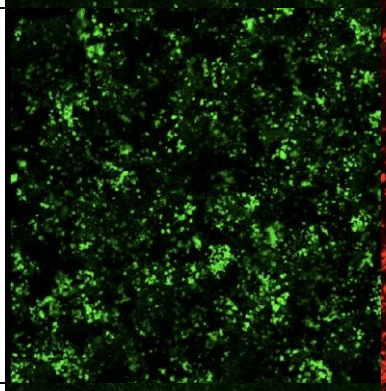
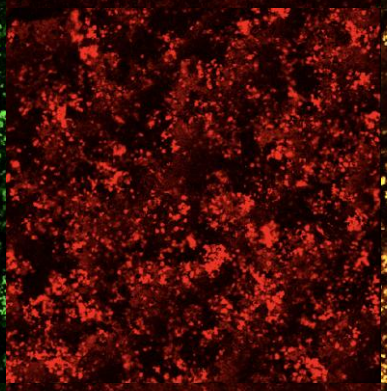
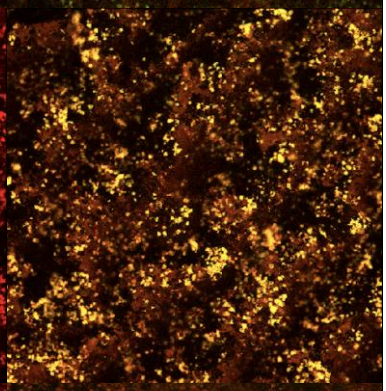
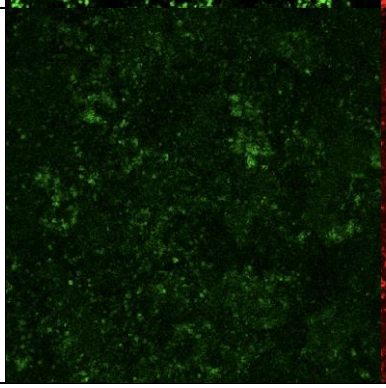
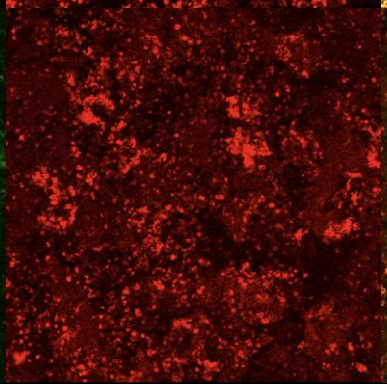
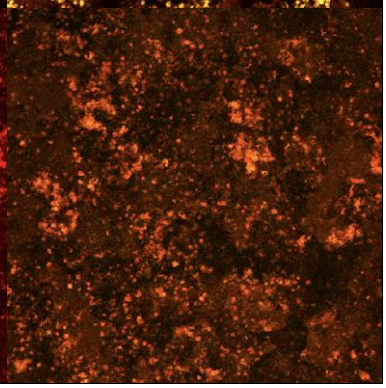
Experimental group	*S. mutans* ATCC 25175 (mm)	Post-hoc	*L. casei* ATCC 4646 (mm)	Post-hoc
e-SDF	28.00 ± 4.47	a	16.00 ± 0.71	a
NuNamel SDF	27.60 ± 4.34	a	15.40 ± 0.55	ab
Advantage Arrest SDF	23.00 ± 3.46	a	14.80 ± 0.45	b
0.2% CHX	12.60 ± 1.34	b	11.60 ± 0.55	c
p-value	<0.001	a > b	<0.001	a > b > c; ab > c

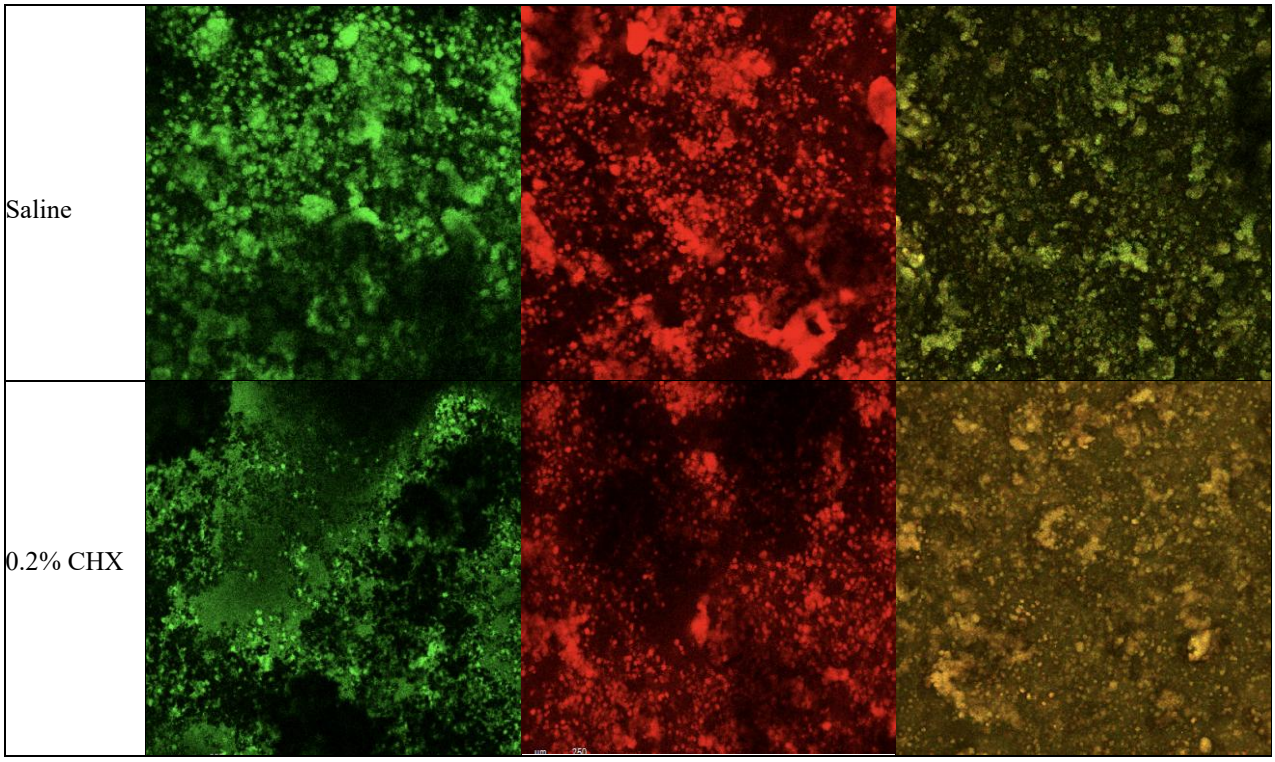
p < 0.05 according to the one-way analysis of variance (ANOVA), followed by the Bonferroni post-hoc test. Different lowercase letters indicate statistically significant differences between groups; groups sharing at least one letter are not significantly different.

3.4. Biofilm viability and biomass

Representative Live/Dead-stained CLSM images are presented in Fig. 2. Significant between-group differences were found in dead biomass (p = 0.049), live biomass (p = 0.001), total biomass (p = 0.006), and the live/dead biomass ratio (p = 0.001; Tables 6 and 7).

Fig. 2 Representative confocal laser scanning microscopy images of Live/Dead-stained dual-species biofilms according to experimental group

Group	Live	Dead	Merged
e-SDF			
NuNamel SDF			
Advantage Arrest SDF			



Green and red fluorescence represented live and dead bacterial cells, respectively. The SDF-treated biofilms exhibited prominent red fluorescence accompanied by lower green fluorescence. In contrast, green fluorescence was more evident in the 0.2% CHX and saline groups, particularly in the saline control

Table 6 Dead, live, and total bacterial biomass across the experimental groups

Group	**Statistic**	**Dead biomass (CH1) (µm³/µm²)**	**Post hoc**	**Live biomass (CH2) (µm³/µm²)**	**Post hoc**	**Total biomass (CH1 + CH2) (µm³/µm²)**	**Post hoc**
Group 1 e-SDF	Mean ± SD	18.32 ± 6.82	a	10.32 ± 6.26	a	28.64 ± 12.76	a
	Median (IQR)	19.25 (15.01–23.95)		10.42 (5.91–13.64)		28.91 (21.50–37.39)	
Group 2 NuNamel SDF	Mean ± SD	25.88 ± 11.77	ab	16.53 ± 9.55	ab	42.41 ± 20.73	a
	Median (IQR)	32.38 (12.48–33.90)		17.92 (8.02–23.81)		50.71 (20.86–57.38)	
Group 3 Advantage Arrest SDF	Mean ± SD	28.64 ± 15.71	ab	16.67 ± 9.35	ab	45.31 ± 24.42	a
	Median (IQR)	27.90 (14.30–44.11)		18.62 (9.41–24.08)		48.49 (23.72–66.34)	

Group 4 Saline	Mean ± SD	43.40 ± 22.37		52.80 ± 22.31		96.21 ± 44.59	
	Median (IQR)	41.24 (24.68–63.25)	b	53.52 (34.16–70.19)	c	94.76 (59.66–133.44)	b
Group 5 0.2% CHX	Mean ± SD	32.30 ± 19.82		22.12 ± 11.54		54.42 ± 29.53	
	Median (IQR)	35.02 (11.65–47.86)	ab	24.11 (9.68–32.30)	b	61.80 (21.32–81.77)	a
p*-value		**0.049**		**0.001**		**0.006**	

**p < 0.05 according to the Kruskal–Wallis test.*

Different lowercase letters within the same post hoc column indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni adjustment (p < 0.05); groups sharing at least one letter are not significantly different. Abbreviations: CH1, dead bacterial biomass; CH2, live bacterial biomass; CHX, chlorhexidine; CLSM, confocal laser scanning microscopy; IQR, interquartile range; SD, standard deviation; SDF, silver diamine fluoride.

Table 7 Live/dead bacterial biomass ratio across the experimental groups

Group	Statistic	Live/dead biomass ratio (CH2/CH1)	Post hoc
Group 1 e-SDF	Mean ± SD	0.51 ± 0.19	
	Median (IQR)	0.55 (0.37–0.62)	a
Group 2 NuNamel SDF	Mean ± SD	0.59 ± 0.25	
	Median (IQR)	0.66 (0.47–0.73)	ab
Group 3 Advantage Arrest SDF	Mean ± SD	0.56 ± 0.25	
	Median (IQR)	0.57 (0.43–0.80)	ab
Group 4 Saline	Mean ± SD	1.28 ± 0.16	
	Median (IQR)	1.26 (1.12–1.45)	c
Group 5 0.2% CHX	Mean ± SD	0.88 ± 0.47	
	Median (IQR)	0.77 (0.45–1.24)	b
p*-value		**0.001**	

**p < 0.05 according to the Kruskal–Wallis test.*

Different lowercase letters within the post hoc column indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni adjustment ($p < 0.05$); groups sharing at least one letter are not significantly different. Abbreviations: CH1, dead bacterial biomass; CH2, live bacterial biomass; CHX, chlorhexidine; CLSM, confocal laser scanning microscopy; IQR, interquartile range; SD, standard deviation; SDF, silver diamine fluoride.

The saline group exhibited significantly greater live biomass than the e-SDF ($p = 0.001$), NuNamel SDF ($p = 0.002$), Advantage Arrest ($p = 0.002$), and 0.2% CHX groups ($p = 0.012$). Live biomass was also significantly lower following e-SDF treatment than following 0.2% CHX treatment ($p = 0.046$). No significant differences in live biomass were detected among the three SDF formulations.

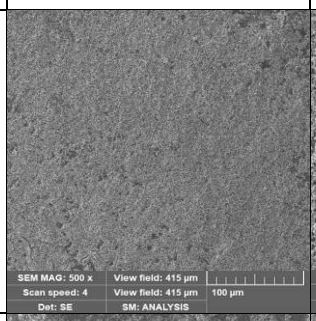
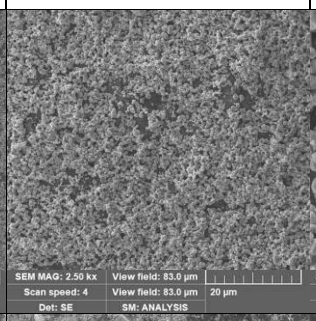
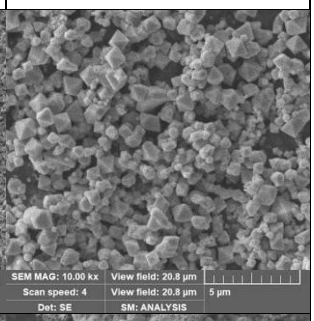
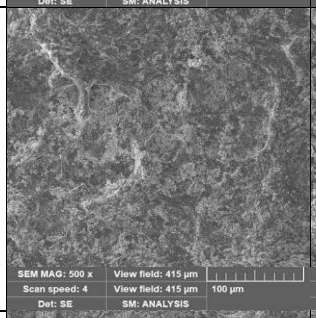
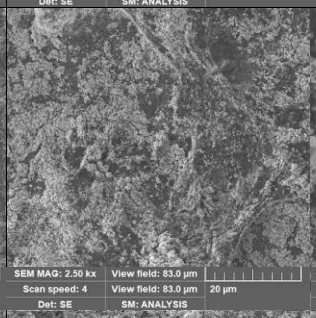
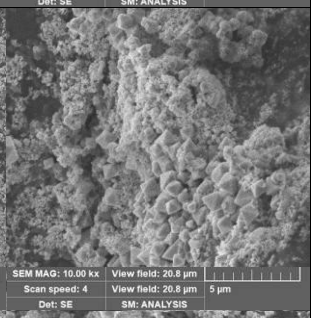
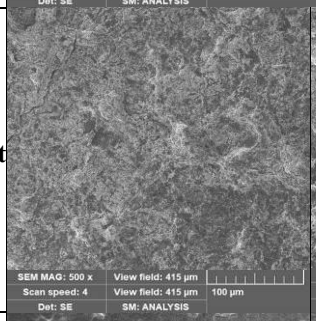
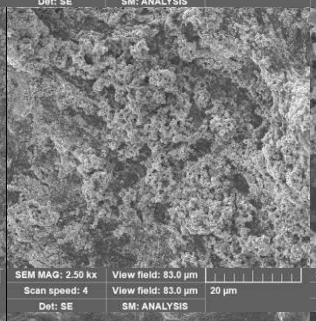
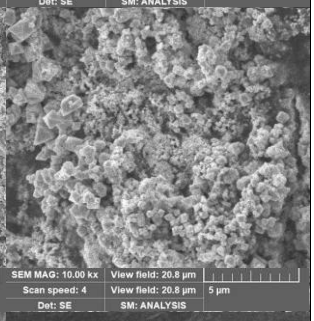
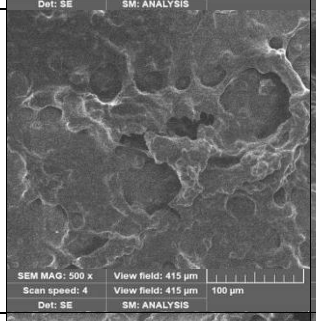
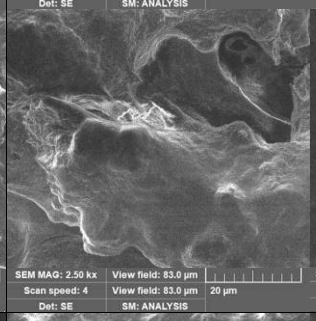
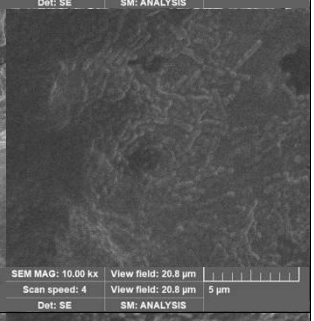
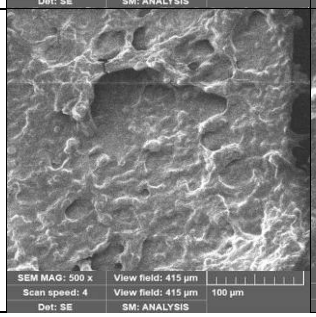
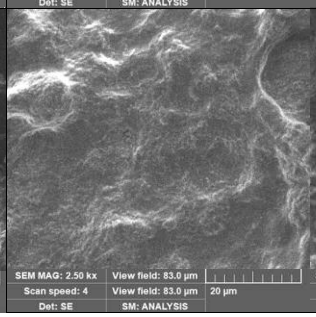
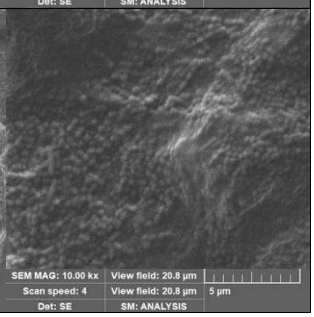
Total biomass was significantly greater in the saline group than in the e-SDF ($p = 0.002$), NuNamel SDF ($p = 0.012$), Advantage Arrest ($p = 0.021$), and 0.2% CHX groups ($p = 0.046$). For dead biomass, the only significant pairwise difference was observed between saline and e-SDF ($p = 0.016$).

The live/dead biomass ratio was significantly higher in the saline group than in the e-SDF, NuNamel SDF, and Advantage Arrest groups (all $p = 0.001$) and the 0.2% CHX group ($p = 0.047$). Moreover, e-SDF produced a significantly lower live/dead ratio than 0.2% CHX ($p = 0.043$). No significant differences were detected among the three SDF formulations.

3.5. SEM evaluation of antimicrobial effects

SEM examination revealed differences in the residual biofilm and dentin surface morphology among the treatment groups (Fig. 3). All three SDF-treated groups exhibited granular and crystalline surface deposits, whereas comparable deposits were not observed following treatment with saline or 0.2% CHX.

380 **Fig. 3** Scanning electron microscopy images of dentin-associated biofilms following antimicrobial treatments

Group	×500	×2500	×10000
Group 1 e-SDF			
Group 2 NuNamel SDF			
Group 3 Advantage Arrest SDF			
Group 4 Saline			
Group 5 0.2% CHX			

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382 *SDF-treated surfaces display granular and crystalline deposits: extensive and relatively homogeneous coverage*

383 *with polygonal and cuboidal structures with e-SDF, less uniformly distributed deposits with localized aggregates*

with NuNamel SDF, and densely packed crystalline aggregates with variable morphology with Advantage Arrest. Saline-treated surfaces show exposed dentinal tubules and a fibrillar morphology, whereas chlorhexidine-treated surfaces retain a relatively continuous fibrillar network. Neither control group exhibits apparent crystalline deposits.

Abbreviations: SEM, scanning electron microscopy; SDF, silver diamine fluoride; CHX, chlorhexidine.

The e-SDF group exhibited extensive and relatively homogeneous surface coverage. At ×10,000 magnification, numerous polygonal and cuboidal crystalline structures were visible across the dentin surface. NuNamel SDF also produced granular deposits, although these were distributed less uniformly and formed localized aggregates separated by areas of visible underlying dentin. Advantage Arrest produced densely packed crystalline aggregates with variable morphology, but their distribution was less homogeneous than that observed with e-SDF.
In the saline group, the dentin surface remained free of crystalline deposits and displayed exposed dentinal tubules and a fibrillar surface morphology. Similarly, the 0.2% CHX group showed no apparent crystalline precipitation and retained a relatively continuous fibrillar network.

4. Discussion

This study compared three commercially available 38% SDF formulations under standardized *in vitro* conditions. All increased dentin surface microhardness relative to artificial caries, although none restored sound-dentin values, and no significant differences were detected among the products. SEM showed SDF-associated deposits and partial occlusion of dentinal tubules. All formulations produced larger inhibition zones than 0.2% CHX against *S. mutans and L. casei*. CLSM also showed lower live biomass and live/dead ratios in the SDF groups than in the saline control. Thus, the products showed comparable remineralization-related performance and marked antimicrobial activity, with limited product-dependent differences in selected antimicrobial outcomes.

The increased microhardness is consistent with interactions between fluoride and the mineral phase of demineralized dentin. SDF may form calcium fluoride-like reservoirs and fluoridated apatite while limiting hydroxyapatite dissolution and collagen degradation [27, 28]. Silver-containing reaction products may further protect the surface. Previous studies have shown that 38% SDF reduces mineral loss and collagen degradation and improves the microhardness and structural integrity of demineralized permanent dentin [28-30], supporting the present findings.

The absence of differences among e-SDF, NuNamel SDF, and Advantage Arrest suggests that their common 38% SDF concentration was more influential than formulation-specific features under the tested conditions. Kamath et al. reported similar fluoride and silver ion release from e-SDF and Advantage Arrest, and Kale et al. found comparable effects on lesion depth and mineral deposition [31, 32]. However, commercial solutions can differ in pH, ion concentration, stability, excipients, storage, and time after opening [33]. The present results therefore indicate comparable performance under this protocol, not physicochemical equivalence.

All SDF groups remained less hard than sound dentin, indicating improvement without complete restoration. Surface microhardness reflects indentation resistance rather than mineral gain alone and is affected by mineral distribution, collagen integrity, tubule orientation, and surface preparation [34-37]. The findings should therefore be interpreted as tissue hardening compatible with remineralization, not regeneration of sound dentin.

SEM complemented the mechanical findings: demineralized dentin was irregular and porous with patent tubules, whereas SDF-treated surfaces showed deposits and varying tubule occlusion. Similar changes have been attributed to calcium-, fluoride-, phosphate-, silver-, and protein-associated reaction products [28, 38]. Such deposits may reduce permeability and provide local ion reservoirs. Nevertheless, SEM cannot determine elemental or crystallographic composition; without compositional analysis, these structures should be described conservatively as SDF-associated deposits. Because SEM was qualitative and included few specimens, visually denser deposition cannot establish superior remineralization.

The antimicrobial experiments assessed planktonic bacteria and a dentin-associated dual-species biofilm. *S. mutans and L. casei were* selected for their acidogenicity, acid tolerance, and roles in caries development; their coexistence can modify biofilm formation and stress responses [39-43]. All SDF products produced larger inhibition zones than CHX against both species. The products did not differ *S. mutans*; *L. casei*, e-SDF produced a larger zone than Advantage Arrest, while other inter-product comparisons were nonsignificant.

SDF antimicrobial activity is mainly attributed to silver and fluoride ions. Silver disrupts membranes, binds thiol-containing proteins, inhibits enzymes, and interferes with DNA replication; fluoride inhibits carbohydrate metabolism and acid production, particularly at low pH [38, 44]. Accordingly, SDF can affect adhesion, growth,

metabolism, and biofilm maturation [19, 38, 44-49]. The present inhibition zones agree with previous diffusion-based findings [50], but zone diameter also depends on agar diffusion. Viscosity, ionic dissociation, and formulation characteristics may influence this assay, which cannot determine minimum inhibitory or bactericidal concentrations.

CLSM provided evidence against an established biofilm. All SDF formulations reduced live biomass and live/dead ratios relative to saline. Only e-SDF produced significantly lower values than CHX for these outcomes; no product differences were found for dead, live, or total biomass. The isolated e-SDF-CHX differences warrant caution because Live/Dead staining primarily reflects membrane integrity, not metabolic activity, reproductive capacity, or cultivability [51]. They do not establish overall clinical superiority.

These CLSM findings agree with studies showing that 38% SDF reduces cariogenic-biofilm viability on dentin [28, 52, 53]. Karaduran et al. reported reduced viability in *S. mutans-L. casei* biofilm and greater activity than CHX, whereas Mei et al. found reduced viable counts and disrupted biofilm architecture [28, 54]. Numerical comparison is limited by differences in species, biofilm age, substrate, exposure, controls, staining, and image analysis, but the overall evidence supports the immediate antibiofilm activity of 38% SDF.

CLSM enabled simultaneous evaluation of biomass and membrane integrity without the dehydration required for SEM [55-57], while SEM supplied complementary surface morphology. SDF-associated deposits, absent after saline and CHX, may retain active compounds at the biofilm-dentin interface. However, SEM cannot distinguish viable cells, and precipitates may obscure bacteria and dentin; these observations are therefore supportive, not direct evidence of antibacterial activity.

This study has limitations. Its *in vitro* design could not reproduce salivary clearance, dietary variation, mechanical loading, host responses, or the diversity and matrix complexity of natural biofilms. The dual-species, 24-h biofilm and single exposure primarily represented an immediate response and did not assess mature biofilms, substantivity, or repeated cariogenic challenges. In addition, independent specimens were used for the five remineralization groups. Because baseline, post-demineralization, and post-treatment hardness were not measured sequentially in each specimen, individual hardness recovery could not be calculated, and natural dentin variation may have contributed to variability.

Surface microhardness did not quantify subsurface mineral distribution, and SEM was qualitative and limited to representative specimens. Deposit composition was not confirmed by elemental or crystallographic analysis. Disk diffusion may have been affected by formulation-dependent diffusion, whereas Live/Dead staining did not assess metabolism or regrowth and may have been influenced by SDF-related optical changes. Finally, permanent third-molar dentin was used; extrapolation to primary dentin, natural lesions, and long-term clinical outcomes requires caution.

Within these limitations, the three 38% SDF formulations increased the microhardness of demineralized dentin, produced SDF-associated deposits and tubule occlusion, and showed marked activity against *S. mutans*, *L. casei*, and their dual-species biofilm. Overall performance was comparable under the tested conditions. Further studies using within-specimen measurements, quantitative mineral and elemental analyses, mature multispecies biofilms, repeated challenges, and clinically relevant substrates should determine whether formulation-specific differences emerge under more complex conditions.

5. Conclusion

Within the limitations of this in vitro study, the three 38% SDF formulations showed no significant differences in dentin surface microhardness or the evaluated dual-species biofilm outcomes. SDF-treated dentin exhibited higher microhardness than untreated artificial caries-affected dentin, although sound-dentin values were not restored. All formulations produced larger inhibition zones than 0.2% CHX against S. mutans and L. casei and reduced live biomass and live/dead biomass ratios compared with saline. Despite differences in selected antimicrobial outcomes, no formulation demonstrated consistent superiority. These findings support the dentin-hardening and antimicrobial potential of the tested formulations, while further investigation is required to establish their long-term effects and clinical relevance.

List of abbreviations

S. mutans: Streptococcus mutans

L. casei: Lacticaseibacillus casei

SDF: Silver diamine fluoride

CLSM: Confocal laser scanning microscopy

SEM: Scanning electron microscopy

CHX: Chlorhexidine gluconate

BHI: Brain–heart infusion

PBS: Phosphate-buffered saline

Declarations

Ethics approval and consent to participate

This study was approved by the Yeditepe University Non-Interventional Clinical Research Ethics Committee (12 June 2026; approval no. 202605Y1142). Written informed consent was obtained from patients for the use of their extracted teeth in research. No teeth were extracted specifically for this study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed 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

HMS and SSK conceptualized and designed the study. HMS performed the experiments and drafted the manuscript. AÇ, MYÜ, and NT contributed to the methodology and interpretation of the findings. All authors critically revised the manuscript, read and approved the final version.

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