Facultad de Odontología

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Effect of fluorinated compounds with silver nanoparticles in dental application

INVESTIGATION

Efecto de compuestos fluorados con nanopartículas de plata en la aplicación odontológica

Efeito de compostos fluorados com nanopartículas de prata em aplicações dentárias

Abstract

Objective: To evaluate the remineralizing, bactericidal, and staining effects of a sodium fluoride solution containing silver nanoparticles, in comparison with the SDI brand silver diamine fluoride treatment. Forty extracted teeth were used 20 as the control group and 20 for testing the fluoride solutions. All samples were demineralized with phosphoric acid, then treated with SDI fluoride and the experimental fluoride on two surfaces of the same tooth, and stored in artificial saliva at 37°C. Mineralization was assessed using the DIAGNOdent 2095 (KaVo) device before treatment and at 1 hour, 24 hours, 7 days, and 14 days post-application. Staining was evaluated through photographs taken with a stereomicroscope. Bacterial growth inhibition was assessed using salivarius and tomato agar inoculated with samples from carious lesions in a pediatric population. The results showed complete remineralization in both treatment groups with no statistically significant differences, and similar bacterial inhibition, though slightly greater with silver diamine fluoride on salivarius agar. Surfaces treated with the experimental fluoride exhibited no clinical staining, while 75% of those treated with silver diamine fluoride showed noticeable dark staining. In conclusion, the experimental fluoride demonstrated effective remineralizing and bactericidal performance without the staining effect, making it a viable alternative.

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Keywords: Silver compounds, nanoparticles, dental remineralization, dental caries.

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Resumen

Objetivo: Evaluar el efecto remineralizante, bactericida y pigmentación producida por una disolución de fluoruro de sodio con nanopartículas de plata comparado con el tratamiento de Fluoruro Diamino de la marca SDI. Se usaron 40 dientes extraídos, 20 como grupo control y 20 para probar las soluciones fluoradas, los cuales se desmineralizaron con ácido fosfórico, después se barnizó la superficie con fluoruro de la marca SDI y fluoruro experimental en dos caras del mismo diente y se almacenaron en saliva artificial a 37°C. Se midió la mineralización con DIAGNOdent 2095 (Kavo) sin tratamiento y después de la aplicación del mismo a 1 y 24 horas, 7 y 14 días. Se comparó la pigmentación por medio de fotografías tomadas con microscopio estereoscópico. Se sembraron en Agar Salivarius y Agar Tomate, muestras obtenidas de lesiones cariosas de una población infantil y en los mismos se realizó una prueba de inhibición de crecimiento bacteriano. Los resultados. muestran remineralización total de las muestras en ambos tratamientos sin diferencia estadística, con similitud de inhibición bacteriana, ligeramente mayor en Fluoruro Diamino en Agar Salivarius. Las zonas tratadas con el fluoruro experimental no muestran pigmentación clínica, 75% de las tratadas con Fluoruro Diamino muestran pigmentaciones obscuras evidentes. En conclusión, el desempeño remineralizante y bactericida del fluoruro experimental es competente sin el efecto pigmentante, lo que lo convierte en una alternativa viable.

Palabras clave: Compuestos de plata, nanopartículas, remineralización dental, caries dental

Resumo

Objetivo: Avaliar o efeito remineralizante, bactericida e de pigmentação produzido por uma solução de fluoreto de sódio com nanopartículas de prata em comparação com o tratamento com Diamino Fluoride da marca SDI. Foram utilizados 40 dentes extraídos, desmineralizados com ácido fosfórico, depois colocados topicamente com Diamino Fluoreto e experimentalmente colocados em dois lados do mesmo dente e armazenados em saliva artificial a 37°C. A mineralização foi medida com o DIAGNOdent2095 (Kavo) sem tratamento e após a aplicação em 1 e 24 horas, 7 e 14 dias. A pigmentação foi comparada por meio de fotografias de microscópio estéreo. Os halos de inibição bacteriana foram testados em culturas de amostras de lesões cariosas de crianças em ágar salivarius e ágar tomate. Os resultados mostram uma remineralização total das amostras em ambos os tratamentos sem diferença estatística, com semelhança de inibição bacteriana, ligeiramente superior no Flúor Diamina em ágar salivarius. As áreas tratadas com o fluoreto experimental não apresentam pigmentação clínica, 75% das áreas tratadas com o fluoreto diamina apresentam pigmentação escura evidente. Em conclusão, o desempenho remineralizante e bactericida do fluoreto experimental é competente sem o efeito de pigmentação, tornando-o uma alternativa viável.

Palavras-chave: Pais, Compostos de prata, nanopartículas, remineralização dentária, cárie dentária

Introduction

Dental caries in children remains a major oral health issue and a significant challenge for dental care. Among the minimally invasive treatments that have proven effective in preventing and arresting dental caries are the application of fluoride solutions such as silver diamine fluoride (SDF) and silver nanoparticle fluoride (SNF).[1]

The combination of fluoride with silver ions has been shown to act synergistically to halt or prevent the progression of caries: fluoride contributes to the remineralization of tooth structure, while silver, due to its antimicrobial properties, inhibits the activity of microorganisms involved in the development of carious lesions.[2,3]

SDF is primarily selected for non-cooperative pediatric patients or those with special needs, where conventional treatments are difficult due to patient-related factors. It is applied topically to the lesion, and it is essential to communicate and obtain consent because of the adverse effect of brown staining at the application site. This rep-

resents a drawback in treating the anterior region of the mouth; therefore, when choosing these treatments, the patient's needs and aesthetic concerns must be taken into account.[4]

SDF promotes remineralization due to its fluoride content (5%) and, because of its high silver content (>20%), leads to the formation of various compounds such as silver phosphate, silver oxide, and silver sulfate—all of which contribute to the staining effect.[5,6]

To avoid this effect, alternatives have been sought to obtain the benefits while minimizing or eliminating the adverse effects. One of the first approaches was the use of potassium iodide to attract the ions responsible for staining into a precipitate. However, results have shown variability depending on the depth of the lesion, and with a temporary effect.[5,7]

Recently, studies have explored various ways in which silver can be combined with fluorinated compounds through the integration of nanotechnology.[3,4,8] The results regarding staining are expected to differ due to the specific characteristics of silver arrangement and its interaction with the medium. It is known that silver in its elemental state has significant resistance to oxidation, which is not the case when present as a salt as in the case of silver nitrates or silver amines. In salt form, silver is ionized, highly reactive, and easily oxidized. Nanoscale interactions modify these oxidation-related properties: due to their small size, nanoparticles have a very high surface-to-volume ratio. In addition, their surface structure is dense and compact, which reduces reactivity and minimizes oxidation without compromising antimicrobial properties.[9,10,11]

In this study, a sodium fluoride gel with silver nanoparticles (SNF) was synthesized as a remineralizing product. A concentration of 2% sodium fluoride was used as a starting point, based on both published data and commercially available formulations in which it is considered a stable compound. For silver, previous publications using concentrations between 1 and 5% were taken as a reference.[12,13] Since the commercial SDI product contains more than 20%, a lower amount of 0.5% was selected due to the use of nanometric size. To facilitate application, carbopol was used to form a gel at different concentrations until achieving the consistency suitable for clinical placement. The objective of this study was to evaluate its antimicrobial and remineralizing efficacy compared to an SDF product from the SDI brand. To this end, inhibition halos (of microbial growth in the culture media used) and mineralization values were measured, and photographs were taken with a stereoscopic microscope to assess the resulting staining.

MATERIALS AND METHOD

The study was approved by the Bioethics Committee of the Faculty of Higher Education Zaragoza and recorded *(FESZ/CE/21-208-05)*.

EXPERIMENTAL COMPOUND SYNTHESIS

A 0.5% solution of silver nanoparticles (Aldrich, CAS 576832) was stabilized in 0.2 mg/mL polyvinylpyrrolidone. Separately, a 2% sodium fluoride solution and a 0.5% carbopol gel were prepared and mixed to obtain the final gel composition.

BACTERIAL INHIBITION TEST

Sixty selective culture media were prepared for *Streptococcus* and *Lactobacillus* 30 Salivarius agar and 30 tomato agar plates. Thirty carious lesion samples were collected from children aged 4 to 7 years using sterile swabs and stored in sterile nutrient broth as a transport medium.

Mass or uniform seeding of the culture media was performed, and three discs imbued with: 1. sterile water, 2. SDF, and 3. SNF were placed on each plate. The plates were stored in anaerobic jars and incubated at 35.5°C for 48 hours.

After the incubation period, the bacterial inhibition halos were measured using the Kirby-Bauer method, assessing the distance between colonies surrounding each disc from edge to edge.

MINERALIZATION AND STAINING TEST

An in vitro study was conducted on 40 natural teeth extracted within the previous three months, cleaned, disinfected, and stored in saline solution until use. The teeth were randomly assigned to two groups: 20 in the control group and 20 in the experimental group. Each tooth was individually labeled with a plastic tag featuring a 2 mm diameter hole, which was used for mineralization measurements and treatment application. The teeth were then stored in artificial saliva at 37°C.

The control group was labeled and stored for measurement without any treatment. In the experimental group, tags were placed on two different surfaces of each tooth, allowing both treatments to be applied to the same sample. Treatments consisted of demineralization with 37% phosphoric acid until values greater than 30 were reached, followed by topical application of the remineralizing agents for one minute: SDF on one side

and SNF on the other. After treatment, the surface was wiped with a dry cotton pellet and stored in artificial saliva at 37°C.

Mineralization values were measured using The KAVO Diagnodent 2095. In the control group, baseline measurements were taken after immersion in artificial saliva at 1 and 24 hours, 7 and 14 days. In the experimental groups, measurements were recorded at baseline, after demineralization, immediately post-treatment, and after immersion at 1 and 24 hours, 7 and 14 days.

STATISTICAL ANALYSIS

IBM SPSS v27 software was used. The Shapiro-Wilk test was applied to determine the normality of the groups (p=0.508), and one-way ANOVA was used to compare the mean values of each treatment. Tukey's multiple comparison test was also performed.

TABLE 1
Promedio en mm del tamaño del halo de inhibición.

	AGAR SALIVARIUS (DS)	AGAR TOMATE MM (DS)
Grupo Control	0	0
Grupo FD	15,45 (3.1)	16 (4.3)
Grupo FN	13,26 (4.8)	15,13 (6.0)

Results

BACTERIAL INHIBITION TEST

Inhibition produced by both treatments was slightly greater in the SDF group, with statistically significant differences observed only in Salivarius agar. The average difference in Salivarius agar was 2.19 mm (p<0.05), while in tomato agar it was 0.87 mm.

MINERALIZATION TEST

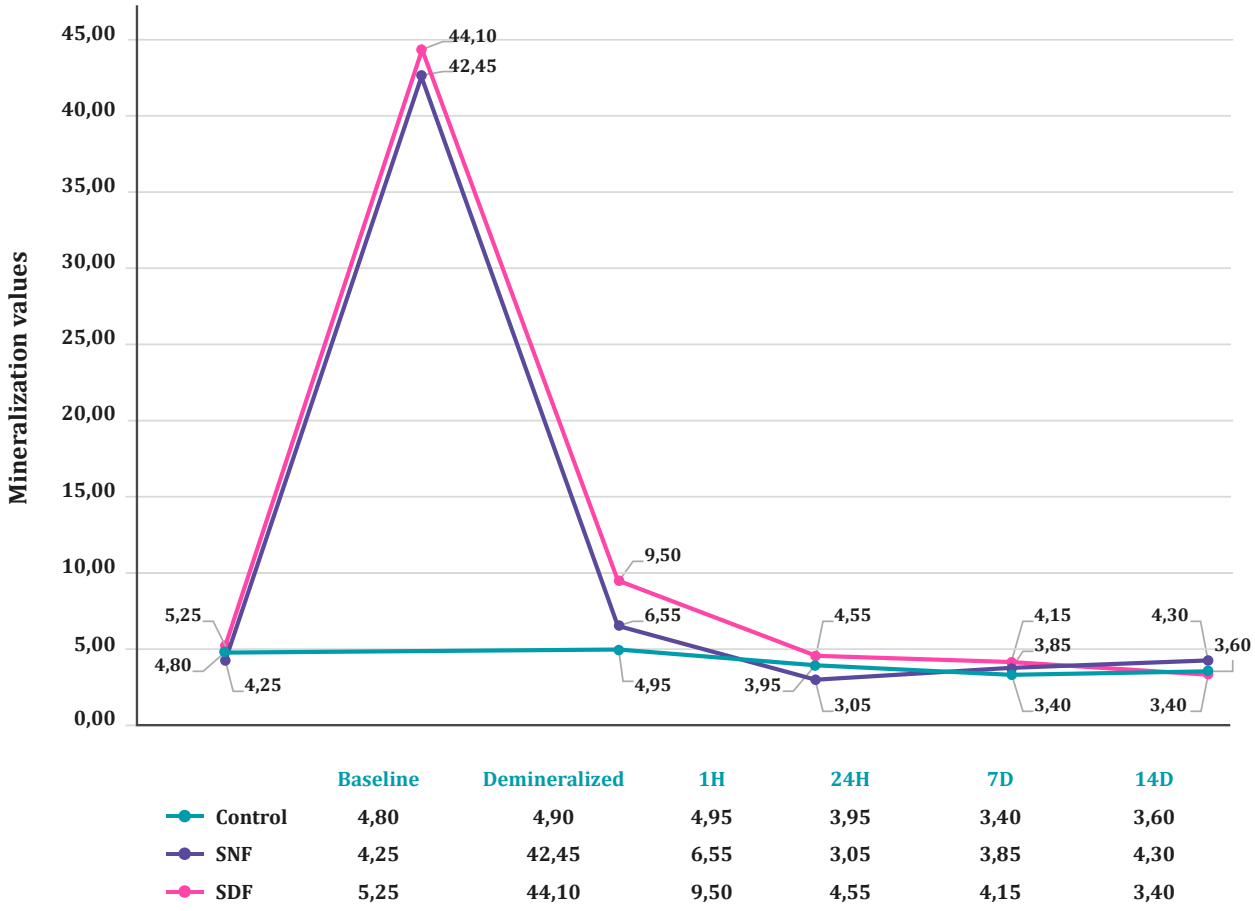
The average mineralization values are shown in Figure 1. Demineralization was greater in the SNF group (mean: 44.1) compared to the SDF group (mean: 42.5). From these values, mineralization was observed in all treated groups, with statistically significant differences (p<0.05) according to ANOVA from 1 hour to 14 days.

All experimental groups achieved successful and complete remineralization, reaching average values consistent with healthy tissue, even after exhibiting high initial demineralization. No statistically significant differences were found between the SDF and SNF experimental groups at 1 hour, 24 hours, 7 days, and 14 days (p>0.05). Although average values dropped after the first hour particularly in the SNF group they remained relatively stable thereafter during immersion, and no statistically significant differences were found when comparing the two treatments from 1 hour to 14 days.

STAINING TEST

Photographs taken 24 hours after application of the compounds show that in the SDF group Figure 2, 75% of the samples presented clinically perceptible dark staining. In photographs taken 14 days after treatment application, 95% of the SDF samples displayed clinically visible brown staining. In the SNF group, 100% of the samples showed no clinically noticeable stains 24 hours after application Figure 3. However, under 1000x magnification using a stereoscopic microscope, 40% of the SNF samples showed grayish spots scattered across the application area, which were not perceptible to the naked eye. The control group exhibited no changes and remained unaltered throughout the study.

After 14 days of immersion, the staining became more clinically evident. In the SDF group, 95% of the samples showed visible dark stains, with only one sample remaining unstained. In the SNF group, there was no evidence of dark staining. Only the circular outline left by acid demineralization was visible, demarcating the application area though in some cases the treated area appeared to blend in, unlike any of the SDF treated samples. Additionally, any pre-existing cracks or fissures in the tooth structure were further accentuated in the SDF group. Figure 4



	Baseline	Demineralized	1H	24H	7D	14D
Control	4,80	4,90	4,95	3,95	3,40	3,60
SNF	4,25	42,45	6,55	3,05	3,85	4,30
SDF	5,25	44,10	9,50	4,55	4,15	3,40

Figure 1 Average mineralization values for the groups at 1-24 hours and 7-14 days.

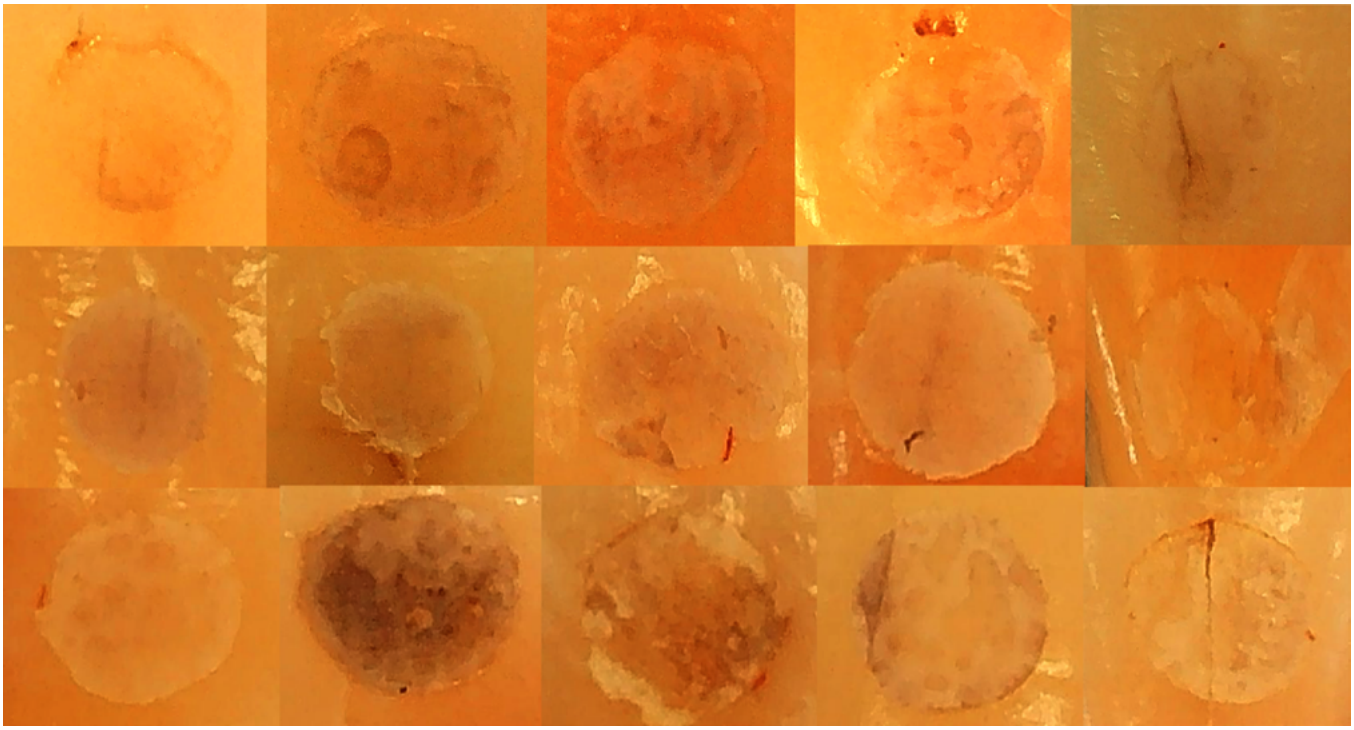


Figure 2 SDF group 24 hours after treatment application (1000x).

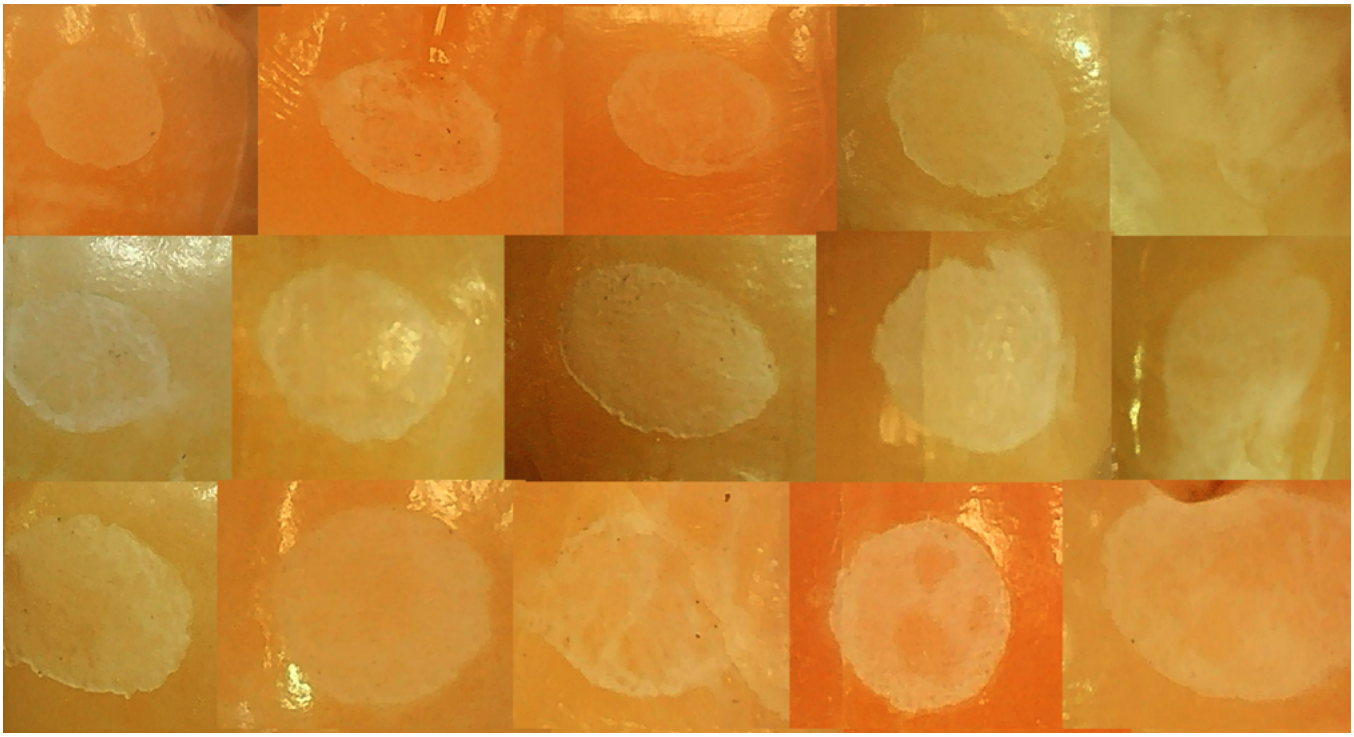


Figure 3 SNF group 24 hour after treatment application (100x).

Discussion

The use of polymer-based formulations has proven effective due to their adhesive properties[14] such as the combination of silver nanoparticles and sodium fluoride proposed in this study. Polymers not only enhance product stability and contact time,[15] but also improve ease of application, consistent with findings by Cardoso et al.[16] who noted that silver nanoparticles can be optimized when combined with polymers such as cationic polysaccharides or chitosan. These materials, like the polyvinylpyrrolidone and carbopol used in this study, are biocompatible, biodegradable, and non-toxic. Other viable alternatives include polyethylene glycol, polymethacrylic acid, and polymethyl methacrylate, allowing for formulation adjustments depending on compound availability.

Regarding silver, the bottom-up synthesis technique is the most common and cost-effective method used in commercial products derived from silver salts, producing nanoparticles ranging in size from 1 to 100 nm.[16]

For many years, preventive treatments for dental caries have focused on remineralization, with fluoride compounds showing strong effectiveness.

Silver diamine fluoride is capable of arresting the caries process, but it produces staining. For this reason, formulations using silver in nanometric form have been explored particularly antimicrobial silver solutions that avoid staining. This aligns with the work of Favaro (2022), who, like in this study, evaluated the remineralizing effects of 2% sodium fluoride combined with silver nanoparticles, achieving promising results in terms of microhardness (as an indicator of mineralization) without negative aesthetic outcomes.[17]

Similarly, Wang (2024) developed a fluoride- and silver-based material by improving silver stability, reporting favorable remineralization outcomes—comparable to the compound proposed in this study as an alternative cariostatic agent that avoids the staining commonly caused by silver diamine fluoride.[18] Salas,[19] in turn, conducted a study applying pit and fissure sealants with and without silver nanoparticles (40–80 nm in size). The samples were analyzed via fluorescence using DIAGNOdent, revealing that sealants with silver nanoparticles resulted in a threefold reduction in fluorescence thus reinforcing their remineralizing potential. This finding is consistent with this study, which demonstrated statistically significant remineralization

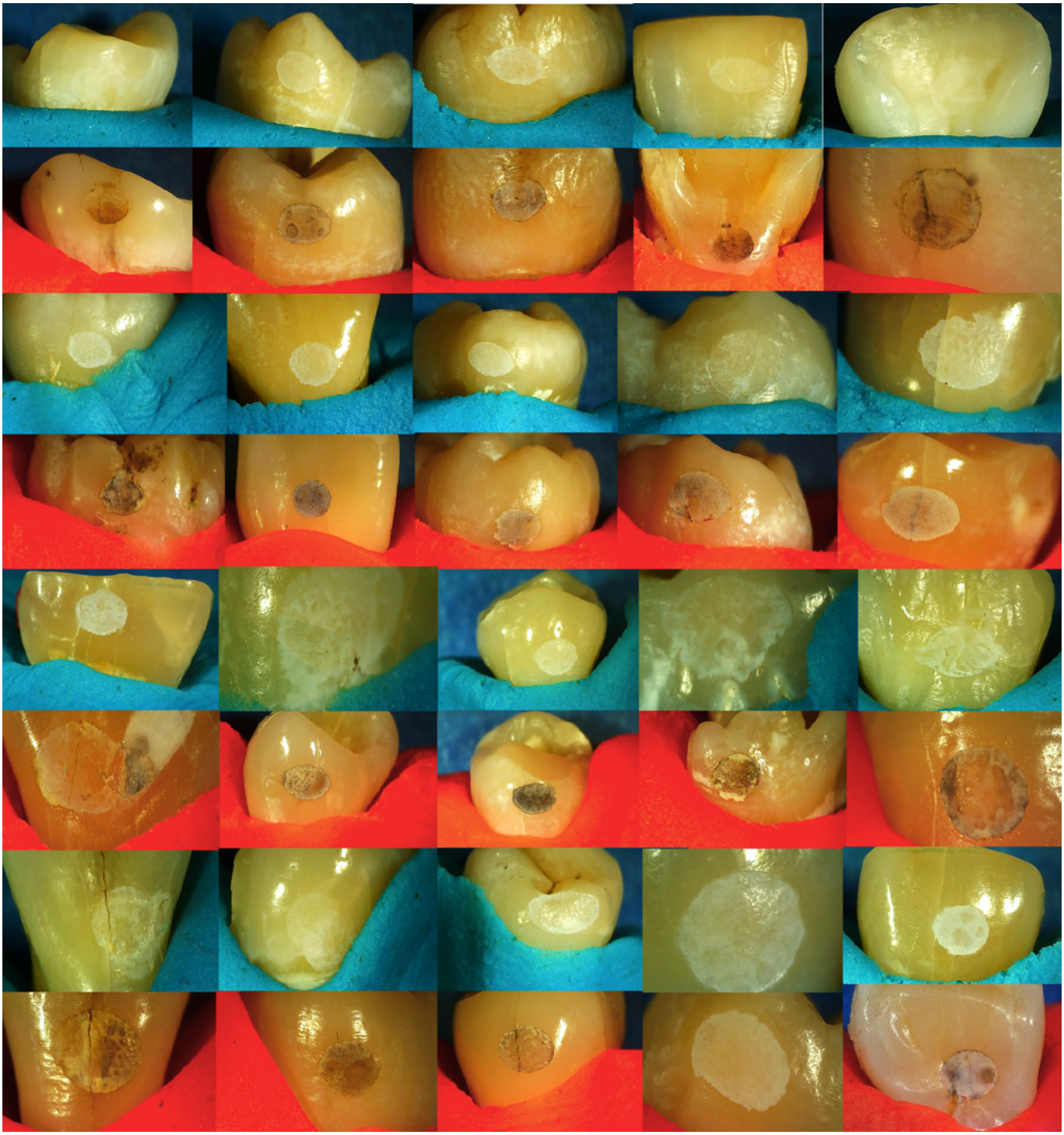


Figure 4 Photographs of the samples 14 days after treatment (100x). The SNF (blue) and SDF (red) treatments are displayed comparatively, one below the other, as they correspond to different surfaces of the same tooth.

($p<0.05$) in all treated groups from 1 hour through 14 days.

Regarding the antimicrobial effect, there is evidence supporting the bactericidal properties of silver nanoparticles, with activity against a wide range of microorganisms including viruses and fungi and particularly demonstrating effectiveness against *Streptococcus mutans*, which is associated with the beginning of caries process. The literature reports that the minimum inhibitory concentration (MIC) against *S. mutans* is 4.86 µg/mL, and the minimum bacteriostatic concentration is 6.25 µg/mL, when using nanoparticles ranging from

40 to 60 nm, respectively.[19,20] Studies such as Morones[21] have evaluated the activity of silver-based compounds, including nanoparticles, and their effects on Gram-negative bacteria within a 1–100 nm size range. These studies observed that nanoparticles adhere to bacterial cell membranes, affecting their function, permeability, respiration, and even penetrating the bacteria. The authors noted that the bactericidal properties of nanoparticles depend on their size, with only those measuring approximately 1–10 nm interacting directly with bacteria.[19] This suggests that the smaller the particle, the more effective the bacterial interaction. Since the nanoparticles in this study fall within the size range reported by the manufacturer (2–100 nm), the compound is considered effective.[22,23] Other works have demonstrated that silver-based compounds also exhibit antibacterial, antifungal, antiviral, anti-inflammatory, tissue-regenerative, antidiabetic, antioxidant, and wound-healing properties.[18, 22, 23] Silver nanoparticles exert antimicrobial effects by inhibiting bacterial growth and metabolism and disrupting transport functions at the cell wall level, ultimately leading to bacterial cell death.[14, 21, 23] The antimicrobial activity of both ionic silver and nanoparticles depends on their bioavailability, the type of target microorganism, and environmental factors that influence bacterial susceptibility such as elevated temperature or alkaline pH.[16, 20] With the particle size used in this study, antimicrobial effects were observed. However, there is a significant positive correlation between the concentration of nanoparticles and the rate of bacterial growth, which is why a concentration of 0.5% was considered appropriate for the development of the experimental formulation.

In this study, the sodium fluoride gel containing silver nanoparticles (SNF) demonstrated antimicrobial efficacy comparable to SDF formulation from SDI. The inhibition halo measurements showed very similar results in tomato agar. However, the bactericidal effect of SNF was slightly lower than that of SDF against certain microorganisms. Despite this, a key advantage of SNF lies in its significantly reduced discoloration.

There is evidence that both silver diamine fluoride and silver nitrate have bactericidal effects against *S. mutans*. Unfortunately, high concentrations, high ionic charge, and silver precipitation are associated with blackish staining and may cause pulp irritation in deep cavities due to dentin penetration. Hernández Sierra evaluated visible changes in extracted teeth using a colorimeter that measures color shifts via refraction. In this experimental setup, teeth were exposed to a toothpaste formed by mixing 98 µg/mL of silver nanoparticles (10^{-3} mol) with a 2% S-97 compound suspended in distilled water. These nanoparticles ranged in size from 40 to 80 nm, and no significant color changes were found a condition similar to that observed with our gel containing <100 nm nanoparticles, where no clinically visible staining occurred, representing a notable advantage.[24]

In this study, dental demineralization was evaluated using fluorescence measurements via DIAGNOdent and microscopy. The value of additional techniques, such as micro-computed tomography (micro-CT) and Fourier-transform infrared spectroscopy (FTIR), is also recognized for a more detailed analysis of perceptible clinical results.[7] Future studies using refractive colorimeters to analyze new samples would also be relevant.

The results obtained with the nanoparticle containing gel were comparable to those of the SDF compound, with the added benefit of not producing clinically visible staining, making it a promising alternative for cariostatic treatment.

FURTHER CONSIDERATIONS AND PERSPECTIVES

The management of dental caries requires comprehensive treatment strategies, and the use of cariostatic agents such as the one proposed in this study may support effective disease control. For improved outcomes, it is advisable to apply the proposed gel following the mechanical removal of infected dentin and subsequent sealing of the cavity with a glass ionomer-based restoration.

One of the complications reported in studies using SDF is marginal staining, which was more pronounced in cases where a stabilizing agent such as potassium iodide (KI) was not applied as a second step.[7]

While some of these considerations fall beyond the scope of this article, the results presented support the continuation of further investigations, including cytotoxicity testing of the compound and the potential use of green-synthesized nanoparticles to evaluate their effects on dentin.

Conclusion

Based on the findings and limitations of this study, it can be concluded that the SNF compound demonstrates mineralization performance comparable to SDF, with a slightly lower bactericidal effect. However, SNF does not cause clinically visible staining, making it a suitable and effective option for application on both anterior and posterior teeth.

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Data availability

All data supporting the findings of this study have been included in the article

Conflict of interest statement

The authors declare no conflict of interest.

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Authorship contribution

NAME AND LASTNAME	ACADEMIC COLLABORATION													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
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Juárez-López María Lilia Adriana	x	x		x		x		x	x			x	x	
Palma Pardínez Rosita				x		x	x		x	x			x	

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