Original Article

Comparison of the Discoloration of Artificially-Induced White Spot Lesions in Primary Teeth Following Treatment with Silver Diamine Fluoride with and without Selenium Nanoparticles: An *in vitro* Study

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ABSTRACT

Background: The application of silver diamine fluoride (SDF) is a popular approach in minimally invasive dentistry. The main limitation of the use of SDF is the dark discoloration of carious lesions due to the rapid oxidation to form Ag_3PO_4. This highlights the need to search for other antimicrobial agents that can provide antimicrobial effects while effectively minimizing SDF-induced discoloration.

Purpose: This study aimed to compare the discoloration of artificially-induced white spot lesions (WSLs) in primary teeth following treatment with silver diamine fluoride (SDF) with and without selenium nanoparticles (SeNPs).

Materials and Method: In this *in vitro* study, 36 primary canine teeth extracted for orthodontic reasons were randomly assigned to three groups (n=12) including (I) 38% SDF, (II) SDF containing SeNPs, and (III) the no-intervention control group. After artificial induction of WSLs in artificial saliva (pH=5 for 96 hours), the abovementioned solutions were applied to the teeth (depending on their group allocation) for 3 minutes, and then rinsed with water. The color parameters were measured immediately, and 14 days after the intervention via a spectrophotometer; then the color change (ΔE) was calculated. The three groups were compared via one-way ANOVA, followed by pairwise comparisons with Tukey's post- hoc test (alpha=0.05).

Results: The ΔE value (after the intervention compared with baseline) was 11.52±4.83 in the SDF, 7.40±3.76 in the SDF/SeNP group, and 0 in the control group. The difference in ΔE was significant among the three groups (p Value<0.001). Pairwise comparisons revealed significantly greater ΔE values in the SDF and SDF/SeNP groups than in the control group (p< 0.001). Additionally, the ΔE of the SDF group was significantly greater than that of the SDF/SeNP group (p= 0.020).

Conclusion: The results of this *in vitro* study revealed significantly lower discoloration of artificially- induced WSLs in primary teeth following treatment with SDF containing SeNP than following treatment with conventional SDF alone.

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Introduction

In pediatric dentistry, the cooperation of children is a key factor in the provision of appropriate treatment. Many children cooperate poorly because of their young age, cognitive problems, or previous negative experiences with dental treatment. In these situations, the use of minimally invasive treatment methods can be a suitable solution [1]. In recent years, minimally invasive dentistry has gained increasing acceptance among dentists. Accordingly, a conservative approach is adopted to assess the caries risk of each patient, with a greater focus on initial prevention. The goal of this biological approach is to preserve the sound tooth structure as well as the demineralized tissue that has the potential for remineralization [2]. Contemporary dentistry focuses on nonrestorative, noninvasive, and microinvasive treatment approaches that stop or reverse the caries process at the lesion level and minimize the loss of sound tooth structure. These treatment approaches include the use of cariostatic agents, sealants, resin infiltrants, fluoride varnishes, toothpastes, and gels [2-3]. The application of silver diamine fluoride (SDF) is also a popular approach in minimally invasive dentistry [3].

SDF, a topical fluoride solution based on metal ions, has attracted much attention as an effective agent for caries inhibition in children and adults. This compound has a pH of 10 and possesses antibacterial properties due to the presence of silver ions. Owing to its alkaline pH, SDF reacts with hydroxyapatite and produces fluorapatite crystals, which are less soluble in acidic environments than hydroxyapatite is. In addition, SDF protects dentine collagen from dissolution and decomposition. The main limitation of the use of SDF is the resulting dark discoloration of carious lesions due to the deposition of silver particles on the carious tooth structure and their rapid oxidation to form Ag3PO4. Therefore, the application of SDF, especially in the esthetic zone and anterior region, is unpleasant for most children and their parents [4–6]. Other limitations of SDF include its metallic taste, short-term black spots on the skin that resolve within 2 to 14 days, and mild painful lesions from accidental contact of the SDF solution with the oral mucosa, which usually resolve within a few days [2].

The application of potassium iodide (KI) is one suggested strategy to reduce dark discoloration following the use of SDF. However, it has been reported that the effect of KI is temporary, and yellow or brown discoloration eventually occurs. A clinical trial revealed that there was no significant difference in the discoloration of carious lesions with or without the use of potassium iodide (KI) during follow-up [7]. On the other hand, there is a concern that the effectiveness of SDF in caries cessation might be reduced if KI is used because when KI reacts with SDF, some of the silver ions react with iodine to form AgI, which is dissolved and removed from the compound [7]. This highlights the need to search for other antimicrobial agents that can provide antimicrobial effects while effectively minimizing SDF-induced discoloration.

Since the main cause of dark discoloration following the use of SDF is the oxidation of silver ions, the use of antioxidant agents (scavengers) can prevent this from occurring. Metal nanoparticles are among these agents. Owing to their strong antioxidant properties, they can prevent the oxidation of silver ions and the resulting discoloration [8].

Advances in nanotechnology have led to the development of metal nanoparticles. The antibacterial properties of metal nanoparticles are well known in the medical field [2-8]. Nanoparticles are more effective than larger particles because of their larger surface area in contact with microorganisms. Metal nanoparticles have attracted the attention of dental researchers because of their antibacterial potential and ability to be used in cariostatic approaches. In addition, they may be able to overcome the clinical limitations of SDF and minimize SDF-induced discoloration of carious lesions [2-8]. Selenium nanoparticles (SeNPs) are among the metal nanoparticles that can act as antioxidants. SeNPs added to SDF can prevent the interaction of silver with oxygen. Thus, instead of forming oxygen-containing Ag3PO4, silver and phosphorus react with fluoride to form silver hexafluorophosphate (AgPF6), which is white (unlike Ag3PO4) and is insoluble (unlike AgI). Therefore, it is not removed and participates in the reaction with hydroxyapatite and strengthens it. Recently, SeNPs have attracted the attention of medical researchers because of their good biocompatibility as well as their antibacterial, antifungal, antiviral, and antioxidant effects. SeNPs have been shown to have antimicrobial effects on numerous bacterial species. On the other hand, the antioxidant effects of SeNPs in combination with topical cariostatic agents have not been fully investigated [2-7]. Although the antibacterial effect, remineralizing potential, and discoloration effect of SeNPs have been previously evaluated, no study has added selenium to SDF solution to examine its effect on SDF-induced discoloration of the tooth structure. A review by Almuqrin *et* *al.* [9] suggested the addition of SeNPs to SDF solution to examine their effect on the degree of discoloration of carious lesions. Therefore, this study aimed to compare the discoloration of artificially-induced white spot lesions (WSLs) in primary teeth following treatment with SDF with or without SeNPs. The null hypothesis of the study was that no significant difference would be found in the color change (ΔE) of WSLs treated with SDF or SDF/SeNPs.

Materials and Method

This *in vitro* experimental study evaluated human primary canine teeth extracted for orthodontic reasons. The study protocol was ethically approved by the University Ethics Committee (IR.MUI.DHMT.REC.1404.087). For the initial assessment, a pilot study was conducted prior to the design of the present study via a similar methodology. 38% SDF alone was applied to one primary canine tooth, and 38% SDF containing SeNPs was applied to another primary canine tooth. The ΔE resulting from the application of SDF containing SeNPs was significantly lower than that caused by the application of SDF alone.

Eligibility criteria

The inclusion criterion was non-carious extraction of primary canine teeth with no history of previous endodontic treatment. Teeth with anomalies, hypoplastic lesions, fractures, restoration, or stainless-steel crowns were excluded. The teeth belonged to children aged 8 to 9. Teeth were collected from the dental school pediatric department.

Sample size

The sample size was calculated to be 12 in each group (a total of 36) according to a study by Aly and Yousry [10], who assumed a ΔE of 9.54±3.1 in the intervention group, 16.45±5.1 in the control group, and a minimum precision (d) of 6, using the sample size calculation formula for independent groups.

Specimen preparation

The teeth were stored in saline solution from the time of extraction until the beginning of the study; the saline solution was replaced weekly. The teeth were placed in artificial saliva with a pH of 5 for 96 hours to create WSLs, and caries progression in the teeth was monitored during this time period [11]. Then, they were vertically mounted in acrylic resin. The teeth were randomly divided into three groups (n=12) by using a table of random numbers:

- Group 1(SDF): 38% SDF solution (Cobalt Company, Iran) was applied to the teeth via a microbrush for 3 minutes [12]. The samples were then rinsed with water for 30 seconds.
- Group 2 (SDF/SeNPs): a synthesized SDF solution containing SeNPs was applied to the teeth for 3 minutes via a microbrush. The teeth were subsequently washed with water for 30 seconds.

This solution was synthesized with the help of a dental material specialist at the Cobalt Company. For this purpose, selenium nanoparticles (SeNPs) with a diameter of 81 nm were added to 38% SDF solution at a concentration of 25 mg/mL.

- Group 3(Control): no intervention was performed on the teeth in this group, and they underwent baseline color assessment with no intervention. The teeth were incubated at 37°C with 100% humidity until follow-up.

Organic extraction of selenium from broccoli (green synthesis)

Broccolis were dried in the shade for 3–4 days. Next, the dried broccoli pieces were ground into a fine powder. Subsequently, 70 mL of distilled water was added to 0.7 g of ground broccoli powder. The mixture was kept at 60–70°C for 10 minutes. The mixture was subsequently boiled and filtered through Whatman No. 1 filter paper. SeNPs synthesized from broccoli extract were added to 30 mM sodium selenite and then centrifuged at 8000 rpm for 10 minutes. The obtained SeNP pellets were powdered in a hot air oven at 70°C for 2 hours and stored in sealed vials for further use [13].

Color measurement

In all three groups, colorimetry was performed in three stages, namely, at baseline (before discoloration), after discoloration, and after 2 weeks [14]. Since the color of the teeth became stable 2 weeks after the application of SDF, the teeth were closely monitored for 2 weeks after SDF application. The color parameters of the teeth were measured via a spectrophotometer (VITA Easyshade; Vident, CA, USA), which was calibrated according to the manufacturer's instructions. Accordingly, the color parameters of L* (lightness), a* (red-green) and b* (blue-yellow) were recorded via the CIE L*a*\b* color system. The ΔE of the teeth was then calculated according to the following formula:

Table 1: Measures of central dispersion for ΔE1 and ΔE2 in the three groups (n=12)

Parameter	Group	Mean	Std. deviation	Minimum	Maximum	95% CI	
						Lower bound	Upper bound
ΔE1	SDF	11.52	4.83	4.89	18.96	8.45	14.60
	SDF/SeNPs	7.40	3.76	2.60	12.82	5.01	9.75
	Control	0	0	0	0	0	0
ΔE2	SDF	13.28	4.81	5.98	20.25	10.22	16.34
	SDF/SeNPs	9.09	3.10	4.01	13.84	7.11	11.06
	Control	0	0	0	0	0	0

ΔE1: Color difference Immediately after the application of solutions compared with baseline; ΔE2: Color difference Two weeks after the application of solutions compared with baseline (SDF: Silver Diamine Fluoride, SeNPs: Selenium Nano-Particles)

$\Delta E=(L1-L2)2+(a1-a2)2+(b1-b2)2$

ΔE was calculated for each group by comparing the color parameters (L, a, b) of each stage with those of the previous stage via the abovementioned formula.

- ΔE1: Color difference of the samples after preparation and application of the solutions compared with the baseline (before the application of solutions)
- ΔE2: Color difference of the samples at 2 weeks after the application of the solutions compared with the baseline value (before the application of solutions)

Data analysis

The data were analyzed via SPSS version 26. One-way ANOVA was used to compare the groups at each time point, while a paired t-test was used for the comparison of groups over time. *p* Value<0.05 was considered statistically significant.

Results

Table 1 presents the measures of central dispersion for ΔE1 and ΔE2 of the three groups. As shown, ΔE1 was the highest in the SDF group and the lowest in the control group. One-way ANOVA revealed a significant difference in ΔE1 among the three groups ($p< 0.001$). Pairwise comparisons via Tukey's test revealed that the mean ΔE1 in both the SDF ($p< 0.001$) and SDF/SeNP ($p< 0.001$) groups was significantly greater than that in the control group. Additionally, the mean ΔE1 in the SDF group was significantly greater than that in the SDF/SeNP group ($p= 0.020$).

ΔE2 was also the highest in the SDF group and the lowest in the control group. One-way ANOVA revealed a significant difference in ΔE2 among the three groups ($p< 0.001$). Pairwise comparisons via Tukey's test revealed that the mean ΔE2 in both the SDF ($p< 0.001$) and SDF/SeNP ($p< 0.001$) groups was significantly greater than that in the control group. Additionally, the mean ΔE2 in the SDF group was significantly greater than that in the SDF/SeNP group ($p= 0.011$).

Table 2 shows the measures of central dispersion for the L* parameter in the study groups immediately and at 2 weeks after the application of the solutions.

Within-group comparisons of ΔE1 and ΔE2 via paired tests are presented in Table 3. As shown, ΔE2 was significantly lower than ΔE1 in the SDF group ($p= 0.044$). However, this difference was not significant in the SDF/SeNP group ($p= 0.166$).

Discussion

This study compared the discoloration of artificially-induced WSLs in primary teeth following treatment with SDF with or without SeNPs. Between-group comparisons revealed significantly lower discoloration both immediately after and 2 weeks after the application of

Table 2: Measures of central dispersion for the L* parameter (lightness) of the three study groups (n=12) immediately and 2 weeks after the application of solutions

Time	Group	Mean	Std. deviation	Minimum	Maximum	95% CI	
						Lower bound	Lower bound
Immediately	SDF	75.93	6.16	70.66	80.85	75.01	79.85
	SDF/SeNPs	78.93	5.86	68.50	85.30	75.20	82.66
	Control	79.40	4.51	71.90	85.60	76.53	82.26
2 weeks	SDF	69.78	5.16	59.10	74.70	64.50	71.06
	SDF/SeNPs	79.43	5.49	68.50	84.20	75.94	82.92
	Control	79.40	4.51	71.90	85.60	76.53	82.26

SDF: Silver Diamine Fluoride, SeNPs: Selenium Nano-Particles

Table 3: Within-group comparison of ΔE1 and ΔE2 via paired t tests

Group	SDF	SDF/SeNPs
ΔE1 (mean±SD)	52.83±11.4	40.76±7.3
ΔE2 (mean±SD)	28.81±13.4	9.3±3.11
Effect Size (95%CI)	0.656 (-0.017- -1.27)	0.428 (-1.01- -0.17)
p Value	0.044	0.166

ΔE1: Color difference Immediately after the application of solutions compared with baseline; ΔE2: color difference two weeks after the application of solutions compared with baseline. SD: standard deviation, SDF: Silver Diamine Fluoride, SeNPs: Selenium Nano-Particles

the solutions in the SDF/SeNP group than in the SDF group. Thus, the null hypothesis of the study was rejected. Within-group comparisons also revealed no significant difference in ΔE2 compared with ΔE1 in the SDF/SeNP group, whereas this difference was significant in the SDF group. These findings highlight the superiority of SDF/SeNPs over SDF alone in terms of causing lower discoloration in WSLs artificially induced in primary teeth.

Conventional SDF remains one of the most effective minimally invasive agents for caries arrest in pediatric dentistry, particularly for young or uncooperative children. However, its major drawback is the black discoloration that results from the oxidation and reduction of silver ions within the tooth structure [15].

Clinicians have attempted to minimize SDF-induced discoloration by applying potassium iodide (KI) after SDF. Although KI can initially reduce dark staining through the formation of silver iodide, the effect appears to be temporary, and discoloration may reappear over time. Moreover, concerns have been raised that KI may reduce the availability of free silver ions and potentially compromise the caries-arresting efficacy of SDF [16].

In contrast, the present study explored an alternative strategy based on antioxidant modulation rather than silver precipitation. The lower ΔE values obtained with SDF/SeNPs suggest that SeNPs may represent a promising adjunct capable of reducing discoloration without relying on the removal of silver ions from the formulation. According to a study by Huang et al. [13], selenium at a concentration of 25 mg/mL is safe for mammalian cells and has acceptable antibacterial and antioxidant effects.

The absence of a significant temporal change in the SDF/SeNP group may suggest that SeNPs produce a more stable optical outcome immediately after application.

SeNPs possess additional characteristics that may be beneficial in cariology, including antibacterial, antifungal, antiviral, and antioxidant effects, together with favorable biocompatibility. Nanoparticles generally exhibit enhanced antimicrobial activity because of their high surface-area-to-volume ratio, which increases their interaction with microorganisms [17]. Consequently, SeNPs may provide dual benefits when incorporated into SDF including reduction of discoloration and reinforcement of antimicrobial efficacy.

Deepak and Priya [18] evaluated the ability of a selenium-containing primer to prevent WSLs, and reported that DenteShield™ selenium-containing primer, with its antimicrobial properties, inhibited biofilm formation and prevented the formation of WSLs without negatively affecting the shear bond strength. Although they evaluated a different variable, their results supported the incorporation of SeNPs in dental materials.

Notably, unlike in previous studies, SeNPs were obtained from broccoli organically through green synthesis in the present study. The production of metal nanoparticles through plant extracts (green synthesis) has been found to be useful in the prevention and treatment of many oral and dental disorders. Many problems, such as drug resistance, may be overcome by using the green synthesis route of nanoparticles [19]. Additionally, silver nanoparticles produced via green synthesis and from plant extracts are more biocompatible and cost-effective than silver nanoparticles produced via chemical methods. Therefore, silver nanoparticles produced from plant extracts can be used as antimicrobial and cariostatic agents in the management of oral diseases, and can also cause less discoloration [20]. Scarpelli *et al.* [21] evaluated the remineralization potential and antimicrobial activity of silver nanoparticles and reported that nanoparticles added to silver particles were able to inhibit 100% of microorganisms at a lower concentration than other antimicrobial agents and caused less discoloration. The results of a systematic review by Khan and Nanmaran [22] on the applications and recent developments of SDF and its effects on primary and permanent teeth revealed that SDF has potential for use in various aspects of dental caries management, endodontic irrigation, and indirect pulp therapy due to its various desirable properties. A clinical study by Ammar *et al.* [23] evaluated the effects of nanosilver fluoride and SDF on dental caries in the primary teeth of children aged 4–6 years, and revealed that SDF had an inhibitory effect on *Streptococcus mutans* and lactobacilli, and decreased carious lesions by two-thirds.

This study had an *in vitro* design, which limits the generalizability of the findings to the clinical setting. The clinical oral environment was not well simulated in this study in terms of pH changes, thermal alterations, the presence of saliva, and oral hygiene practices by patients. Thus, future *in vitro* studies are needed to better simulate the clinical setting and obtain more generalizable results. Clinical trials are also needed to verify the present findings. The efficacy of the addition of other nanoparticles, such as nanohydroxyapatite, to SDF should also be investigated.

Conclusion

The results of this *in vitro* study revealed significantly lower discoloration of artificially-induced WSLs in primary teeth following treatment with SDF containing SeNPs than following treatment with conventional SDF alone.

Conflict of Interest

The authors declare no conflict of interest in this study.

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