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Dentin Staining Caused by Nano-silver Fluoride: A Comparative Study

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Clinical Relevance

The new formulations of nano-silver fluoride caused less dentin staining than the already available commercial agents 35% silver fluoride and silver diamine fluoride at 30% and 38%.

SUMMARY

The objective of this study was to evaluate the dentin staining potential of nano-silver fluoride (NSF 600 and 1500 ppm) compared with the following commercially available cariostatic agents: Advantage Arrest (Elevate Oral Care, West Palm Beach, FL, USA), Riva Star (SDI, Victoria, Australia), and Cariestop (Biodinâmica, Paraná, Brazil). Seventy-five extracted human molars were sectioned at the cementoenamel junction, and the occlusal enamel was removed for exposure of coronary dentin. The samples were divided among the five agents tested (n=15). The dentin staining (ΔE/ΔL) was analyzed with a digital spectrophotometer (VITA Easyshade, VITA Zahnfabrik, Bad Säckingen, Germany) at three different time points (before application, after two weeks, and after four weeks). Photographic images were also performed. The Kruskal-Wallis and Mann-Whitney tests compared the mean ΔE and ΔL values between groups. The NSF 600 and 1500 ppm resulted in the smallest color change (ΔE=1.02 and 1.53) and dentin staining after four weeks (ΔL=−0.76 and −1.2). The new formulations differed significantly from the commercial cariostatic agents ($p<0.001$). NSF might be an alternative to silver diamine fluoride since it does not compromise esthetics.

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INTRODUCTION

Dental caries is the most prevalent oral disease in the world, affecting all countries.[1,2] Oral hygiene is the most efficient method to prevent and control the disease.[3] However, several therapies have been proposed to reverse the carious process, including the topical application of fluoride, chlorhexidine, fluoride varnish, and silver diamine fluoride (SDF).[4-6] The last is a conservative, efficient, and less invasive alternative,[7-10] but its application can stain the tooth, compromising esthetics.[11-13]

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The mechanism of action of SDF consists of the interaction of silver ions (Ag+) with the sulfhydryl groups of proteins and DNA of bacteria. This alters hydrogen bonding and thereby inhibits respiratory processes, cell wall synthesis, and cell division, which cause the death and eventual lysis of the cell.[14-16] However, remnant silver particles can precipitate and form a layer of silver phosphate on the carious dentin.[17,18]

The application of potassium iodide (KI) immediately after the use of SDF has been proposed to minimize this adverse effect.[11,17] In addition, new formulations based on silver nanoparticles also have been evaluated for their use in stain reduction.[19,20]

Smaller silver particles would be more efficient because of their greater surface area per volume, increasing the contact with microbial cells.[19,21-24] A new formulation, called nano-silver fluoride (NSF), contains silver nanoparticles, chitosan, and fluoride. This compound thus combines preventive and antimicrobial properties and promises not to cause tooth staining because of the size of its particles and because it does not undergo oxidation.[19,21,25,26] This new substance is safe to be used in humans, and its production costs are low.[25,27] In addition, it has shown good results in laboratory and clinical studies.[19,21,25-27] However, it remains to be established whether NSF causes less staining of permanent teeth.

Therefore, the objective of this study was to evaluate the dentin staining caused by NSF compared with other cariostatic agents (30% and 38% SDF and 35% silver fluoride). The null hypothesis was that there is no difference in the dentin staining between the cariostatic agents tested.

METHODS AND MATERIALS

Selection of the Sample

The sample calculation was based on the results of Sayed and others.[28] The mean ΔE and standard deviations at 14 days in the dark for SDF and SDF+KI were used for calculation. Sample calculation was performed using BioEstat 5.3 (Instituto de Desenvolvimento Sustentável Mamirauá, Manaus, Brazil). A 4.96 minimal difference was considered between mean ΔE of the treatment groups with a standard error of 0.55; six treatment groups were considered with an alpha type error of 0.05 and a beta power of 0.80. To note significant differences, the ideal sample size per group was four. A sample size of 15 was used to compensate for possible outlier values that might lead to sample loss.

Seventy-five permanent human molars extracted for therapeutic reasons were used. The teeth were examined under a stereomicroscope (40×). Carious teeth, teeth with restorations, and teeth with fractures, cracks, or fissures in the coronary portion were excluded.

Sample Preparation

The teeth were disinfected with 0.5% chloramine-T solution at 4°C for one week (ISO 11405:2003) and stored in distilled water (4°C) until the beginning of sample preparation. The teeth were cleaned with periodontal curettes, followed by prophylaxis with a pumice slurry and brush. The samples were cross-sectioned at the cementoenamal junction to access the pulp chamber. After cleaning the pulp chamber, the occlusal enamel was removed with a diamond disc in a cutting machine under refrigeration (Isomet, Buehler Ltd, Lake Bluff, IL, USA). The samples were embedded in PVC cylinders with acrylic resin with the occlusal surface facing upward.

Sample Treatment

The samples were randomly allocated into five groups according to the type of treatment (n=15). The products were applied as recommended by the manufacturers (Table 1). One drop of each agent was applied on the dentin surface followed by an active application with a microbrush for one minute. After treatment with the agents, the samples were placed in hermetically sealed containers and kept in artificial saliva (1% carboxymethylcellulose, 0.12% potassium chloride, 0.005% magnesium chloride, 0.18% methylparaben, and 100 mL of deionized water)[29-31] and changed every seven days. At each evaluation period, the samples were removed from artificial saliva, washed with 50 mL of distilled water, and dried with a gentle air jet.

Before the four-week assessment, samples were submitted to a brushing cycle. The brushing was performed in a simulated brushing machine (ElQuip, São Paulo, Brazil). Samples were fixed to the mechanical devices, and Colgate Classic Clean soft bristle toothbrushes (Colgate-Palmolive, São Paulo, Brazil) were used. Brushing was performed only with distilled water, and no abrasive agent was used. A load of 200g was applied with brushing speed of 250 cycles per minute for three minutes. This step aimed to analyze if brushing can reverse the staining caused by agents.

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Table 1: *Materials, Composition, and Application Mode as Recommended by the Manufacturer*

Cariostatic Agent	Composition	Application
NSF 600: nano-silver fluoride, 600 ppm (CETENE, Pernambuco, Brazil	Silver nanoparticles, chitosan and sodium fluoride	Active application of one drop of the agent with a microbrush for 1 min
NSF 1500: nano-silver fluoride, 1500 ppm (CETENE, Pernambuco, Brazil)	Silver nanoparticles, chitosan, and sodium fluoride	Active application of one drop of the agent with a microbrush for 1 min
SDF 30: silver diamine fluoride, 30% (Cariestop, Biodinâmica, Paraná, Brazil)	Hydrofluoric acid, silver nitrate, ammonia hydroxide, and deionized water	Active application of one drop of the agent with a microbrush for 1 min
SDF 38: silver diamine fluoride, 38% (Advantage Arrest, Elevate Oral Care, Florida, USA)	Aqueous silver diamine fluoride, deionized water, and FD&C Blue 1	Active application of one drop of the agent with a microbrush for 1 min
SF: silver fluoride 35% (Riva Star, SDI, Victoria, Australia)	Silver particles, iodine, and fluoride + potassium iodide	Application of one drop of the content of the silver capsule with a specific applicator brush for 1 min; application of one drop of the content of the green capsule for 1 min

Color Measurement

Three color measurements were performed per sample: at baseline (0; before application of the cariostatic agent), after two weeks, and after four weeks (after a brushing cycle) from application. Dentin staining was determined based on the parameters of the CIELAB system (L*a*b*), in which L* corresponds to lightness and the mean value ranges from 0 (black) to 100 (white). The value of a* corresponds to the red-green axis and b* to the yellow-blue axis. Dentin staining was determined through ΔE ($\Delta E=[(L^*_2-L^*_1)^2+(a^*_2-a^*_1)^2+(b^*_2-b^*_1)^2]^{1/2}$) and ΔL ($\Delta L^*=L^*_2-L^*_1$). The degree of staining was evaluated within the following time points: ΔL, zero to two weeks and zero to four weeks.

Objective color measurement was performed with the VITA Easyshade device. Two-color measurements per block were obtained at each time point. The analyzed area was standardized using a custom thermo-formed tray with a 1-mm acetate plate. A hole was made at the central area of the tooth corresponding to the diameter of the spectrophotometer tip.

The mean ΔE and ΔL values were compared between groups using the Kruskal-Wallis test, followed by the Mann-Whitney test ($p=0.05$).

Photographic Images

To observe the dentin staining, the samples were photographed at the same time intervals (baseline, two weeks, and four weeks) using an EOS Rebel T6 digital camera (Canon, Tokyo, Japan), EF 100-mm f/2.8 Macro USM Lens (Canon), and MR-14EX II Macro Ring Lite Flash (Canon). Established parameters were used to standardize image acquisition: ISO 100, F22 aperture, and TTL flash mode at maximum power. The photographs were obtained at the same time of day (14:00 hours). A 40-cm distance between the lens and samples was established. The samples were placed on a white surface near the window with natural light, and the photographs were taken.

RESULTS

The staining of all experimental groups at different time points is shown in Table 2. Differences were observed for ΔE between Advantage Arrest and Cariestop groups at the intervals of zero to two weeks ($p=0.045$) and zero to four weeks ($p=0.041$). However, when ΔL was evaluated separately, differences were observed only between zero and four weeks ($p=0.031$).

Riva Star resulted in similar ΔE and ΔL as NSF 600 at the interval of zero to two weeks ($p=0.713$ and $p=0.724$, respectively). At the interval of zero to four weeks, more significant modifications were observed for Riva Star when compared with the NSF 1500 and NSF 600 groups. However, the intensity was still lower than the one observed for the Advantage Arrest and Cariestop groups.

Samples treated with NSF 1500 exhibited more significant staining than those treated with NSF 600 at the interval of zero to two weeks. At four weeks, the results obtained for the two groups were close to zero, indicating a small variation. No statistically significant differences were observed between these groups in the ΔE and ΔL evaluation ($p=0.081$ and $p=0.270$, respectively).

The photographic images can be seen in Figure 1. After two weeks, all samples exhibited some degree of staining when compared with the baseline assessment. However, after the brushing cycle and

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Table 2: *Mean (Standard Deviation) of Dentin Staining ΔE and ΔL According to the Cariostatic Agent and Assessment Time (n=15)*[a]

Cariostatic Agent	$\Delta E_{0\text{-}2w}$	$\Delta L_{0\text{-}2w}$	$\Delta E_{0\text{-}4w}$	$\Delta L_{0\text{-}4w}$
Advantage Arrest	49.22 (8.62) A	−47.62 (8.67)A	52.95 (7.47) A	−51.69 (6.73) A
Cariestop	41.56 (9.32) B	−40.01 (9.87) A	47.15 (7.85) B	−45,11 (8.10) A
NSF 1500: nano-silver fluoride, 1500 ppm	17.97 (3.37) C	−18.95 (7.84) B	1.53 (0.89) C	−1.2 (1.08) C
NSF 600: nano-silver fluoride, 600 ppm	7.35 (3.23) D	−6.55 (3.36) C	1.02 (0.77) C	−0.76 (6.17) C
Riva Star	8.25 (4.49) D	−7.76 (6.17) C	30.27 (8.24) D	−29.93 (9.36) B

[a] *0-2w = 0 to 2 wk; 0-4 w = 0 to 4 wk. Negative values indicate staining. Different letters indicate significant differences between groups (columns) (Mann-Whitney test).*

color measurement after four weeks, the color was reestablished in the NSF 600 and NSF 1500 groups compared with baseline.

DISCUSSION

The hypothesis that no difference exists in the dentin staining between the cariostatic agents evaluated was rejected. Evaluation of ΔE and ΔL after the brushing cycle at four weeks showed that the nanoparticle formulations resulted in less staining of the teeth ($p<0.001$).

SDF is a cariostatic agent that has been investigated for years because of its proven clinical efficacy.[7,32] The good results obtained with this agent have encouraged studies aiming at the reduction of its adverse effects.[33] Parental acceptance was evaluated in New York City about the use of SDF for the treatment of caries in schoolchildren.[12] In that study, 32.5% of the participants classified the staining in posterior teeth as unacceptable, and 70.3% classified the esthetics of treated anterior teeth as unacceptable. These data suggest that, although effective, the use of SDF is limited and encounters resistance due to esthetic concerns.

The application of KI immediately after the use of SDF has been proposed to minimize the staining.[1,17] An *in vitro* study of extracted human molars evaluated tooth staining after treatment with SDF or SDF+KI by digital spectrophotometry and visual evaluation after four weeks of follow-up. The authors observed that the combined application of KI reduced or even prevented tooth staining, with the groups treated only with SDF being visibly darker (more significant variation in ΔL).[17] Once in contact with the silver particles, KI gives rise to silver iodide, which has a white-yellow color.[18] These results are consistent with the present study. Riva

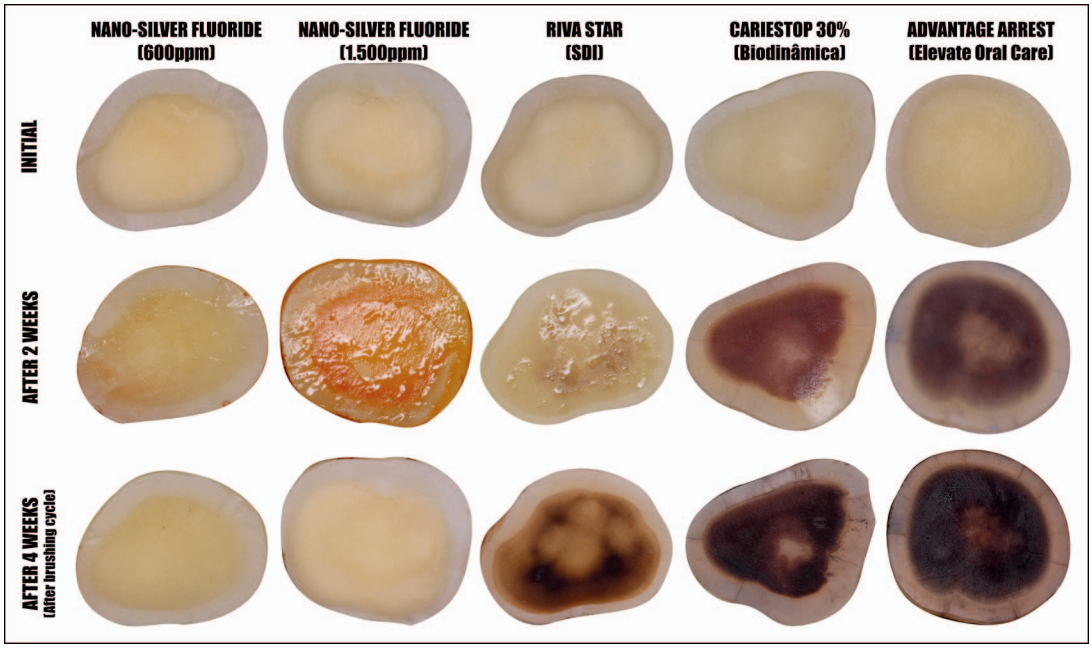

Figure 1. *Photographic images of the observed dentin staining*

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Star (SDI) consists of two capsules: one containing silver fluoride and the other KI. This agent resulted in less staining of the teeth after four weeks when compared with Advantage Arrest (SDF 38%) and Cariestop (SDF 30%), which do not contain KI ($p<0.001$). However, a certain degree of dentin staining that could compromise esthetics could still be observed for Riva Star (Figure 1).

This staining is the result of the precipitation of silver particles through the formation of a layer of silver phosphate on the carious dentin followed by oxidation of the particles.[17,18] Patel and others,[18] who evaluated staining as a function of time, found that the black staining of carious dentin was clinically noticeable within two minutes after application of the agents. A relatively constant increase in the level of staining was observed after five minutes, and a deep black stain was detected on the dentin and enamel four to six hours after application. These results agree with the findings of the present study, in which time was the determinant factor for the extent of dentin staining in the case of the commercial agents. Regarding the experimental NSF formulations, the ΔE and ΔL data and photographic findings indicated minimal staining after four weeks. NSF does not form oxides on contact with the oxygen of the medium and therefore does not cause dentin staining.[25]

Evaluation after two weeks showed that NSF 600 and NSF 1500 caused a yellowish stain on the teeth, resulting in a decrease in lightness in these groups (−6.55 and −18.95, respectively). However, the brushing cycle seems to have removed the stained layer as a film, and the undesired staining effect and lightness returned to the baseline values (−0.76 and −1.2, respectively). On the other hand, all other groups reduced luminosity even after the brushing cycle. The presence of chitosan in the composition may have favored the formation of this film. Chitosan is a cationic biopolymer that is obtained by the deacetylation of chitin and is the second most abundant polymer found in nature.[35] In an aqueous medium, chitosan tends to agglomerate and adhere to the surface.[34] However, this film is easily removed, even with gauze. Samples were submitted to a short brushing cycle (three minutes) simulating clinical conditions to standardize the removal of the superficial pigments. When added to NSF, chitosan acts as a nanoparticle carrier. In addition, chitosan has a synergistic effect due to its antibacterial and remineralizing properties.[19,36]

Studies that evaluate tooth color variations, either bleaching or staining potential, have reported the results using ΔE and ΔL.[11,17,18,37,38] Both measures were used in this study, with approximate results. This finding may be related to the fact that the cariostatic agents cause dental darkening. Therefore, the luminosity (L*) was the color axis of the CIELAB system that mostly varied since it considers the variation between white and black.[37]

A digital spectrophotometer was used for the evaluation of dentin staining since the perception of colors by visual analysis is prone to subjective interpretation and varies among different observers. Visual color evaluation can be affected by factors such as lighting and human physiological variables (fatigue, age, and emotional state), while the spectrophotometer provides precise and accurate color measurements of teeth.[39,40] A previous clinical study compared three different methods for color evaluation (visual, spectrophotometer, and digital photography) of the right upper incisors of 50 patients. The authors observed a high agreement rate between digital methods and concluded that digital photography is a reliable method for color selection.[41] Hence, in the present study, dentin staining was objectively evaluated (spectrophotometer) and visually registered with photographs.

In clinical studies evaluating tooth staining caused by cariostatic agents, the researchers respond objectively only with yes/no (does cause or does not cause staining).[18,25] The photographic analysis allows the evaluation over time through visual monitoring as well as analysis of the gray scale.[18] Despite the illustrative nature of the photographic assessments in the present study, the findings were consistent with the quantitative approach to color measurement with a digital spectrophotometer.

CONCLUSIONS

The new formulations (NSF 600 and 1500 ppm) caused less dentin staining than the already available commercial agents 35% silver fluoride, 30% SDF, and 38% SDF. NSF might be an alternative to SDF since it does not compromise esthetics.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ethics Committee on Research

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Involving Humans of the University of Pernambuco. The approval code issued for this study is 2.577.182.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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