Research Article

Effect of three chemical agents on stain removal from dentin caries lesions treated with silver diamine fluoride

Basílio Rodrigues Vieira[a], Sara Brito Silva Costa Cruz[a], Cristiane Araújo Maia Silva[a], Frank Lippert[b], Adam Kelly[b], Anderson Takeo Hara[b], Frederico Barbosa de Sousa[c]

[a] Graduate Program in Dentistry, Health Sciences Center, Federal University of Paraiba, João Pessoa, Paraiba, Brazil.

[b] Department of Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, Indianapolis, IN, USA.

[c] Department of Morphology, Health Sciences Center, Federal University of Paraiba, João Pessoa Paraiba, Brazil.

Short Title: Whitening agents on SDF-treated dentin caries

Corresponding Author:

Full name: Frederico Barbosa de Sousa

Department: Department of Morphology, Health Sciences Center,

Institute/University/Hospital: Federal University of Paraiba

Street Name & Number: Cidade Universitaria s/n

Telephone number: 55 83 32167254

City, State, Postal code, Country: João Pessoa, Paraiba, 58051-900, Brazil

E-mail: fredericosousa@hotmail.com

Number of Tables: 2.

Number of Figures: 2.

Word count: 4.208.

Keywords: Silver Diamine Fluoride; Root caries; Tooth Bleaching;

Abstract

Introduction: this in vitro study aimed to evaluate the effect of three different chemical agents on stain removal and mineral uptake of artificial dentin caries (ADC) lesions treated with silver diamine fluoride (SDF). Methods: baseline L*a*b* values were determined in polished human permanent dentin blocks, and ADC lesions were induced with an acid gel for 1 week. Samples were assigned to four groups: three groups where half of each sample received SDF (30% SDF for 3 min), while the other half received SDF followed by a bleaching treatment protocol (garlic extract, bentonite, or 35% hydrogen peroxide). The fourth group had one SDF-treated half and one half without SDF. Colour changes (ΔE) were measured by spectrometry, and transversal microradiography was used to quantify integrated mineral loss (ΔZ) 24h after treatment (SDF or SDF+bleaching). A two-way mixed ANOVA was applied. 30% Results: SDF application increased mineral uptake by ADC ($p = 0.001$). The type of chemical agent evaluated ($p<0.0001$), time ($p=0.01$) and their interaction ($p<0.0001$) bleached the ADC treated with SDF. However, 35% hydrogen peroxide was the only compound with a bleaching effect ($p < 0.001$), without returning to baseline colour. None of the compounds altered the mineral uptake effect of SDF ($p = 0.30$). Conclusion: this in vitro study showed mineral uptake effect in ACD within 24h after SDF application and the ability of hydrogen peroxide to partially remove (reduction of 24%) the staining caused by SDF without affecting its mineral uptake effect.

Introduction

Silver diamine fluoride ($Ag(NH_3)_2F$) (SDF) is among the noninvasive approaches available for managing untreated caries [1]. SDF is a basic solution (pH of 10-12) containing silver, fluoride, and ammonia. Silver acts as an antimicrobial, while fluoride is present in high concentration (44,800 ppm F) to promote remineralization. Such products have the ability to reduce the growth of cariogenic bacteria, prevent degradation of collagen in dentin, inhibit demineralization, and promote remineralization of enamel and dentin [2]. The main reaction products of SDF with teeth and saliva are fluor-hydroxyapatite, calcium fluoride, silver chloride [3], and silver phosphate (Ag_3PO_4), the latter being responsible for the darkening effect on hard dental tissues [4].

The main limitation of SDF use in the management of caries lesions is darkening of the dental hard tissues [5]. When it comes to managing caries lesions in children, a high degree of unsatisfaction in relation to tooth darkening caused by SDF application has been reported from both parents and patients [6]. Due to this frequent complaint, new strategies for improving the aesthetic aspect of SDF-treated teeth have been devised. The use of potassium iodide (KI) has been recommended after the use of SDF to reduce the degree of dark staining [7]. According to some reports, immediately after the application of KI, there was a considerable decrease in the dark stains caused by Ag_3PO_4 [8]. However, systematic reviews have not provided scientific support for the whitening effect of SDF-treated caries lesions [9-10].

Formation of silver phosphate requires interaction of SDF with phosphate ions. Considering a solubility product of 8.89×10^{-17} for silver phosphate, ~ 0.04 mM of phosphate are required in the liquid phase to reach equilibrium [11]. Saliva provides a relatively high amount of inorganic phosphorus (2-22 mM) [12], thus favoring silver phosphate precipitation and, hence, the darkening of SDF-treated caries lesions. Most of the available evidence from in vitro studies related to the effect of whitening agents on the stain removal from SDF-treated caries lesions rely on methods that did not provide sources of phosphate in the liquid phase contacting caries lesions after SDF application [2, 8, 13-16], thus underestimating the darkening effect cause by SDF application. Another gap in the literature is the lack of evidence regarding the effect of whitening agents on the mineral uptake effect of SDF.

The formation of silver phosphate could be hampered by products that bind with silver, such as bentonite (clay composed predominantly of the montmorillonite clay mineral, from the smectite group) and garlic extract. Bentonite's mechanism of action involves reacting with electrostatic charges. The negatively charged flat surface of bentonite adsorbs positively charged species. This agent can also indirectly adsorb some phenolic compounds by binding to proteins that have been complexed with the phenolic compounds, thereby promoting a clarifying effect. Bentonite is a good adsorbent, abundantly available in nature, with a low cost and high availability. Its uses include agglomeration of iron ore particles, bleaching of oils, clarification of beverages, soil waterproofing, cosmetic products, and toothpaste [17]. Natural bentonite (not thermally activated and not acid-activated) has been shown to present high adsorption capacity of silver ions [18]. Garlic is a natural compound whose composition contains phenols, which have been shown to adsorb silver ions from aqueous solutions [19], and garlic has been shown to remove heavy metals (such as copper) from aqueous solutions [20]. But no studies have tested the effects of such agents on stain removal from SDF-treated caries lesions.

Considering the gaps in the scientific literature mentioned above, the present study aimed at evaluating the effect of three different chemical agents on the colour and mineral uptake effect of ADC lesions treated with SDF.

Materials and Methods

A total of 60 extracted human molars teeth were selected from a tooth-bank at the Oral Health Research Institute, Indiana University School of Dentistry. The human dentin samples used in this study were obtained from teeth collected from dental practice clinics across the USA over several years. Based on the local Institutional Review Board (Indiana University IRB # NSO 911-07), this study was approved and exempted from requiring written informed consent from the donors. This research complied with the guidelines for human studies and was conducted ethically in accordance with the Declaration of Helsinki. In summary, all the methodology presented in this study was detailed and systematically organized in a flowchart (shown in Fig. 1.).

Sample preparation

Root dentin blocks (4×4×2 mm) were cut from the buccal and lingual faces of permanent human molars using a low-speed water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). They were flattened using a sequence of 500, 1200, 2400, and 4000 grit silicon carbide paper (MDFuga, Struers Inc., Cleveland, OH, USA), polished with 1-µm diamond suspension (Struers Inc.) and sonicated sonicated in 2% Micro 90 cleaning solution (International Products, Burlington, NJ, USA) for 3 min and rinsed with DI water after completion of the polishing procedures. The specimens were stored under wet conditions at 4 °C in a refrigerator (Kenmore; Whirlpool, Benton Harbor, MI, USA).

Color analysis

The L*a*b* (Commission Internationale de l'Eclairage) values were collected for each specimen. The measurements were made using a spectrophotometer, Minolta Chroma Meter CR-241

(Minolta Camera Co., Osaka, Japan) with an aperture size of 0.3 mm (smaller measurable circle). The equipment was previously calibrated before each use. The color measurements were taken initially after the sample preparation, after the demineralization cycle (caries induction), after 24 hours of silver diamine fluoride use, and 24 hours after the bleaching treatment. All measurements were repeated three times. The mean values of L*a*b* were measured, and the color change (ΔE = {(ΔL*)2 + (Δa*)2 + (Δb*)2}1/2) was calculated after demineralization (ΔE_{Demin}: demineralization-initial), after the use of SDF (ΔE_{SDF}: staining-demineralization), and after bleaching ($\Delta E_{Bleaching}$: staining-bleaching). The examiner who collected L*a*b* was aware of the group of each sample, but the examiner who calculated color outcomes was blinded regarding study groups.

Simulation of artificial dentin caries lesion

Prior to the experiment, the samples were fixed on resin sticks to provide support for subsequent procedures. Artificial dentin caries lesions were induced by placing the specimens in a demineralizing solution of carboxymethylcellulose (CMC), as described by Lippert et al. [21] and adjusted for dentine (shorter demineralization time). Briefly, the specimens were demineralized for 7 days in a solution containing 0.1 M lactic acid, 4.1 mM Ca (as $CaCl_2$-2H2O), 8.0 mM PO_4 (as KH_2PO_4), and 1.0% w/v CMC (Sigma-Aldrich Co., St. Louis, MO, USA), with the pH adjusted to 5.0, at 37 °C. The solution was not renewed, but the specimens were evaluated daily to ensure total contact with the solution. Color analysis was performed following the demineralization process.

Group allocation

After demineralization, the specimens were randomly divided into four groups. One side of all samples received the application of 8.15 mg of 30% silver diamine fluoride (Cariestop®, Biodinâmica, Brazil) for 3 minutes. The other side received the same application of 30% silver diamine fluoride for 3 minutes, followed by one of the proposed bleaching protocols, namely:

- Group I (garlic extract, n=19): the commercially available plant extract (garlic oil, by Naiak, Brazil) was applied and remained in contact with the dental surface for 3 minutes;
- Group II (bentonite, n=19): a commercially available natural bentonite (Tersil CBM, by Biovital, Brazil) used as paste was applied and remained in contact with the dental surface for 3 minutes;
- Group III (hydrogen peroxide, n=18): it received treatment with 35% hydrogen peroxide according to the manufacturer's instructions for time (45 minutes);
- Group IV (no treatment, n=8): A group with half treated with 30% SDF and half with no treatment (only artificial dentin caries lesion) was created to test the efficacy of treatment in dentin mineral uptake.

The untreated sample was sliced into two halves, as previously mentioned. A plastic film was placed to cover the half side that did not receive the bleaching treatment. Figure 2 shows a picture of a sectioned specimen from group IV and the space where the plastic was inserted. Subsequently, the samples were immersed in artificial saliva (2.20 g/l gastric mucin, 1.45 mM $CaCl_2 \cdot 2H_2O$, 5.40 mM KH_2PO_4, 28.4 mM NaCl, 14.9 mM KCl, pH 7.0) [22] 37° C for 24 hours. Then, all samples were washed with distilled water, slightly dried, and had their color measured again using a spectrophotometer, with the same initial.

Mineral loss and lesion depth analysis

The specimens were mounted on acrylic rods and sectioned with a hard tissue microtome (Silverstone-Taylor Hard Tissue Microtome, Series 1000 Deluxe, USA). Two dentin sections (100 ± 10 µm) were obtained from each side of the sample (bleached and unbleached). The sections were kept moist and mounted on glass plates and then digitally radiographed on the Inspektor TMRD1 (Inspektor Research Systems BV, Amsterdam, Netherlands) with the following parameters: 20 kV, 30 mA, at a distance of 42 cm, for 5 minutes. For each section (bleached and unbleached), an area of approximately 400 × 400 µm that represented the entire lesion area and did not contain any cracks, debris, or other alterations was selected for analysis. Integrated mineral loss (ΔZ) is calculated by multiplying the lesion depth (L) by the mineral loss at that depth. The lesion depth is defined as the distance from the outer surface of the tissue to the point where the mineral content is reduced to 5% compared to sound tissue. In the case of dentin, lesion depth can be determined by tracing the percent mineral volume profile toward the depth of the dentin in each dentin section. In this regard, sound dentin is defined as having about 48% mineralization. Subsequently, the images were analyzed by an examiner who was blinded regarding study groups.

Statistical analysis

The groups were tested for normality by assessing kurtosis and skewness. Data presenting kurtosis and skewness within -2 to 2 of the optimal value (-2 to 2 for skewness and 1 to 5 for kurtosis) were considered normally distributed. The homogeneity of variances was tested by calculating the ratio between the highest and lowest variances in the group, and variances were considered homogeneous when the ratio was ≤ 10. A two-way mixed model ANOVA test was performed with a 5% one-tailed significance level in R Studio software, using the ANOVA_test function from the Rstatix package and the eta.F function from the MOTE package. The factors included a paired factor (occlusal and cervical halves of the sample) and a non-paired factor (type of bleaching agent), creating the mixed model. Pos-hoc analyses were performed in R software using paired t test (two-tailed significance level of 5%) calculated by the function ttest of the package stats, and Cohen's d effect size and its 95% confidence interval were calculated using the function cohens_d of the package effectsize.

To compute probabilities to support either the null or the alternative hypotheses, Bayes factors (ratio of the probability of the alternative hypothesis by the probability of the null hypothesis; BF_{10}) were computed in R software, using the functions anovaBF (for 2-way mixed model Anova) and ttestBF (for paired t test) of the package BayesFactor. A BF_{10} of 5, for instance, means that the alternative hypothesis is 5 times more likely than the null hypothesis, while BF_{10} of 0.2 means that the null hypothesis is 5 times (1/5) more likely than the alternative hypothesis [23].

Results

SDF treatment resulted in a reduction in mineral loss (ΔZ) compared to 'no treatment' in group IV (p = 0.001). ΔZ values (mean ± standard deviation; vol%min×µm) were: 1319 ± 706 (SDF) and 2223 ± 751 (no treatment).

The color change measurement data are presented in Table 1. Significant differences in $\Delta E_{bleaching}$ between groups (p< 0.001) were observed, with strong evidence for the alternative hypothesis (BF_{10} << 100). The paired independent variable (factor 2) indicated significant differences (p-value of 0.01) in $\Delta E_{bleaching}$, but with a low support for the null hypothesis (BF_{10} of 0.46). A statistically significant interaction between the study factors (p<0.001) was observed, with strong evidence for the alternative hypothesis (BF_{10} << 100). Hydrogen peroxide was the only compound capable of significantly altering the color, with partial removal (~24% reduction) of the darkening effect.

Data on the mineral uptake effect are shown in Table 2. The results of the two-factor ANOVA test on ΔZ indicate that there was no significant effect of the type of bleaching agent (group; p = 0.30) or sample pairing (time: upper half representing before bleaching and lower half representing after bleaching) on the mineral uptake effect (p=0.14), with moderate evidence for the null hypothesis (BF_{10} of 0.17 and 0.20). Furthermore, the interaction between the type of bleaching agent and time also had no significant effect (p = 0.14), with a strong support for the null hypothesis (BF_{10} of 0.030). The pair wise comparisons on the effect of bleaching agents on ΔZ suggest that the bleaching agent did not impair the mineral uptake effect of SDF. The Bayes factor provided only low support for the null hypothesis in all pair wise comparisons.

Discussion/Conclusion

The present in vitro study showed, for the first time in the literature, the effects of bleaching agents on the stain removal and mineral uptake effect of ADC treated with SDF followed by exposure to saliva. The importance of including salivary exposure after SDF application relies on the fact that exposure to saliva is a key event required for achieving proper mineral uptake effect (from calcium and phosphate ions that combine with fluoride to form fluor-hydroxyapatite and calcium fluoride) and darkening (from phosphate to form silver phosphate) of dentin from SDF.

The in vitro model used here, including both bleached and non-bleached groups, was able to induce a large mineral uptake effect and a large darkening effect on ADC 24 h after SDF application. Considering that dentin mineral uptake is associated with obliteration of dentinal tubules [24], which, in turn, reduces tooth pain in dentin, our results of a large mineral uptake effect after 24h of SDF treatment is consistent with the clinical reduction of tooth sensitivity detected 24h after SDF treatment of cervical dental defects with exposed dentin in adults [25]. Areas with blocked dentinal tubules, forming radiopaque lines under microradiography as described previously [26], were detected in sound dentin but not in the ADC lesion area (Fig. 2E-F). This suggests that the decrease in ΔZ in SDF-treated lesions was not solely due to the blocking of dentinal tubules. Instead, it is more likely that the decrease resulted from the combined effect of both blocking of dentinal tubules and mineral uptake by intertubular dentin. Darkening of natural dentin caries after 24h of SDF application has also been reported as a common finding in SDF-treated dentin caries [25]. Thus, our in vitro model can be considered to have reasonably mimicked what occurs in vivo.

Regarding the efficacy of chemical agents in removing these stains, the results showed that under the experimental conditions, 35% hydrogen peroxide was the only compound among those studied to have the ability to remove the stain significantly ($p = 0.0008$, power = 96.8%) when applied immediately after SDF, but the post-bleaching color did not return to baseline values and remained relatively dark.

Some points deserve attention when comparing our study with previous ones: type of dental tissue, presence of saliva, and interference of whitening in the mineral uptake effect of SDF. Rafiee et al. [16] studied enamel instead of dentin, which may overestimate the whitening potential of H_2O_2, since darkening by SDF is greater in active carious lesions in dentin than in enamel [1]. Rafiee et al. [16] evaluated the impact of the application of carbamide peroxide (10%) with or without potassium iodide (KI) to remove SDF stains on deciduous teeth enamel and found that there was no difference in the groups in color quality (all appeared lighter). In Rafiee et al. [16] study, there was no artificial saliva present, which may have caused lighter stains and once again overestimated the potential of H_2O_2 in removing stains caused by SDF. Several in vitro studies that analyzed the role of KI in removing stains caused by SDF also did not include artificial saliva [8, 13-15], a fact that may have also overestimated the role of KI in removing stains caused by SDF. Therefore, the present in vitro study is closer to reality by providing a source of phosphorus/phosphate similar to the in vivo environment.

Al-Angari et al. [27] also used 40% H_2O_2 as a bleaching agent after the use of SDF. The compound also showed positive results in reducing staining. However, in the study, there was no exposure to artificial saliva, and the application of H_2O_2 occurred after staining by SDF and not immediately. In the present study, the application of bleaching agents occurred immediately after SDF application, which is a situation closer to the reality found in clinical practice. Al-Angari et al. [27] evaluated the role of the mineral uptake effect of SDF but did not perform this analysis after the bleaching step to assess whether H_2O_2 action could interfere with the mineral uptake effect.

Thus, the joint analysis of color change, followed by the ΔZ change presented in this study, is innovative in the literature. Alternative hypotheses that the tested products affect mineral uptake in artificial carious lesions in dentin after the use of SDF were rejected. Another point to highlight is that this was the first study to show that significant mineral uptake effect on active dentin carious lesions treated with SDF can occur in a short period (24 hours after exposure to saliva).

One limitation of this study is the lack of analysis of the obliteration of dentinal tubules by SDF, which would be one of the main actions in the treatment of dentin sensitivity, since such a compound has increasingly indications and approvals for this clinical situation. The use of hydrogen peroxide in vivo might cause tooth sensitivity, and this must be investigated in future investigations. The follow-up period of the samples after mineral uptake, as well as adding more applications of H_2O_2, is also a limitation of this study and the other in vitro studies evaluated [16, 27]. Longer follow-up periods can help better understand the dynamics of the stain removal process caused by SDF. Finally, some fluoride ions might have been released from the SDF-treated samples and diffused into the artificial saliva affecting the group not treated with SDF, as shown recently [28]. However, it was shown that the release of fluoride from SDF-treated dentin caries specimens affected the severity (ΔZ and lesion depth) of adjacent untreated artificial dentin caries specimens after 48 h, but not after 24h, of further exposure to an in vitro cariogenic challenge. Here, ΔZ was measured after 24h of exposure to saliva. In addition, our group without SDF treatment and with salivary exposure presented lesion severity (ΔZ) lying between that reported for ADC created after 4 days and before 8 days in a similar in vitro cariogenic model without salivary exposure prior to TMR [29]. Thus, the available evidence suggests that any crossover effect of fluoride released by SDF might have not affected ΔZ significantly in our study.

Finding bleaching agents that are low cost, widely available, and do not cause harm to the mineral uptake effect is extremely important for the widespread use of SDF. This factor justified the choice of bentonite and garlic extract, which have bleaching properties in other aspects of the industry [30, 31]. However, for stain removal in teeth treated with SDF, such compounds did not promote significant reduction in the darkening resulting from the application of SDF.

The justification for garlic extract and bentonite not having a bleaching effect on silver diamine fluoride may be related to the chemical composition of these agents and their ability to interact with

SDF. As previously pointed out, garlic extract is rich in sulfur compounds and allicin, which are known for their antimicrobial and antioxidant properties [30]. However, these compounds may not have the ability to interact with SDF to produce a bleaching effect. On the other hand, bentonite is a clay that has been used in various bleaching procedures, including dental bleaching [31], being able to absorb impurities and stains, but it may not have the ability to chemically interact with SDF to bleach it.

Another additional justification for the garlic extract and bentonite not working to whiten SDF may be related to their ability to penetrate into the micro fissures and porosities present on the material's surface. SDF is a highly penetrative material that is capable of filling in porous areas of dental tissues affected by caries [1]. However, the presence of porosities and microfissures on the surface may hinder the penetration of whitening agents, such as garlic extract and bentonite, into these areas. On the other hand, hydrogen peroxide is capable of penetrating into the porosities and microfissures of carious lesions, contributing to an increase in the depth of demineralization [32], which may have caused the whitening action.

When interpreting the relevance of the results of this study, it is important to consider the myriad of clinical factors that can directly or indirectly affect SDF pigmentation and the whitening action of the studied chemical agents. Therefore, there is a need for more studies that control these factors in an isolated and interactive manner, especially by conducting a longitudinal follow-up of the samples. Future studies in this area should also focus on reducing experimental errors.

Based on the results obtained in the present in vitro study, 35% hydrogen peroxide was the only agent with a bleaching effect on SDF-treated ADC, with no deleterious effect on the mineral uptake effect on dentin, but the post-bleaching color was considerably dark after bleaching, indicating that removing dark staining caused by SDF application is quite challenging. Development of new strategies and products for bleaching SDF-treated dentin caries are required to achieve a reasonably esthetic outcome after SDF application.

Statement of Ethics

The human dentin samples used in this study were obtained from teeth collected from dental practice clinics across the USA over several years. Based on the local Institutional Review Board (Indiana University IRB # NSO 911-07), this study was approved and exempted from requiring written informed consent from the donors. This research complied with the guidelines for human studies and was conducted ethically in accordance with the Declaration of Helsinki.

Conflict of Interest Statement

Authors declare no conflict of interest.

Funding Sources

The first author covered with his own financial resources his visiting researcher activities at the Oral Health Research Institute of the Indiana University School of Dentistry, and the materials and equipment used in this study were provided by the Oral Health Research Institute of the Indiana University School of Dentistry.

Author Contributions

Basílio R. Vieira participated in all the operative activities and wrote the manuscript. Sara B. S. C. Cruz and Cristiane A. M. Silva performed the study design and model proposal. Adam Kelly was present supervising the laboratory steps. Frank Lippert performed the analysis of the data from the TMR and revised the manuscript. Anderson T. Hara and Frederico B. de Sousa conceived the idea, analyzed data, and revised the manuscript.

Data Availability Statement

The data generated or analyzed during this study consist mainly of images and numerical values acquired during the experimental phase. The most representative images and values (shown in the tables) of which are included in this article. Further inquiries can be directed to the corresponding author.

References

1. Zheng FM, Yan IG, Duangthip D, Gao SS, Lo ECM, Chu CH. Silver diamine fluoride therapy for dental care. Jpn Dent Sci Rev. 2022;58(1):249-257.
2. Zhao IS, Gao SS, Hiraishi N, Burrow MF, Duangthip D, Mei ML, Lo EC, Chu CH: Mechanisms of silver diamine fluoride on arresting caries: a literature review. Int Dent J. 2018;68:67–76.
3. Mei ML, Nudelman F, Marzec B, Walker JM, Lo ECM, Walls AW, Chu CH. Formation of Fluorohydroxyapatite with Silver Diamine Fluoride. J Dent Res. 2017;96(10):1122-1128.
4. Crystal YO, Niederman R. Evidence-Based Dentistry Update on Silver Diamine Fluoride. Dent Clin North Am. 2019;63(1):45-68.
5. Lo ECM, Duangthip D. Non-restorative Approaches for Managing Cavitated Dentin Carious Lesions. Springer International Publishing AG, part of Springer Nature 2019 S. C. Leal, E. M. Takeshita (eds.), Pediatric Restorative Dentistry. 2019:141-160.
6. Llodra JC, Rodriguez A, Ferrer B, Menardia V, Ramos T, Morato M. Efficacy of silver diamine fluoride for caries reduction in primary teeth and first permanent molars of schoolchildren: 36- month clinical trial. J Dent Res. 2005;84:721-724.
7. Knight GM, McIntyre JM. Mulyani. The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine. Aust Dent J 2006;51:42–45.
8. Patel J, Anthonappa RP, King NM. Evaluation of the staining potential of silver diamine fluoride: in vitro. Int J Paediatr Dent. 2018; 28(5):514-22.
9. Roberts A, Bradley J, Merkley S, Pachal T, Gopal JV, Sharma D. Does potassium iodide application following silver diamine fluoride reduce staining of tooth? A systematic review. Aust Dent J. 2020;65(2):109-117.
10. Haiat A, Ngo HC, Samaranayake LP, Fakhruddin KS. The effect of the combined use of silver diamine fluoride and potassium iodide in disrupting the plaque biofilm microbiome and alleviating tooth discoloration: A systematic review. PLoS One. 2021;16(6):e0252734.
11. Rumble, J. CRC Handbook of Chemistry and Physics (99 ed). CRC Press. Pp 5-189. ISBN 1138561630.
12. Cury JA, Tenuta LMA, Tabchoury CPM. Bioquímica oral. 3ª ed. São Paulo: Editora Santos; 2018.
13. Nguyen V, Neil C, Felsenfeld J., Primus C. Potassium Iodide. The Solution to Silver Diamine Fluoride Discoloration? Adv Dent Oral Health. 2017;5(1):555655.
14. Zhao IS, Mei ML, Burrow MF, Lo EC, Chu CH. Effect of Silver Diamine Fluoride and Potassium Iodide Treatment on Secondary Caries Prevention and Tooth Discolouration in Cervical Glass Ionomer Cement Restoration. Int J Mol Sci. 2017;18(2):340.

15. Detsomboonrat P, Thongmak P, Lertpayab P, Aiemsri W, Sooampon S. Optimal concentration of potassium iodide to reduce the black staining of silver diamine fluoride. J Dent Sci. 2022;17(1):300-307.
16. Rafiee A, Memarpour M, Benam H. Evaluation of bleaching agent effects on color and microhardness change of silver diamine fluoride-treated demineralized primary tooth enamel: An in vitro study. BMC Oral Health. 2022;22(1):347.
17. Nones J, Riella HG, Trentin AG, Nones J. Bentonite protects neural crest stem cells from death caused by aflatoxin B1: Effects of bentonite on different cell types: A brief review. Appl Clay Sci. 2015;104(1):119-127.
18. Hefne JA, Mekhemer WK, Alandis NM, Aldayel OA, Alajyan T. Removal of silver (I) from aqueous solutions by natural bentonite. J King Saud Univ. Sci. 2010;*22*(1): 155-176.
19. Cantuaria M L, de Almeida Neto A F, Nascimento ES, Vieira MG. Adsorption of silver from aqueous solution onto pre-treated bentonite clay: complete batch system evaluation. J Clean Prod. 2016; 112*:* 1112-1121.
20. Chowdhury A, Bhowal A, Datta S. Equilibrium, thermodynamic and kinetic studies for removal of copper (II) from aqueous solution by onion and garlic skin. Water. 2012; 4: 37-51.
21. Lippert F, Butler A, Lynch RJM, Hara AT. Effect of fluoride, lesion baseline severity and mineral distribution on lesion progression. Caries Res. 2012;46(1):23-30.
22. Al Dehailan L, Martinez-Mier EA, Lippert F. The effect of fluoride varnishes on caries lesions: an in vitro investigation. Clin Oral Investig. 2016;20(1):1655–1662.
23. Gronau Q.F., Alexander Ly & Wagenmakers E.J. (2020). Informed Bayesian t-Tests. The American Statistician, 74(2), 137-143.
24. Schüpbach, P., Lutz, F., & Guggenheim, B. Human root caries: histopathology of arrested lesions. Caries Res. 1992;26(3), 153-164.
25. Castillo JL, Rivera S, Aparicio T, Lazo R, Aw TC, Mancl LL, Milgrom P. The short-term effects of diammine silver fluoride on tooth sensitivity: a randomized controlled trial. J Dent Res. 2011;90(2):203-8.
26. Bjørndal L, Thylstrup A. A structural analysis of approximal enamel caries lesions and subjacent dentin reactions. Eur J Oral Sci. 1995; 103(1), 25-31.
27. Al-Angari SS, Lippert F, Platt JA, Eckert GJ, González-Cabezas C, Li Y, Hara AT. Bleaching of simulated stained-remineralized caries lesions in vitro. Clin Oral Investig. 2019;23(4):1785-1792.

28. Romero MJRH, Lippert F. Indirect caries-preventive effect of silver diamine fluoride on adjacent dental substrate: A single-section demineralization study. Eur J Oral Sci. 2021;129(1):e12751.
29. Lippert F, Churchley D, Lynch RJ. Effect of lesion baseline severity and mineral distribution on remineralization and progression of human and bovine dentin caries lesions. Caries Res. 2015; 49(5): 467-476.
30. Ferreira DG. Dentífricos branqueadores: composição, efeitos, eficácia e cosméticos de venda livre em Portugal [tese]. Lisboa: Universidade Fernando Pessoa; 2017.
31. Zhu XY, Zeng YR. Garlic extract in prosthesis-related infections: a literature review. J Int Med Res. 2020;48(4):300060520913778.
32. Briso AL, Gonçalves RS, Costa FB, Gallinari Mde O, Cintra LT, Santos PH. Demineralization and hydrogen peroxide penetration in teeth with incipient lesions. Braz Dent J. 2015;26(2):135-40.

Figure Legends

Fig. 1. Flowchart with the steps performed in the study methodology.

Fig. 2. Photographs of samples containing the fissure section dividing the areas with SDF (top half) and the paired half (bottom half: SDF + garlic extract in A; SDF + bentonite paste in B; SDF + hydrogen peroxide in C; no SDF treatment in D). The plastic film was inserted into the sectioned space created between them. E, typical aspect of SDF-treated ADC lesion under microradiography. F, details of inserts in E, showing natural sclerotic (blocked) dentinal tubules in sound dentin (top) but not in the subsurface dentin caries lesion (bottom).

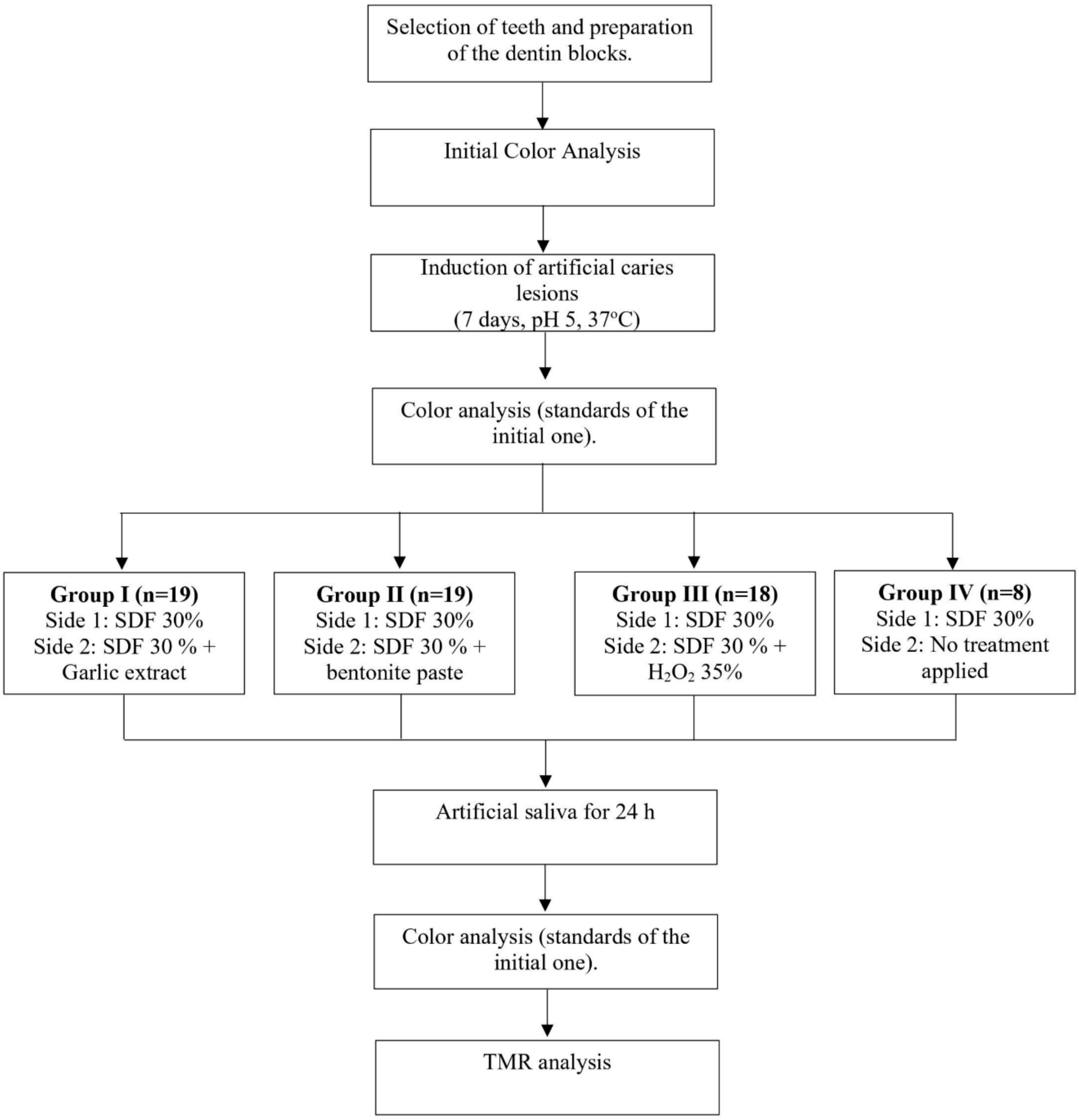
Selection of teeth and preparation of the dentin blocks.
Initial Color Analysis
Induction of artificial caries lesions
(7 days, pH 5, 37°C)
Color analysis (standards of the initial one).
Group I (n=19)
Side 1: SDF 30%
Side 2: SDF 30 % + Garlic extract
Group II (n=19)
Side 1: SDF 30%
Side 2: SDF 30 % + bentonite paste
Group III (n=18)
Side 1: SDF 30%
Side 2: SDF 30 % + H_2O_2 35%
Group IV (n=8)
Side 1: SDF 30%
Side 2: No treatment applied
Artificial saliva for 24 h
Color analysis (standards of the initial one).
TMR analysis

Figure 1.

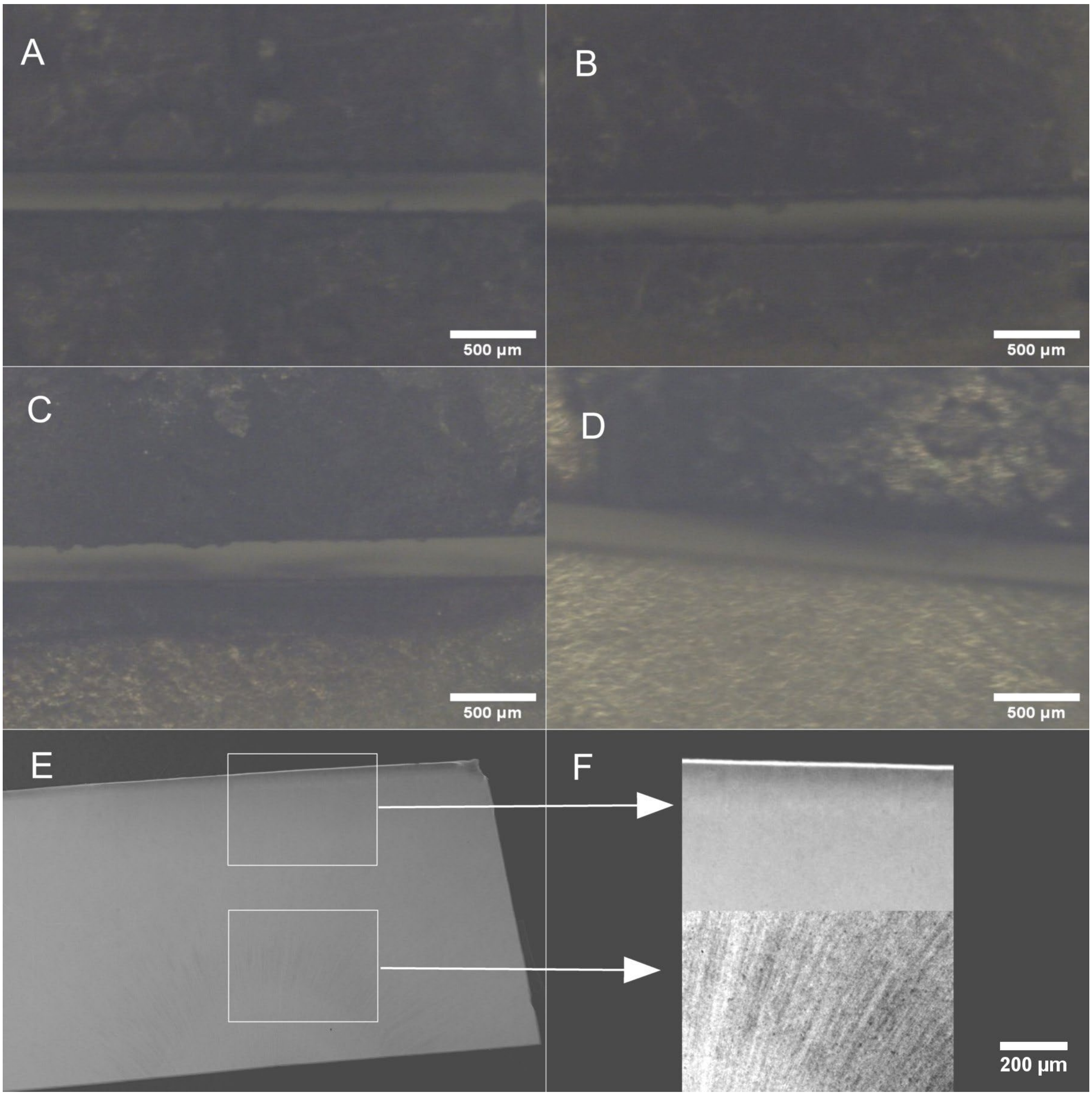

Figure 2.

Table 1. Color change data: descriptive statistics, results from two-way mixed model ANOVA test and analyses of paired groups from two-tailed paired t-test.

Descriptive statistics

Group (N)	**ΔE_{caries} (mean ± SD)[1]**	**ΔE_{SDF} (mean ± SD)[2]**	**$\Delta E_{bleaching}$ (mean ± SD)[3]**
Garlic extract (19)	6.46 ± 1.92	44.51 ± 11.19	45.26 ± 11.38
Bentonite (19)	6.71 ± 2.14	37.86 ± 11.48	38.88 ± 12.29
Hydrogen peroxide (18)	5.77 ± 2.48	36.02 ± 10.81	28.76 ± 11.92
No treatment (08)	5.62 ± 1.71	38.08 ± 13.90	-

Results from two-way mixed model ANOVA test

Factor	**p value**	**Effect size eta squared (IC 95%)**	**BF_{10}**
Bleaching agent	2.34×10^{-7}	0.44 (0.22 to 0.61) - high	89051547.00
Sample pairing	0.01	0,12 (0.01 to 0.31) – low	0.46
Interaction	6.35×10^{-5}	0.31(0.10 to 0.49) - high	61583361.00

Results of analyses of paired groups

Group (N)	**Hedge's g (95% CI)**	**p value (statistical power)**	**BF_{10}**
SDF x SDF+ Garlic extract (19)	0.06 (-0.63;0.74)	0.81 (4.0 %)	0.24
SDf x SDF + Bentonite (19)	0.19 (-0.49;0.88)	0.41 (11.0 %)	0.33

SDF x SDF + Hydrogen peroxide (18)	0.96 (0.22;1.71)	0.0008 (96.8 %)	47.42

[1]difference between sound dentin and after caries lesion creation

[2]difference between sound dentin and SDF-treated lesions

[3]difference between sound dentin and bleached SDF-treated lesions

Table 2. Mineral uptake data: descriptive statistics on ΔZ statistics for both the experimental (ΔZexp) and control (ΔZctr) groups, results of two-way mixed model ANOVA test and analyses of paired groups from two-tailed paired t-test.

Descriptive statistics

Group	N	ΔZexp[1] mean (± SD)	ΔZctr[2] mean (± SD)
Garlic extract	19	1288 ± 789)	1604 ± 1085)
Bentonite	19	1105 ± 802)	1345 ± 575)
Hydrogen peroxide	18	1704 ± 1086)	1216 ± 543)

Results from two-way mixed model ANOVA test

Factor	p value	Effect size eta squared (IC 95%)	BF_{10}
Bleaching agent	0.30	0.05 (0.00 a 0.19) – low	0.17
Sample pairing (before x after bleaching)	0.68	0.003 (0.00 a 0.10) – low	0.20
Interaction	0.14	0.08 (0.00 a 0.23) - low	0.03

Results of analyses of paired groups

Group (N)	Hedge's g (95% CI)	p value (statistical power)	BF_{10}
SDF x SDF+ Garlic extract (19)	-0.27 (-0.73; 0.18)	0.2407 (19.31 %)	0.45

SDf x SDF + Bentonite (19)	-0.23 (-0.69; 0.22)	0.3161 (14.94 %)	0.38
SDF x SDF + Hydrogen peroxide (18)	0.38 (-0.09; 0.85)	0.1103 (33.92 %)	0.78

[1]Mineral loss in the specimen half subjected to treatment after SDF application

[2]Mineral loss in the specimen half not subjected to treatment after SDF application