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A comparative analysis of staining propensity of SDF, SDF with potassium iodide and SDF with glutathione biomolecule on demineralized enamel: An *in-vitro* study

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Abstract
Aim: To evaluate and compare the staining propensity of SDF, SDF with Potassium iodide and SDF + Glutathione biomolecule on demineralized enamel.
Study Design: Forty-five teeth were selected and stratified to receive either 38% SDF; 38% SDF + KI or 38% SDF + 20% GSH. The analysis for staining (ΔE) was done using a UV- Vis Spectrophotometer and was recorded at different intervals for one month. Data analysis was done using Kruskal- Wallis test, Post-hoc analysis (Dunn) and a repeat-measures analysis of variance (RM-ANOVA).
Results: SDF caused maximum staining. SDF + KI staining was significantly less ($P<0.05$) compared to SDF at 1 week, 2 weeks and one month. SDF + GSH showed least staining at 5 minutes and 24 hours among the three groups but the staining increased gradually and had no statistical difference after 1 week, 2 weeks and one month when compared to other groups.
Conclusion: Application of KI following SDF helps in reducing the staining. Addition of GSH had no significant effect.

Keywords: silver diamine fluoride, SDF staining, SDF potassium iodide, SDF glutathione, caries arrest, SDF black staining, SDF caries arrest, SDF discolouration, SDF incipient caries

1. Introduction

Silver compounds have been commonly used in medicine and dentistry since the beginning of the 19th century.[1] In the late 1960s, Silver Diamine Fluoride (SDF) was developed in Japan by Reichi Yamaga, and colleagues, combining the antibacterial properties of silver ions and the caries preventive effects of fluoride [2].

SDF is a colourless, alkaline solution (pH 8-9) which contains silver and fluoride, which forms a complex with ammonia [3]. SDF is reported to release two-three times more fluoride than sodium fluoride, stannous fluoride, acidulated phosphate fluoride and varnishes [4]. SDF has several advantages such it arrests carious lesions, prevention of caries, arrests root caries, desensitizes sensitive teeth and also in dilution can be used as a root canal irrigant [5, 6]. Although, the higher number of desirable properties, SDF causes black staining of carious enamel and dentin. SDF on application to the carious forms silver phosphate which is believed to be yellow in colour and gradually turns dark [7]. The black staining caused as a side effect of SDF application has been an aesthetic concern to children and their parents. Due to the lower acceptance of black staining caused by SDF, the caries arresting property of SDF often gets disregarded by many parents and clinicians [8].

To increase the acceptance of SDF among patients, parents and clinicians, there is a need to modify SDF which will result in no or negligible staining, thus retaining the benefits of all the properties of SDF as well as increasing the acceptance of SDF.

Potassium iodide (KI) has been applied additionally over SDF to reduce the staining caused by SDF [7]. Also, glutathione (GSH) mixed with SDF has been assessed for the staining caused by SDF on bovine enamel and dentine [9].

There is lack of evidence of role of glutathione in reducing the staining caused on demineralized enamel of human teeth. Therefore, the aim of this study was to evaluate and compare the staining propensity of SDF, SDF with KI and SDF + GSH on demineralized enamel.

The objectives of the study were

- To evaluate the staining propensity of 38% SDF on demineralized enamel 5 minutes, 24 hours, 1 week, 2 weeks and one month after application.
- To evaluate the staining propensity of 38% SDF + KI on demineralized enamel 5 minutes, 24 hours, 1 week, 2 weeks and one month after application.
- To evaluate the staining propensity of 38% SDF + GSH on demineralized enamel 5 minutes, 24 hours, 1 week, 2 weeks and one month after application.
- To compare the staining propensity of 38% SDF, 38% SDF+KI, 38% SDF + GSH on demineralized enamel 5 minutes, 24 hours, 1 week, 2 weeks and one month after application.

2. Materials and Methods

2.1 Study Design

An *in-vitro* study to evaluate and compare the staining caused by SDF, SDF and KI and SDF + GSH with the help of UV-Vis Spectrophotometer.

2.2 Sample Preparation

The study was conducted in the Department of Pediatric and Preventive Dentistry after obtaining approval by the Institutional Review Board. Forty-five (N=45) premolars were collected from the Department of Oral and Maxillofacial Surgery. Circular plastic sticker was applied either on the buccal or the lingual surface of the tooth, the rest of the tooth surface was coated with an acid resistant nail varnish. After drying of the nail varnish, the stickers were removed leaving a circular window without nail varnish on it. The teeth were immersed in a demineralizing solution (2.2mM $CaCl_2$, 2.2mM KH_2PO_4, 0.05M Acetic acid, pH adjusted to 4.4 1M KOH) for 96 hours. 10 ml of the demineralizing solution was used per sample [10]. After sample preparation, baseline colour measurements (T_0) were taken of all the samples with the help of a UV- Vis spectrophotometer. The samples were divided randomly into three groups as per the material to be used.

Table 1: Categorization of materials to the respective groups.

Group	Material	Sample Size
Group 1	38% SDF	n= 15
Group 2	38% SDF + KI	n= 15
Group 3	38% SDF + 20% GSH	n= 15

2.2 Procedure of application of material

Group 1

Teeth were washed and dried before the application of SDF. Applicator tip was dampened with 38% SDF and was applied over the demineralized enamel surface for about one minute. After one minute of application the residues were washed off and the teeth were immersed in artificial saliva.

Group 2

Similar to group 1, the teeth were washed and dried and were followed by the application of 38% SDF application. After one minute of SDF application, 10% KI solution with the help of applicator tip was applied over the demineralized enamel surface. On application of KI over the surface which previously received 38% SDF, until creamy white precipitates turned clear. The residues were washed off and were immersed in artificial saliva.

Group 3

In this group, 38% SDF and 20% GSH were mixed vigorously in a beaker with the help of a stirrer in a laboratory until the solution was clear without any precipitates. The mixed solution was applied on the demineralized enamel with an applicator tip for about one minute. After the application of 38% SDF + 20% GSH, the residues were washed off and were immersed in artificial saliva.

2.3 Assessment of colour change

A spectrophotometer (Cary Series UV- Vis Spectrophotometer, Agilent Technologies) was used to measure the colour coordinates (L* a* b*). All the specimens underwent colour measurement prior to the application of SDF and these were considered as the baseline values (T_0). Subsequent colour measurements were taken 5 minutes (T_1), 24 hours (T_2), 1 week (T_3), 2 weeks (T_4) and one month (T_5) after application of SDF, SDF + KI and SDF with Glutathione. Colour changes were characterized using the Commission Internationale d'Eclairage L* a* b* colour space (CIE L* a* b*). The colour difference (ΔE) was calculated according to the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{[1, 2]}$$

where L* is the colour value (lightness), a* and b* denote chromacity and ΔL*, Δa* and Δb* are the changes in L*, a* and b*, respectively between the initial time point and the next point being compared.

2.4 Statistical Analysis

Data were entered into Microsoft Office Excel (version 2013) in a spreadsheet. Data were entered and checked for errors and discrepancies. Data analysis was done using windows based 'MedCalc Statistical Software' Version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017).

Data for change in colour intensity (ΔE) was expressed as mean, median and standard error of mean (SEM). 95% confidence intervals (C.I.) was presented as appropriate. The change in colour intensity (ΔE) was compared between the three groups at each time period using Kruskal- Wallis test with group as the main factor. Post-hoc analysis (Dunn) was used for pair-wise comparisons. A repeat-measures analysis of variance (RM-ANOVA) was used for overall comparison of ΔE with the group as the main factor and time period as repeat measure factor. All the tests were done using two - sided test at alpha 0.05. Thus, the criterion for rejecting the null hypothesis was a 'p' value of <0.05.

3. Results

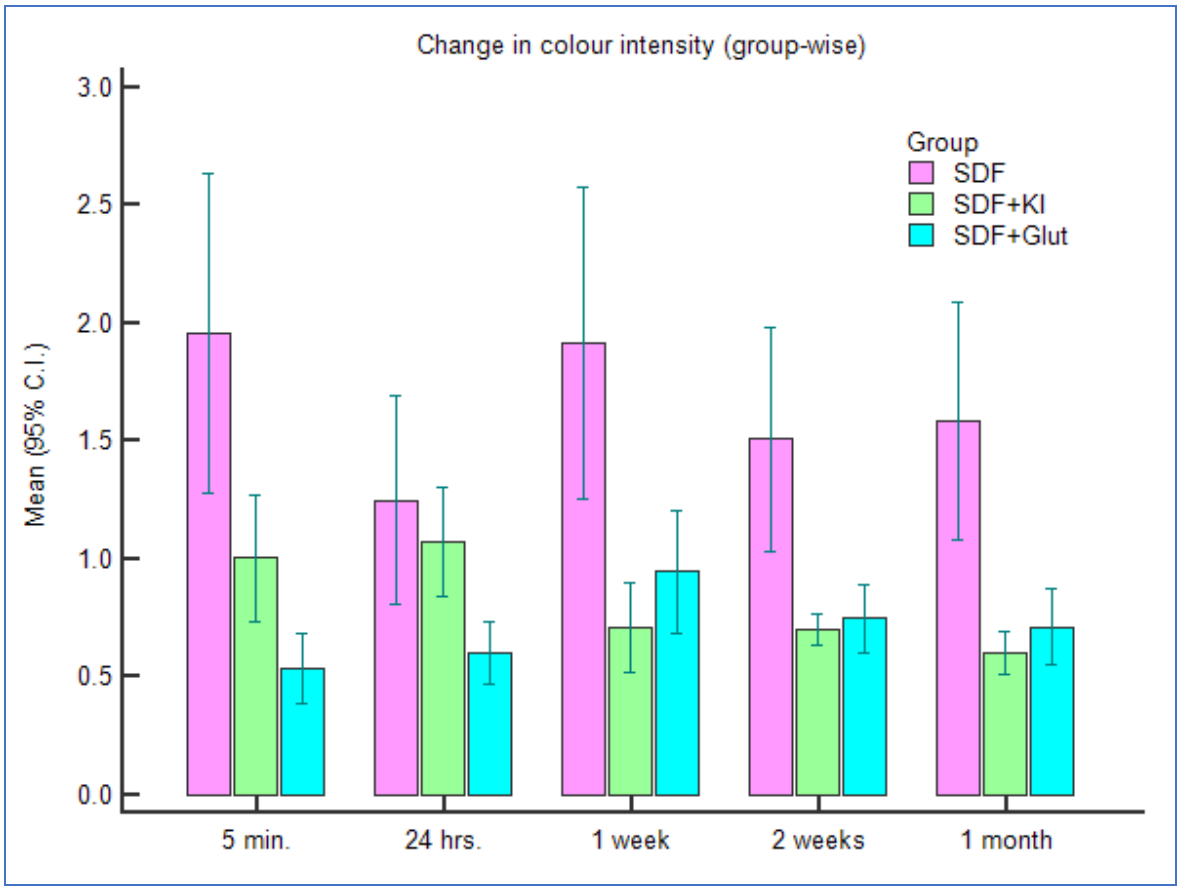
Table 2 shows the mean values of change in colour intensity at different time intervals for all the three groups.

Table 2: Comparison of mean of colour change at different time interval using repeated measures of ANOVA.

Time / Groups	ΔE_1	ΔE_2	ΔE_3	ΔE_4	ΔE_5	Between group 'P' value for Repeated Measures ANOVA	Within group 'P' value for Repeated Measures ANOVA
Group 1 (SDF)	1.95785847	1.24977828	1.919089636	1.509571555	1.587172052	P<0.001*	P<0.001*
Group 2 (SDF + KI)	1.006571163	1.072432889	0.713117433	0.703971584	0.606618894		
Group 3 (SDF + GSH)	0.53781148	0.603756653	0.946834872	0.747984425	0.71376697		

*Statistically Significant P<0.001

The change in the colour intensity were compared using Kruskal- Wallis test and Post Hoc Dunn test for pair wise comparison amongst the three groups, at T_1, group 3 showed lower value and there was statistically significant difference when compared with group 1 ($p< 0.05$); however there was no statistical difference between group 1 and 2 and between group 2 and 3. At T_2, group 3 showed lower degree of staining and there was statistically significant difference when compared with group 1 and group 2 ($p< 0.05$); however comparison of group 1 and 2 showed no statistical difference. At T_3, T_4 and T_5 group 2 showed lesser degree of staining and was statistically significant when compared with group 1 ($p< 0.05$); comparison between group 1 and 3 and between group 2 and 3 showed no statistically significant difference in the change of colour intensity.



Graph 1: Bar graph depicting all the mean values of change in colour intensity with standard deviation in all three groups at different time intervals.

4. Discussion

SDF has been proved to be highly effective in arresting dental caries in primary teeth [6]. SDF also has a potential in preventing dental caries [11, 12]. Systematic review and meta-analysis conducted by Gao *et al* (2016) concluded that 38% SDF resulted in 81% arrest in active carious lesions in primary teeth [6].

The mechanism of action of SDF is believed to be due to the antimicrobial activity against the cariogenic bacteria, remineralisation of carious dentin, inhibits demineralisation of enamel and dentin and preservation of collagen from degradation in demineralised dentin [13].

Several studies have found that removal of caries is not required before the application of SDF. Application of SDF is a non- invasive, cheaper and an easier method to treat caries [14]. SDF, however, causes black staining of the carious lesions which often causes dissatisfaction among patients.

Upon application of SDF on the tooth, it reacts with the structure of the tooth, it releases a large quantity of free silver ions. When these ions are reduced, they aggregate and precipitate resulting in formation of black stains [9]. The black staining is believed to be caused by the formation of silver phosphate [7]. Silver phosphate crystals are yellow in colour and gradually turn dark especially on exposure to light [9].

Application of potassium iodide following SDF application has been studied and found to reduce the staining potential of SDF. KI reacts with the free silver ions to produce silver iodide which is a creamy white precipitate [7].

Sayed *et al* (2018) [9] introduced mixture of SDF with GSH to inhibit the staining. GSH is a tri-peptide biomolecule which

contains a thiol group (-SH) which has a high affinity for adsorption onto metal surfaces. GSH coats the silver particles and reduces aggregation of silver particles as well as it controls the rate at which silver ions are released which possibly can be a reason to reduce the colour change caused by SDF [9].

In this study, enamel surface was artificially demineralized using an acidic solution (2.2mM $CaCl_2$, 2.2mM KH_2PO_4, 0.05M Acetic acid, pH adjusted to 4.4 1M KOH).[10] The reason for selecting artificially demineralized enamel was to assess the staining property of SDF when used as a preventive agent in arresting incipient carious lesions.

In this study, SDF group showed the highest amount of staining at all time intervals. The change in the colour intensity in SDF + KI was not statistically significant when compared with SDF at 5 minutes and 24 hours post application; however at one week, two weeks and a month post application it showed a lesser degree of staining which was statistically significant when compared with SDF group. The change in colour intensity in SDF + GSH group was lower than SDF group after 5 minutes, 24 hours however there was no statistically significant difference at one week, two weeks and a month after the application. On comparison of SDF + KI and SDF + GSH, there was a statistically significant difference between the two groups at 24 hours, SDF + GSH showing a lesser value however there was no statistically significant difference at 5 minutes, one week, two weeks and a month after application of material.

In this study, staining of demineralised enamel with SDF was observed. Staining of carious lesion has already been reported in the literature. In this study SDF + KI group showed the least amount of staining at the end of one month which is consistent with earlier observations in the literature.[7,15]

Although there was less staining in SDF + GSH group, the difference was not statistically significant when compared with SDF alone at the end of one month as the intensity of staining gradually increased in the teeth treated with SDF + GSH over a period of one month. This finding could be attributed to the homeostasis property of GSH that is it controls the rate of release of silver ion and might have caused a delayed increase in colour intensity [16]. Findings from the current study contrasts from the observations made by Sayed *et al* (2018) [9]. This can be attributed to a longer follow up as compared to the aforementioned study.

SDF application followed by KI can be beneficial when aesthetics is a concern, but clinical trials are required for its recommendation in clinical practice. Also, the effect of KI on the antimicrobial and caries arresting properties of SDF should be studied.

In this study, evident black staining was observed on the root surface of the teeth. The staining seen on the root surface of the teeth was visibly more evident than the staining seen on the crown of the tooth. However, analysis of the stains on the root surface was not done as it was out of the scope of this study.

This study has certain limitations. The observations in this study are limited by the *in vitro* methodology. The dynamic nature of the oral cavity is difficult to replicate, such as the impact of brushing, saliva, presence of chromogenic and non-chromogenic bacteria and mastication and to study its impact on the staining. In this study, teeth were continuously immersed in a stationary artificial saliva which also has a different chemical nature. This study has only assessed the effect of KI and GSH on staining caused by SDF but the study did not address their effect on the cariostatic property of SDF.

Despite the above limitations, this study is one of its kind as no study has been reported assessing the staining caused by SDF and the above formulations on demineralized enamel and has reported for further need for research.

5. Conclusion

Based on the observations of this study, following are the specific conclusions drawn with respect to each one of the study objectives:

1. Staining caused by 38% SDF was evident as early as 5 minutes after application, there was a slight reduction in the intensity of staining after 24 hours, staining increased rapidly till 1 week following which there was a steady increase in the intensity of staining. The change in colour intensity at every time interval was statistically significant.
2. Application of KI following SDF application helped in reducing the staining caused by SDF. Intensity of staining was comparable to SDF post application, but the intensity of staining decreased over a period of one month and the reduction was statistically significant.
3. Mixing of SDF with GSH helped in reducing the intensity of staining initially, but the intensity of staining increased gradually and the increase in the intensity of staining was statistically significant at every time interval.
4. SDF + KI resulted in reduction of staining when compared with SDF alone, but was statistically non significant when compared with SDF + GSH. SDF + GSH was effective in reducing the colour change initially but the intensity kept increasing gradually, it had no significant difference when it was compared with SDF alone and with SDF + KI from 1 week onwards.
5. We observed staining of demineralized enamel with SDF. SDF + KI was effective in reducing the intensity of staining. SDF + GSH was effective initially but the intensity of staining increased gradually.

6. Recommendations

Based on our findings we recommend

- An in-vivo study with a randomized controlled trial design comparing the potential of staining of SDF and SDF + KI on incipient caries in primary as well as in permanent teeth can confirm the observations made by us with respect to the staining potential.

7. Clinical Significance

There is a paradigm shift in the management of dental caries, SDF has emerged as a caries arresting agent; however, it leads to black staining of the carious lesion. Application of KI helps in reducing the staining caused by the SDF; and can potentially improve its acceptance in clinical situations.

8. References

1. Peng JY, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: A review. Journal of dentistry 2012;40(7):531-41.
2. Hu S, Meyer B, Duggal M. A silver renaissance in dentistry. European Archives of Paediatric Dentistry. 2018;19(4):221-7.
3. Rosenblatt A, Stamford TC, Niederman R. Silver diamine fluoride: a caries “silver-fluoride bullet”. Journal of dental research 2009;88(2):116-25.
4. Greenwall-Cohen J, Greenwall L, Barry S. Silver diamine fluoride-an overview of the literature and current

clinical techniques. British Dental Journal. 2020;228(11):831-8.
5. Shah S, Bhaskar V, Venkatraghavan K, Choudhary P, Trivedi K. Silver diamine fluoride: A review and current applications. Journal of Advanced Oral Research. 2014;5(1):25-35.
6. Gao SS, Zhao IS, Hiraishi N, Duangthip D, Mei ML, Lo EC, Chu CH. Clinical trials of silver diamine fluoride in arresting caries among children: A systematic review. JDR Clinical & Translational Research 2016;1(3):201-10.
7. Patel J, Anthonappa RP, King NM. Evaluation of the staining potential of silver diamine fluoride: *in vitro*. International journal of paediatric dentistry 2018;28(5):514-22.
8. Crystal YO, Janal MN, Hamilton DS, Niederman R. Parental perceptions and acceptance of silver diamine fluoride staining. The Journal of the American Dental Association 2017;148(7):510-8.
9. Sayed M, Matsui N, Hiraishi N, Nikaido T, Burrow MF, Tagami J. Effect of glutathione bio-molecule on tooth discoloration associated with silver diammine fluoride. International journal of molecular sciences 2018;19(5):1322.
10. Gopalakrishnan VL, Anthonappa RP, King NM, Itthagarun A. Remineralizing potential of a 60-s *in vitro* application of Tooth Mousse Plus. International journal of paediatric dentistry 2017;27(5):356-63.
11. 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. Journal of dental research 2005;84(8):721-4.
12. Braga MM, Mendes FM, De Benedetto MS, Imparato JC. Effect of silver diammine fluoride on incipient caries lesions in erupting permanent first molars: a pilot study. Journal of Dentistry for Children 2009;76(1):28-33.
13. Zhao IS, Gao SS, Hiraishi N, Burrow MF, Duangthip D, Mei ML *et al*. Mechanisms of silver diamine fluoride on arresting caries: a literature review. International dental journal. 2018;68(2):67-76.
14. Crystal YO, Marghalani AA, Ureles SD, Wright JT, Sulyanto R, Divaris K, *et al*. Use of Silver Diamine Fluoride for Dental Caries Management in Children and Adolescents, Including Those with Special Health Care Needs. Pediatric dentistry 2017;39(5):135.
15. Nguyen V, Neill C, Felsenfeld J, Primus C. Potassium iodide. The solution to silver diamine fluoride discoloration?. Health 2017;5(1):555655.
16. Taglietti A, Diaz Fernandez YA, Amato E, Cucca L, Dacarro G *et al*. Antibacterial activity of glutathione-coated silver nanoparticles against gram positive and gram negative bacteria. Langmuir 2012;28(21):8140-8.