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Effect of Potassium Iodide and Glutathione on Color Change and Remineralization Potential Induced by Silver Diamine Fluoride Application

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Abstract

BACKGROUND: To evaluate the effect of applying potassium-iodide (KI) and glutathione (GSH) on silver-diamine fluoride (SDF) induced demineralized dentin discoloration as well as their effect on its remineralization potential.

MATERIALS AND METHODS: To examine color change, cervical dentinal demineralized cavities were performed mesially and distally in 16 human premolars. Glass-ionomer restoration (GIC) was applied and allocated to four groups I-IV according to the following pre-treatments: No pre-treatment, SDF, SDF+KI, SDF+20% (wt) GSH. Spectrophotometric evaluation of samples at time intervals: 1,7 and 14 days of GIC application. To examine the effect of remineralization, 21 bovine dentin blocks were divided into groups I-III: SDF, SDF + KI, and SDF + GSH. Vickers microhardness was measured, before and after demineralization and after 7 days of treatment.

RESULTS: Spectrophotometric results after 14 days for groups I-IV were: 1.29±0.18, 12.24±0.19, 2.19±0.32 and 4.76±0.19 respectively. Groups III and IV showed significant reduction in ΔE compared with Group II, although they showed significant increase in ΔE compared with group I ($p<0.001$). KI showed better management of color changes than GSH. The microhardness test results after treatment application to demineralized dentin for Groups I-III were: 30.81±20.87, 30.59±16.42, 24.69±13.21, respectively. All groups showed significantly increased microhardness of demineralized dentin ($P\leq0.05$), which was comparable to that of Group I.

CONCLUSION: Application of KI and GSH after SDF significantly minimized color changes without affecting the remineralizing effect of SDF.

KEYWORDS: Silver diamine fluoride; Remineralization; Discoloration; Potassium iodide; Glutathione

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Introduction

Dental caries is one of the most prevalent health conditions worldwide, despite efforts to control it [1]. Untreated dental caries lead to general health problems, including pain, poor quality of life, and reduced productivity [1,2]. Cariology research currently focuses on the effectiveness of medical treatments for caries arrest and remineralization rather than the conventional invasive drill-and-fill approach for caries management [3].

Atraumatic Restorative Technique (ART) is considered a minimal intervention method for caries management using hand instruments for carious tissue removal and restoring decayed tooth structure by high viscosity glass ionomer cement

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restoration (HVGIC) [4]. This technique showed less dental anxiety than the traditional drill-and-fill approach [5]. As a modification to this technique for a better prognosis, it was suggested to pre-treat the remaining carious tooth structure with SDF prior to HVGIC restoration application. This modification was named Silver Modified Atraumatic Restorative Technique (SMART) [6,7]. As lately, SDF gained attention for arresting and remineralizing carious dental lesions [8]. In 2014, the FDA approved SDF for caries arrest in both dentitions [9]. Furthermore, no need to remove carious dental tissues before SDF application [10], which made its use simple, economical and patient-friendly [11]. Previous studies have shown a promising effect of SDF in the control of carious dentin demineralization [12,13].

Despite the advantages of SDF, yet it has limited desirability owing to its dark-staining effect [14]. Even application of glass ionomer restoration couldn't effectively mask this dark staining effect [15]. The purpose of this study was to reduce this discoloration without affecting the remineralization potential of the SDF. Potassium iodide (KI) application after SDF was suggested, which resulted in a creamy yellowish-white silver iodide (AgI) precipitate by binding to free silver ions from the SDF [16]. However, it was shown that the demineralized dentin microhardness was significantly decreased by reducing the silver ion count as a result of KI addition to SDF [17,18]. Therefore, in this in vitro study, we tested glutathione (GSH), a relatively unpopular new material for discoloration management. Glutathione (GSH) is an intracellular non-protein thiol (NPSH) antioxidant that acts as a metal chelator, which may decrease free silver ion aggregation by coating its particles without affecting its count [19].

Accordingly, this study aimed to evaluate the effect of applying KI and GSH on SDF induced tooth discoloration as well as their effect on SDF remineralization potential.

Material and Methods

Color change spectrophotometric evaluation

Sixteen sound-extracted permanent human premolars were used in this study. Premolars were collected from the Oral and Maxillofacial Surgery Department of the Faculty of Dentistry at Ain Shams University. The Ain-Shams University Faculty of Dentistry Ethical Committee approved the protocol for teeth collection and storage (Approval No. RECEM 011910). The teeth were rinsed with deionized water and cleaned with a periodontal curette to remove any soft tissue debris. Teeth with stains and extreme visual discoloration were excluded, such that teeth were visually with homogenous color. The teeth were then polished using a polishing cup and a pumice. Standardized 2 mm deep square cavities of size 4 × 4 mm were made on the mesial and distal sides of each premolar at the cemento-enamel junction using tungsten carbide bur flat fissure (FG 557-Prima dental-UK) and NSK handpiece high speed under copious amount of water. Each premolar was completely covered with a clear nail varnish (YOLO, A.R.E), except for the mesial and distal cavity windows, which were kept unprotected. Additionally, the apical foramen was occluded externally with the varnish for no contamination. This resulted in 32 specimen, which were then distributed randomly into four experimental groups (n= 8) according to the treatment protocol (Figure 1). Each group had 8 samples (which means 4 teeth), such that the same treatment is applied to both sides of the tooth, avoiding contamination of variable treatments during storage.

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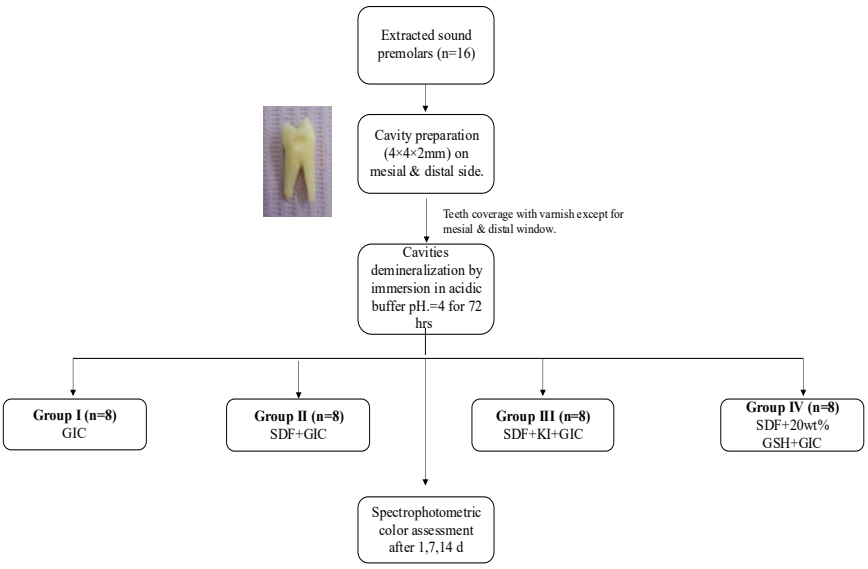

Figure 1. *Flow chart for Experimental design of color change assessment*

Each tooth, including two specimens (one on each side), was separately immersed in an acidic buffer solution for 72 h in a separate container at room temperature. The pH was adjusted by the addition of 1 M KOH to maintain a pH of 4. The demineralizing solution was changed every 24 hours [20]. As according to the pilot study done, the demineralization depth was average 150 ± 15 µm measured at Oral Pathology Department, faculty of dentistry, Ain-Shams University with polarized light microscope with magnification power X10 using a digital camera (EOS 650D, canon, Japan) which was mounted on a light microscope (BX60, Olympus, Japan).

Thirty-two specimens were allocated randomly to four treatment groups (n=8). In Group I, the cavities of demineralized dentin were filled with GIC restorations without pre-treatment. While in Group II 38% SDF solution was applied for 1 minute with a micro-brush according to the manufacturer's instructions, Group III was treated with the SDF solution as mentioned previously followed by immediate application of saturated KI solution until the creamy white precipitate became clear and in Group IV demineralized dentin was treated by a mixture done by mixing 38% SDF solution with 20% (wt) GSH until the mixture became clear with no precipitates and then applied by a microbrush for one minute. Then all cavities in Groups II, III, IV were then washed with copious amounts of distilled water for 30s and dried with oil-free compressed air and filled with GIC restoration (Table 1). All previous work was done by a single experienced practitioner for standardization. Then all the samples were stored individually in a deionized solution.

Color assessments of the specimens (n=32) were recorded at three-time intervals: baseline after 24 h of GIC restoration application, 7 days, and then 14 days. Color changes were recorded using a spectrophotometer (Agilent Cary 5000-USA) on the GIC restoration surface at the National Institute of Standards (NIS-Egypt-Giza). It was calibrated and used in the standard mode in the visible wavelength region (380nm to 780nm) for all samples. Computed Cie Lab values were measured under Illuminate D65 with a 10 degrees standard observer. The Cie Lab coordinates L* a* b*, hue and chroma of each sample at variable time interval was evaluated. Then the ΔL, Δa, Δb between different time intervals of each sample was calculated and hence the ΔE color change between time intervals was measured. Finally, the mean and standard deviation SD of each group was statistically evaluated.

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Table 1: List of materials used, chemical composition, and manufacturer.

Material	Composition	Manufacture
Demineralizing solution	• 2.2 mM $CaCl_2$, 2.2 Mm KH_2PO_4, 50 mM Acetic Acid • pH 4	Manufactured in pharmaceutical lab.
38% SDF solution (Riva star)	• ($AgNH_2F$) solution contains 25% silver, 62% water, 5% fluoride, 8% amine • pH 10	SDI, Bayswater, Australia
Saturated potassium iodide (Riva star)	• contains 1 g of potassium iodide per millilitre.	SDI, Bayswater, Australia
Glutathione GSH (Sigma-Aldrich)	• L-Glutathione reduced	St. Louis, MO, USA
Glass ionomer cement restoration (Fuji™ IX GP FAST capsule A3)	• Aluminofluorosilicate glass, polyacrylic acid, distilled water, poly-carboxylic acid	GC International, Tokyo, Japan

Remineralization and microhardness evaluation

Bovine teeth were collected from cattle slaughtered in the food-manufacturing industry. None of the animals were harmed to participate in this study. Twenty-one bovine dentin samples (6×6×2 mm) were prepared from the labial and lingual surfaces of the cervical portion of incisor roots. The samples were then cut using a low-speed disc *(Komet, Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany)*, and a straight handpiece (S 11 LG, W&H, Bùrmoos, Austria) with copious amounts of coolant. Specimens were embedded in acrylic resin (Acrostone, Cold Cure, Egypt), and the surfaces were successively ground flat using 150-1200 grit silicon carbide papers (English SiC waterproof abrasive sandpaper) under running water. All previous work was done by a single experienced operator.

First, the microhardness of sound specimens was measured. All samples were subjected to demineralization in the same manner as previously described. The microhardness of the specimens was measured after demineralization. The samples were randomly divided into three groups based on the treatment applied. Group I received 38% SDF solution as the control group, Group II received SDF followed by KI solution, and Group III received a mixture of SDF with 20%(wt) GSH. All treatments were performed by a single experienced practitioner as described previously. Each group was stored in a container of deionized water for one week. The surface microhardness was then re-measured for all the samples.

Microhardness measurements were performed at Dental Research Centre (DR centre-Egypt-Cairo) using a Vickers diamond microhardness tester (Wilson 1102/1202 Buehler MicroMet 6000 series, Germany) in Vickers hardness units (VHN). The microhardness measurements were taken at three different points 0.5 mm apart from the adjacent indentation at each dentin sample. Each measurement was carried out using a 100 g (HV 0.1) load for a dwell time of 10 s. Mean of three indentations made on each surface

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for each dentine disc was used for statistical analysis.

Statistical analysis

Numerical data are presented as both, the mean and standard deviation (SD). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check the data distribution to assess the normality. All data presented a normal parametric distribution, such that they were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons. While repeated measures were analyzed using ANOVA followed by Bonferroni post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$. Statistical analysis was done using the R statistical analysis software, version 4.1.3 [21]. Cohen's d was calculated and interpreted based on Lakens' formula and thresholds using the online resource available at https://effect-size-calculator.herokuapp.com/ [22]. The R package MBESS was used to calculate the confidence intervals (CI) for Cohen's d using the noncentral *t* method as in Table 3&4, 6&7 [23].

Results

Spectrophotometric results

The results showed that the highest value of color change ΔE was found in SDF group II, followed by SDF + GSH group IV, and SDF + KI group III, while the lowest value was found in Group I at all time intervals. There was a significant interaction between the time and group. Post-hoc pairwise comparisons showed that the values of the different groups were significantly different from each other ($p<0.001$), as shown in Table 2, 3, & 4.

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Table 2: Mean, Standard deviation (SD) values of color change (ΔE) for different groups.

Interval	Color change (ΔE) (Mean ± SD)				p-value
	Group I	Group II	Group III	Group IV	
Baseline-1 week	0.95 ± 0.16^{Db}	7.64 ± 0.10^{Ab}	1.73 ± 0.67^{Cb}	3.24 ± 0.18^{Bb}	<0.001*
1-2 weeks	0.36 ± 0.03^{Dc}	4.67 ± 0.24^{Ac}	0.78 ± 0.19^{Cc}	1.67 ± 0.14^{Bc}	<0.001*
Baseline-2 weeks	1.29 ± 0.18^{Da}	12.24 ± 0.19^{Aa}	2.19 ± 0.32^{Ca}	4.76 ± 0.19^{Ba}	<0.001*
p-value	<0.001*	<0.001*	<0.001*	<0.001*	

Different upper and lowercase superscript letters indicate a statistically significant difference within the same horizontal row and vertical column respectively*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

Table 3: Effect sizes (95% confidence intervals) of the intergroup comparisons of color change

Interval	First group	Second group	Cohen's d	95% confidence interval		Magnitude
				Lower	Upper	
Baseline-1 week	Group (I)	Group (II)	-47.4	-67.85	-30.65	Large
	Group (I)	Group (III)	-1.51	-2.72	-0.44	Large
	Group (I)	Group (IV)	-12.714	-18.5	-8.42	Large
	Group (II)	Group (III)	11.66	7.71	16.95	Large
	Group (II)	Group (IV)	28.57	18.81	40.92	Large
	Group (III)	Group (IV)	-2.91	-4.55	-1.56	Large
1-2 weeks	Group (I)	Group (II)	-23.83	-34.14	-15.92	Large
	Group (I)	Group (III)	-2.92	-4.56	-1.57	Large
	Group (I)	Group (IV)	-12.23	-17.77	-8.1	Large
	Group (II)	Group (III)	16.99	11.32	24.38	Large
	Group (II)	Group (IV)	14.44	9.59	20.74	Large
	Group (III)	Group (IV)	-5.04	-7.5	-3.13	Large
Baseline-2 weeks	Group (I)	Group (II)	-55.94	-80.05	-36.17	Large
	Group (I)	Group (III)	-3.28	-5.05	-1.84	Large
	Group (I)	Group (IV)	-17.73	-25.43	-11.81	Large

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	Group (II)	**Group (III)**	36.11	23.33	51.69	**Large**
	Group (II)	**Group (IV)**	37.22	24.05	53.28	**Large**
	Group (III)	**Group (IV)**	-9.233	-13.46	-6.047	**Large**

|d| < 0.2 "negligible", |d| < 0.5 "small", |d| < 0.8 "medium", otherwise "large".

Table 4: Effect sizes (95% confidence intervals) of the intragroup comparisons of color change

Group	First interval	Second interval	Cohen's d	95% confidence interval		Magnitude
				Lower	Upper	
Group (I)	**Baseline-1 week**	**1-2 weeks**	4.556	2.35	7.88	**Large**
	Baseline-1 week	**Baseline-2 weeks**	-1.775	-3.214	-0.74	**Large**
	1-2 weeks	**Baseline-2 weeks**	-6.407	-10.996	-3.416	**Large**
Group (II)	**Baseline-1 week**	**1-2 weeks**	14.36	7.893	24.076	**Large**
	Baseline-1 week	**Baseline-2 weeks**	-26.932	-45.105	-14.423	**Large**
	1-2 weeks	**Baseline-2 weeks**	-31.088	-52.058	-15.39	**Large**
Group (III)	**Baseline-1 week**	**1-2 weeks**	1.715	0.604	3.201	**Large**
	Baseline-1 week	**Baseline-2 weeks**	-0.779	-1.742	0.035	**Medium**
	1-2 weeks	**Baseline-2 weeks**	-4.763	-8.189	-2.52	**Large**
Group (IV)	**Baseline-1 week**	**1-2 weeks**	8.655	4.726	14.538	**Large**
	Baseline-1 week	**Baseline-2 weeks**	-7.301	-12.472	-3.969	**Large**
	1-2 weeks	**Baseline-2 weeks**	-16.459	-27.58	-9.067	**Large**

|d| < 0.2 "negligible", |d| < 0.5 "small", |d| < 0.8 "medium", otherwise "large".

Microhardness tests

As shown in Table 5, 6, & 7, T1 showed no significant difference between the different groups ($p>0.05$), indicating the demineralization phase of all samples. On the other hand, at the T2 interval, the SDF + KI Group II showed a significantly higher value ($p \leq 0.05$) than the SDF + GSH Group III, while no significant difference ($p>0.05$) was observed between the two groups and SDF Group I.

Table 5: Mean, Standard deviation (SD) values of micro-hardness for different groups.

Interval	Micro-hardness (mean±SD)			p-value
	Group I	Group II	Group III	
T0	53.66 ± 5.22^{Ba}	57.06 ± 10.34^{ABa}	60.29 ± 7.73^{Aa}	**0.034***
T1	34.46 ± 10.72^{Ac}	34.24 ± 6.72^{Ac}	35.05 ± 3.93^{Ac}	**0.884ns**
T2	46.26 ± 4.89^{ABb}	47.15 ± 5.22^{Ab}	43.34 ± 3.46^{Bb}	**0.024***
p-value	**<0.001***	**<0.001***	**<0.001***	

Different upper and lowercase superscript letters indicate a statistically significant difference within the same horizontal row and vertical column respectively*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

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Table 6: Effect sizes (95% confidence intervals) of the intergroup comparisons of microhardness.

Interval	First group	Second group	Cohen's d	95% confidence interval		Magnitude
				Lower	Upper	
T0	Group (I)	Group (II)	-0.39	-1.47	0.654	Small
	Group (I)	Group (III)	-0.94	-2.11	0.133	Large
	Group (II)	Group (III)	-0.33	-1.404	0.71	Small
T1	Group (I)	Group (II)	0.02	-1.024	1.072	Negligible
	Group (I)	Group (III)	-0.07	-1.12	0.98	Negligible
	Group (II)	Group (III)	-0.14	-1.19	0.905	Negligible
T2	Group (I)	Group (II)	-0.16	-1.22	0.878	Negligible
	Group (I)	Group (III)	0.65	-0.407	1.759	Medium
	Group (II)	Group (III)	0.805	-0.26	1.95	Large

|d| < 0.2 "negligible", |d| < 0.5 "small", |d| < 0.8 "medium", otherwise "large".

Table 7: Effect sizes (95% confidence intervals) of the intergroup comparisons of microhardness.

Group	First interval	Second interval	Cohen's d	95% confidence interval		Magnitude
				Lower	Upper	
Group (I)	T0	T1	1.98	0.76	3.75	Large
	T0	T2	1.27	0.34	2.53	Large
	T1	T2	-1.23	-2.53	-0.25	Large
Group (II)	T0	T1	2.28	0.96	4.23	Large
	T0	T2	1.05	0.13	2.23	Large
	T1	T2	-1.87	-3.53	-0.72	Large
Group (III)	T0	T1	3.58	1.7	6.5	Large
	T0	T2	2.46	1.05	4.58	Large
	T1	T2	-1.95	-3.66	-0.78	Large

|d| < 0.2 "negligible", |d| < 0.5 "small", |d| < 0.8 "medium", otherwise "large".

Discussion

The silver-modified atraumatic restorative technique (SMART) offers an alternative minimal interventional treatment for caries management by arresting caries progression using SDF and restoring tooth form by HVGIC [7,24,25]. However, one of the major drawbacks of the SDF treatment is its black staining effect on the carious tooth structure, which has limited its acceptance by aesthetically concerned patients and accordingly its general use [26,27]. This discoloration is due to the reaction of SDF with carious dentin resulting in the production of silver phosphate (Ag_3PO_4), silver chloride, and free metallic silver. Ag_3PO_4 is yellow when it is first formed but readily turns black under the influence of reducing agents or sunlight. This black impermeable layer prevents the progression of caries [12,13,28,29]. Finding a solution for the dark staining without affecting SDF's biological effect on carious dentin would provide a great opportunity for the universal use of SDF. Potassium iodide was introduced commercially as a solution to reverse this discoloration but unfortunately controversies regarding its effect on the antibacterial and remineralization potential of the SDF [30-33]. Introducing an alternative option for discoloration management was in need. For that reason, glutathione biomolecule was suggested as an alternative to reduce teeth discoloration without affecting the

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biological efficiency of the SDF [19]. There is limited evidence regarding efficiency of GSH compared to commercially available KI. Accordingly, this study aimed to evaluate the efficiency of GSH compared to KI on remineralization potential and teeth discoloration induced by SDF treatment of demineralized dentine.

High viscosity Glass-ionomer restoration was used as simulation for clinical application of SMART technique as it offers chemical bonding to dentine with good cavity seal, remineralizing effect, biocompatibility, ease of application, and low cost [34]. For standardization of tested variables, packable self-cure glass ionomer was applied to avoid any further discoloration that may be caused by the light curing; as the interaction products of both SDF and KI (Ag_3PO_4 and AgI) are photosensitive [14,15]. Cavities were done at the cemento-enamel junction for location standardization and for supporting SDF use in root caries arrest, as previously proposed in other studies due to its effectiveness and ease of application [35-39].

Spectrophotometer was used for evaluation of the teeth discoloration as one of the most commonly used tools to assess the color change [40]. In Group II, the color change with time was the highest among all groups as shown in table 2. GIC restoration had limited discoloration masking effect [15]. While Group III & IV showed significant reduction in color change relative to Group II at all time intervals (Table 2). Still, they had significant change of color with time. As in Group III, upon application of KI immediately after SDF, a bright yellow silver iodide (AgI) compound was formed [31,32,41]. However, this yellow color soon turned dark and became darker with time. This could be justified by the amount of KI solution that wasn't enough to interact with all the free silver ions, or may be attributed to the high photosensitivity of AgI that readily dissociates into black metallic silver again by light exposure [27]. On the other hand, in Group IV glutathione biomolecules reduced discoloration owing to its high adsorption affinity onto metallic surfaces which in turn might have decreased silver particle aggregation. Additionally, GSH has a haemostatic effect which might have controlled the rate of silver ion release [42]. Application of a mix of 20%(wt) GSH with SDF prior GIC restoration in SMART for color management was new [19]. In Group I color change was visually undetectable, but it was significant along with time. This may be attributed to the degradation of the metal-polyacrylate bonds as an inherent property in GIC restorations, causing lack of color stability [43].

Accordingly, it was assumed that the percentage of GSH was not sufficient to completely coat all the excess silver particles to efficiently reduce the color change. Pilot trials to dissolve more percentage of glutathione powder in 38% SDF solution resulted in mixture with precipitates. However, GSH ability to decrease the color changes of dentin may encourage further studies to better understand simpler ways of increasing the GSH concentration in SDF solutions. According to the results of the current study, KI is more effective than 20%(wt) GSH in reducing the color changes of demineralized dentin. This finding disagree with a pilot study that was done after aging and found no significant difference in color change between application of SDF only and adding KI to it prior to GIC restoration [30].

Regarding the microhardness results, bovine teeth were reported to resemble human teeth in multiple features including dentin surface properties [44]. It offered more homogeneity in respect to mineral composition compared with human teeth which is beneficial for invitro studies [45]. The base line of the demineralized dentine was standardized without any significant difference between the randomly distributed dentine discs. The different groups showed a significant increase in the microhardness of demineralized dentin, with no significant difference between the different treatments (Table 5). This can be explained by an increase in the mineral content of dentin by extrafibrillar and intrafibrillar mineral formation by the deposition of spherical grains, which were

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confirmed by SEM investigations in a previous study [46]. Our results supported the assumptions in previous studies that silver has no role in the SDF remineralizing effect [18]. This justifies that addition of KI to the SDF did not affect its remineralization properties. The increase in dentin surface hardness after the application of previous treatments was due to the recovery of nanomechanical properties of the demineralized dentin surface by crystal precipitation [47]. Meanwhile, our results contrast those of other study that showed that KI application significantly reduced the hardness effect of the SDF compared with the unaccompanied SDF. In this latter study, micro-computed tomography (micro-CT) and Fourier transform infrared (FTIR) spectroscopy were used for indirect measurement of remineralization potential [31].

As an in-vitro study, there are some accompanied limitations, as the artificial demineralization, which was created by an acidic solution rather than combining the bacterial caries model as in real. This method is extensively used in research due to its simplicity and reproducibility [20]. In addition, we were focused mainly on the anti-demineralizing effects of SDF. Moreover, the usage of deionized water as a storage medium between variable time intervals after treatment application, was another limitation as ionic concentrations were kept at unrealistic low levels than that of viable dentinal fluid [48], which may affect discoloration intensity by further interacting with SDF. In vitro studies are different from the more complex clinical situation. Such that the results cannot be directly extrapolated to the in-vivo condition. Another limitation was the inability to dissolve more than 20%(wt) of glutathione powder in SDF solution, whether using a magnetic stirrer or glass slab and spatula, since exceeding the 20%(wt) caused white precipitations in mixture.

Accordingly, in this in-vitro study we were concerned with managing the SDF induced discoloration, in a trial to motivate SDF application as a liner beneath final restoration, in deep carious lesions.

Conclusion

Within the limitations of this in-vitro study, 20%(wt) GSH is a promising solution, but with less efficiency than KI, for management of SDF induced teeth discoloration without compromising the remineralization potential. Further studies with higher concentrations of GSH are recommended.

References

1. Kassebaum NJ, Smith AGC, Bernabé E, et al. Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors. *J Dent Res*. 2017;96(4):380-387. doi:10.1177/0022034517693566
2. Martins MT, Sardenberg F, Bendo CB, et al. Dental caries remains as the main oral condition with the greatest impact on children's quality of life. *PLoS One*. 2017;12(10):e0185365. Published 2017 Oct 5. doi:10.1371/journal.pone.0185365
3. Ricketts D, Lamont T, Innes NP, Kidd E, Clarkson JE. Operative caries management in adults and children. *Cochrane Database Syst Rev*. 2013;(3):CD003808. Published 2013 Mar 28. doi:10.1002/14651858.CD003808.pub3
4. Leal S, Bonifacio C, Raggio D, Frencken J. Atraumatic Restorative Treatment: Restorative Component. *Monogr Oral Sci*. 2018;27:92-102. doi:10.1159/000487836
5. Frencken JE, Leal SC, Navarro MF. Twenty-five-year atraumatic restorative treatment (ART) approach: a comprehensive overview. *Clin Oral Investig*. 2012;16(5):1337-1346. doi:10.1007/s00784-012-0783-4
6. Ballikaya E, Ünverdi GE, Cehreli ZC. Management of initial carious lesions of hypomineralized molars (MIH) with silver diamine fluoride or silver-modified atraumatic restorative treatment (SMART): 1-year results of a prospective, randomized clinical trial. *Clin Oral Investig*. 2022;26(2):2197-2205. doi:10.1007/s00784-021-04236-5
7. Modasia, R., Modasia, D. Application of silver diamine fluoride as part of the Atraumatic Restorative Technique . *BDJ Student* **28**, 42–43 (2021).

Dentistry 3000
Vol 12 No 1 (2024) DOI 10.5195/d3000.2024.402

https://doi.org/10.1038/s41406-021-0199-1

8. Santos VE Jr, Vasconcelos Filho A, Targino AG, et al. A new "silver-bullet" to treat caries in children--nano silver fluoride: a randomised clinical trial. *J Dent*. 2014;42(8):945-951. doi:10.1016/j.jdent.2014.05.017
9. Contreras V, Toro MJ, Elías-Boneta AR, Encarnación-Burgos A. Effectiveness of silver diamine fluoride in caries prevention and arrest: a systematic literature review. *Gen Dent*. 2017;65(3):22-29.
10. Chu CH, Lo EC, Lin HC. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. *J Dent Res*. 2002;81(11):767-770. doi:10.1177/0810767
11. Johhnson B, Serban N, Griffin PM, Tomar SL. Projecting the economic impact of silver diamine fluoride on caries treatment expenditures and outcomes in young U.S. children. *J Public Health Dent*. 2019;79(3):215-221. doi:10.1111/jphd.12312
12. Mei ML, Ito L, Cao Y, Li QL, Lo EC, Chu CH. Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation. *J Dent*. 2013;41(9):809-817. doi:10.1016/j.jdent.2013.06.009
13. Mei ML, Ito L, Cao Y, Lo EC, Li QL, Chu CH. An ex vivo study of arrested primary teeth caries with silver diamine fluoride therapy. *J Dent*. 2014;42(4):395-402. doi:10.1016/j.jdent.2013.12.007
14. Duangthip D, Fung MHT, Wong MCM, Chu CH, Lo ECM. Adverse Effects of Silver Diamine Fluoride Treatment among Preschool Children. *J Dent Res*. 2018;97(4):395-401. doi:10.1177/0022034517746678
15. Hamdy D, Giraki M, Abd Elaziz A, Badran A, Allam G, Ruettermann S. Laboratory evaluation of the potential masking of color changes produced by silver diamine fluoride in primary molars. *BMC Oral Health*. 2021;21(1):337. Published 2021 Jul 9. doi:10.1186/s12903-021-01697-8
16. Patel J, Anthonappa RP, King NM. Evaluation of the staining potential of silver diamine fluoride: in vitro [published online ahead of print, 2018 Jul 4]. *Int J Paediatr Dent*. 2018;10.1111/ipd.12401. doi:10.1111/ipd.12401
17. Hamama HH, Yiu CK, Burrow MF. Effect of silver diamine fluoride and potassium iodide on residual bacteria in dentinal tubules. *Aust Dent J*. 2015;60(1):80-87. doi:10.1111/adj.12276
18. Sorkhdini P, Gregory RL, Crystal YO, Tang Q, Lippert F. Effectiveness of in vitro primary coronal caries prevention with silver diamine fluoride - Chemical vs biofilm models. *J Dent*. 2020;99:103418. doi:10.1016/j.jdent.2020.103418
19. 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. *Int J Mol Sci*. 2018;19(5):1322. Published 2018 Apr 29. doi:10.3390/ijms19051322
20. Zhang X, Neoh KG, Lin CC, Kishen A. Remineralization of partially demineralized dentine substrate based on a biomimetic strategy. *J Mater Sci Mater Med*. 2012;23(3):733-742. doi:10.1007/s10856-012-4550-5
21. Team, R. C. (2021). R: A language and environment for statistical computing v. 3.6. 1 (R Foundation for Statistical Computing, Vienna, Austria, 2019). Retrieved from https://www.r-project.org
22. Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in Psychology, 4*(863). https://doi.org/10.3389/fpsyg.2013.00863
23. Kelley, K. (2007). Methods for the Behavioral, Educational, and Social Sciences: An R package. *Behavior Research Methods, 39*(4), 979–984. https://doi.org/10.3758/BF03192993
24. Bridge G, Martel AS, Lomazzi M. Silver Diamine Fluoride: Transforming Community Dental Caries Program. *Int Dent J*. 2021;71(6):458-461. doi:10.1016/j.identj.2020.12.017
25. Natarajan D. Silver Modified Atraumatic Restorative Technique: A Way towards "SMART" Pediatric Dentistry during the COVID-19 Pandemic. *Front Dent*. 2022;19:12. Published 2022 Mar 12. doi:10.18502/fid.v19i12.9215
26. Peng JJ, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: a review. *J Dent*. 2012;40(7):531-541. doi:10.1016/j.jdent.2012.03.009
27. Garg S, Sadr A, Chan D. Potassium Iodide Reversal of Silver Diamine Fluoride Staining: A Case Report. *Oper Dent*. 2019;44(3):221-226. doi:10.2341/17-266-S
28. Mei ML, Nudelman F, Marzec B, et al. Formation of

Dentistry 3000
Vol 12 No 1 (2024) DOI 10.5195/d3000.2024.402

Fluorohydroxyapatite with Silver Diamine Fluoride. *J Dent Res*. 2017;96(10):1122-1128. doi:10.1177/0022034517709738

29. Lou YL, Botelho MG, Darvell BW. Reaction of silver diamine [corrected] fluoride with hydroxyapatite and protein [published correction appears in J Dent. 2012 Jan;40(1):91-3]. *J Dent*. 2011;39(9):612-618. doi:10.1016/j.jdent.2011.06.008
30. Miller MB, López LA, Quock RL. Silver diamine fluoride, potassium iodide, and esthetic perception: An in vitro pilot study. *Am J Dent*. 2016;29(5):248-250.
31. 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. Published 2017 Feb 6. doi:10.3390/ijms18020340
32. 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. doi:10.1111/adj.12743
33. Nguyen V, Neill C, Felsenfeld Dds J, Primus Phd C. Potassium Iodide. The Solution to Silver Diamine Fluoride Discoloration? *Adv Dent & Oral Health*.2017;5:1–6. doi:10.19080/ADOH.2017.05.555655
34. Lohbauer U. Dental Glass Ionomer Cements as Permanent Filling Materials? —Properties, Limitations Future Trends. *Materials (Basel)*. 2009;3(1):76-96. Published 2009 Dec 28. doi:10.3390/ma3010076
35. Zhang W, McGrath C, Lo EC, Li JY. Silver diamine fluoride and education to prevent and arrest root caries among community-dwelling elders. *Caries Res*. 2013;47(4):284-290. doi:10.1159/000346620
36. Göstemeyer G, Kohls A, Paris S, Schwendicke F. Root caries prevention via sodium fluoride, chlorhexidine and silver diamine fluoride in vitro. *Odontology*. 2018;106(3):274-281. doi:10.1007/s10266-018-0341-x
37. Tan HP, Lo EC, Dyson JE, Luo Y, Corbet EF. A randomized trial on root caries prevention in elders. *J Dent Res*. 2010;89(10):1086-1090. doi:10.1177/0022034510375825
38. Grandjean ML, Maccarone NR, McKenna G, Müller F, Srinivasan M. Silver Diamine Fluoride (SDF) in the management of root caries in elders: a systematic review and meta-analysis. *Swiss Dent J*. 2021;131(5):417-424.
39. Hiraishi N, Sayed M, Takahashi M, Nikaido T, Tagami J. Clinical and primary evidence of silver diamine fluoride on root caries management. *Jpn Dent Sci Rev*. 2022;58:1-8. doi:10.1016/j.jdsr.2021.11.002
40. Kim-Pusateri S, Brewer JD, Davis EL, Wee AG. Reliability and accuracy of four dental shade-matching devices. *J Prosthet Dent*. 2009;101(3):193-199. doi:10.1016/S0022-3913(09)60028-7
41. Patel J, Anthonappa RP, King NM. Evaluation of the staining potential of silver diamine fluoride: in vitro [published online ahead of print, 2018 Jul 4]. *Int J Paediatr Dent*. 2018;10.1111/ipd.12401. doi:10.1111/ipd.12401
42. Taglietti A, Diaz Fernandez YA, Amato E, et al. Antibacterial activity of glutathione-coated silver nanoparticles against Gram positive and Gram negative bacteria. *Langmuir*. 2012;28(21):8140-8148. doi:10.1021/la3003838
43. Hotwani K, Thosar N, Baliga S. Comparative in vitro assessment of color stability of hybrid esthetic restorative materials against various children's beverages. *J Conserv Dent*. 2014;17(1):70-74. doi:10.4103/0972-0707.124154
44. Fonseca RB, Haiter-Neto F, Carlo HL, et al. Radiodensity and hardness of enamel and dentin of human and bovine teeth, varying bovine teeth age. *Arch Oral Biol*. 2008;53(11):1023-1029. doi:10.1016/j.archoralbio.2008.06.012
45. Ghisi AC, Kopper PM, Baldasso FE, et al. Effect of superoxidized water and sodium hypochlorite, associated or not with EDTA, on organic and inorganic components of bovine root dentin. *J Endod*. 2015;41(6):925-930. doi:10.1016/j.joen.2015.01.039
46. Banerjee A, Frencken JE, Schwendicke F, Innes NPT. Contemporary operative caries management: consensus recommendations on minimally invasive caries removal. *Br Dent J*. 2017;223(3):215-222. doi:10.1038/sj.bdj.2017.672
47. Cai J, Burrow MF, Manton DJ, Tsuda Y, Sobh EG, Palamara JEA. Effects of silver diamine fluoride/potassium iodide on artificial root caries lesions with adjunctive application of proanthocyanidin. *Acta Biomater*. 2019;88:491-502. doi:10.1016/j.actbio.2019.02.020

Dentistry 3000
Vol 12 No 1 (2024) DOI 10.5195/d3000.2024.402

48. Shellis RP. Effects of a supersaturated pulpal fluid on the formation of caries-like lesions on the roots of human teeth. *Caries Res*. 1994;28(1):14-20. doi:10.1159/000261614