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Comparing the Effect of Potassium Iodide and Glutathione on Color Stability and Shear Bond Strength of Glass Ionomer to Silver Diamine Fluoride Treated Carious Dentine in Primary Teeth: An In-Vitro Study

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Aim: The aim of the study is to compare the color stability after application of Potassium Iodide (KI) versus Glutathione Biomolecule (GSH) to silver diamine fluoride (SDF) treated carious dentine surface of primary teeth. And to assess shear bond strength of SDF treated carious primary dentin to glass ionomer after application of KI and GSH.

Materials and methods: Total of 54 teeth were collected after extraction from British University clinic, 24 used for the color stability test and 30 for shear Group 1 SDF, Group 2 SDF+ KI, Group 3 SDF + GSH. For color test carious teeth were selected and multiple intervals were measured before material application, immediately after, 7days, and 10 days. For shear test artificial demineralization was preformed then material application and glass ionomer application by teflon mold then left in artificial saliva for 7 days then subjected to shear testing. Tests were conducted in the British University and Ain Shamis Universities and the National institute of Standards.

Results: Color change data were analyzed using two-way mixed model ANOVA followed by simple effects comparisons revealing GSH masked discoloration of SDF followed by KI group and least SDF group. Shear bond strength data were analyzed using one-way ANOVA followed by Tukey's post hoc test revealing no statistical significance between GSH and KI group, but statistical significance from SDF group.

Conclusion: GSH had superior results in masking of SDF discoloration and comparable shear bond strength to KI group and can therefore be used as an alternative to KI.

Keywords: Keywords: Silver Diamine Fluoride, Potassium Iodide, Glutathione Biomolecule, Caries

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Introduction

Dental caries is the most prevalent dental illness that is faced by humans worldwide. [1] It is especially a concern in pediatric patients, this is due to the morphological differences between deciduous and permanent teeth.

Early childhood caries refers to children who are 71 months or younger and have one or more primary teeth that are decayed, missing, or filled. [2]Treatment of dentinal caries has evolved. Historically the concept of drill and fill was the most appropriate. Then soon it evolved to minimally invasive approaches. The application of Silver Diamine Fluoride (SDF) was initially introduced in 1970. [4] Drs Nishino and Yamaga in Japan used it to halt dental decay. [3]They devised this method to merge the effects of fluoride ions and silver ions to obtain its benefits on desensitizing hypersensitive dentin, preventing secondary caries following restorations, and preventing and arresting dental caries in children. [3]

Potassium iodide (KI) has long been used to overcome the black staining that results after SDF application. It has also been proven to be very effective in masking discoloration as opposed to SDF alone. This can be achieved by vigorously mixing KI powder with SDF until a creamy white precipitate results. Although KI treatment has minimal restrictions, the staining decrease is only transient, and tooth surface darkening still happens. [6]

Glutathione (GSH) biomolecule integration into SDF has recently demonstrated encouraging outcomes in terms of reducing SDF discoloration. GSH is a naturally occurring material found in the human body. It consists of a metal ion and non-protein thiol group found inside of cells. GSH functions as an antioxidant chelator. It is normally given to patients as a supplement for liver function. As for its use in dentistry, GSH was added to SDF to prevent staining potential. This mixture subsequently reduced the SDF-related color alterations. Due to the coating action of the thiol groups in the cysteine that bind to the silver in SDF therefore hindering the tooth discoloration process. [7]

In vitro study by Sayed et al. (2018) assessed the impact of GSH on the decreasing dentine and enamel discoloration following the application of 38% SDF solution. One hundred twenty specimens of bovine teeth were used, and they were split into three groups: (1) SDF only (control); (2) SDF with KI solution; and (3) SDF combined with 20% GSH by weight. Sayed intended to utilize a novel, as-yet-untested substance to cover up the SDF discoloration. They came to the conclusion that the GSH biomolecule, particularly on enamel and to a lesser extent on dentine, had an impact on reducing discoloration of tooth surface following application of SDF.[7,8]

According to Gupte Manal et al. 2021 who tested staining propensity of SDF with KI and with GSH it was concluded that KI helped reduce staining while GSH had no significant effect and based on her recommendation this study will further investigate the qualities of GSH in comparison to the well-known KI. [9]

Also, Kamble et al. 2021 examined the effects of KI and GSH on tooth discoloration following the application of SDF. They found that GSH was successful in minimizing SDF discoloration, but that KI significantly reduced discoloration as time elapsed, indicating it as being more effective.[10]

Usually after Applying SDF and the subsequent discoloration pediatric dentist may apply a restorative material to aid in masking of the discoloration and as a restorative option. Ideally it would be preferred to have a material that can do both simultaneously, and for this reason Hamdy et al. 2021, tested composite versus glass

ionomer. The conclusion reached was that all materials mask the color initially, but only composite maintained the masking by aging. As a result, it was selected in this study to further investigate Glass ionomer cement, since it is highly recommended for use in pediatric patients.[11]

Due to the gap of knowledge and difference in opinions it was decided to investigate GSH and compare it to KI not only in masking of SDF induced discoloration but also in comparing its shear bond strength with glass ionomer which is the treatment of choice in pediatric patients.

Materials and Methods

Ethical consideration

This research protocol was exempted prior to the conduction of the study by the Ethical Committee of Faculty of Dentistry, Ain Shams University FDASU-RECEM022114.

Study Design

This study is an In-Vitro study, conducted at the Faculty of Dentistry Ain Shamis University, Pediatric Dentistry and Dental Public Health Department. The Faculty of Pharmacy, Ain Shamis University. The Nano-technology Research center, British University in Egypt. The Faculty of Pharmacy, British University in Egypt. Finally, the National Institute of Standards in Cairo.

Teeth Selection

Teeth were examined both through visual inspection and light microscope with magnification 10x- 1000x to determine specimen eligibility for the tests.

Teeth Disinfection

Thymol disinfection solution was prepared at the Faculty of Pharmacy British University in Egypt. All collected samples were disinfected in thymol solution 0.1% then air-dried using air water syringe.

Sample size estimation

For Color stability

In order to apply a statistical test of the null hypothesis—that there is no difference in color stability between the investigated groups—a power analysis was created with sufficient power. The anticipated sample size (n) was a total of eighteen samples, based on the results of Sayed et al.[7]

By using an alpha level of 0.05, a beta of 0.2, or power=80%, and an effect size (f) of (0.811) computed. The sample size computation was done with G*Power 3.1.9.7.

For shear bond strength

In order to do a statistical test of the null hypothesis—which states that there is no difference in shear bond strength between the investigated groups—a power analysis was created with sufficient power. With an alpha threshold of 0.05, a beta of 0.2, meaning power of 80%, and an effect size (f) of (0.682) determined by using the data from Zhao et al. 2019[12], the expected sample size (n) was twenty-four samples. To calculate the sample size, G*Power 3.1.9.7 was used.

Study Procedure

For color testing

Twenty- four carious teeth were extracted due to exfoliation or medical purposes and used for the study; acrylic resin cylinders 3 cm in height and 2 cm in diameter was used to mount teeth to ease their cutting and handling. They were then kept at room temperature in distilled water. Water-cooled carborundum discs (6911 HK, Komet Dental, Germany) attached to a low-speed motor (Ultimate 450, NSK, Germany) at a speed of 6000 rpm were used to grind the enamel surrounding a carious lesion to expose the dentine and create a smooth occlusal surface

of dentine. Next, using the same disc, carious dentin slices (5 x 5 mm) were removed from the tooth in order to standardize size. The samples were then randomly allocated into 3 groups, each with 8 teeth, based on the treatment method and the manufacturer's guidelines.

Group AC: In accordance with the manufacturer's instructions, 38% SDF was applied to the tooth surface, agitated with a micro-brush for one minute, left for two minutes, then washed with a large amount of distilled water for thirty seconds.[11]

Group BC: As directed by the manufacturer, SDF was applied using a micro-brush to the tooth surface from blue bottle. Afterwards, a saturated KI solution was applied using a different micro-brush from green bottle until the creamy white precipitates turned clear. The area was then thoroughly cleaned for 30 seconds with abundant distilled water. [11]

Group CC: SDF was combined with 20% GSH powder by weight and thoroughly mixed until the solution was clear and free of precipitates. Then applied in accordance with the manufacturer's recommendations, much like group AC. [7]

For every specimen, a single operator measured color three times throughout each time interval, recording the mean results. The intervals measured were as follows: baseline (before to solution application), right after solution application, and after seven days and after ten days to evaluate color stability over time.

A spectrophotometer (JP7200F, Juki, Tokyo, Japan) with a wavelength range of 380–780 nm was used to record color. The three-dimensional L*a*b* CIELAB color space system (L*a*b*) was used to explain each hue. L* symbolizes brightness ranging from dark (0) to brilliant (100), a* explains red (+a*) to green (−a*), and b* depicts yellow (+b*) to blue (−b*). For every specimen, a single operator measured color three times throughout each time interval, recording the mean results. The variation in color (ΔE) of each specimen between baseline and each time-interval point was calculated.

For shear testing

Thirty human primary molars that were free of caries were gathered. Using a low-speed saw equipped with a diamond blade, specimens were prepared by removing coronal enamel and revealing a flat horizontal dentine surface (ISOMET 4000; Buehler, Lake Bluff, Germany)[27]. Cold-cured acrylic was used to embed all dentine specimens. Under running water, the dentine specimen's surfaces were polished using sandpaper of micro-fine 2000-grit. For seven days at 25 °C, all samples were submerged in a demineralizing solution made at Ain Shamis University's Faculty of Pharmacy (pH 4.4, 50 mM acetate, 2.2 mM KH2PO4, 2.2 mM CaCl2). [12] Next, groups were assigned at random.

AS: SDF (positive control group): In accordance with the manufacturer's recommendations, a 38% SDF solution (Korea) was applied to the demineralized surfaces and agitated with a micro-brush for one minute, then left for two minutes and washed with a large amount of distilled water for thirty seconds. [12]

For group BS: SDF + KI (Riva Star, SDI, Bayswater, Australia) was applied to the demineralized surfaces. The demineralized surfaces were topically treated with a 38% SDF solution, and then the treatment site was treated with a saturated KI solution until the creamy white color turned transparent. In accordance with the manufacturer's recommendations, distilled water was used to thoroughly wash the treated surfaces. [12]

For group CS: SDF was combined with 20% glutathione by weight and vigorously stirred until the mixture was clear and free of precipitates. then applied in line with the

manufacturer's directions, much like group AS. [7]

All samples were immersed in artificial saliva after 30 minutes at room temperature for 24 hours.[12] Preparation of artificial saliva was at the Faculty of Pharmacy, Ain Shamis University. It was composed of 4200mg/L NaHCO3, 500mg/L NaCl and 200mg/L KCL, and the pH was adjusted to 7.4 using a pH meter and left for 24 hours after treatment and before Glass ionomer restoration. [13]

Shear bond test was preformed after 7 days of restoration to ensure to complete setting and reach optimal strength.[29]

A universal testing machine with a flat edge loading head (3345; Instron, England) was used to perform the Shear Bond test. The machine's upper movable head was equipped with a uni-beveled chisel with a 0.5 mm width blade. The chisel blade was placed as close to the Glass Ionomer restoration interface as possible, and it applied compression force via a crosshead speed of 1.0 mm/min until the specimen failed. The shear bond strength was measured in MPa was determined by dividing the force necessary for failure (Newton) by the surface area (mm2). Results computed with Blue Hill Universal program.

Failure mode was conducted using scanning electron microscope (Thermo Scientific Quattro Scanning Electron Microscope; Columbus, OH, USA).

Results

Statistical analysis

Fisher's exact test was used to examine the frequencies and percentages of the categorical data. The mean and standard deviation (SD) figures were used to display numerical data. By examining the data distribution and applying Shapiro-Wilk's and Levene's tests, respectively, they were examined for normality and variance homogeneity. They were discovered to have uniform variations among groups and to be regularly distributed. The two-way mixed model ANOVA was used to examine the color change data. This was followed by simple effects comparisons using the pooled error term from the ANOVA model and p-value correction through the False Discovery Rate (DFR) method. Tukey's post hoc test was performed after a one-way ANOVA was used to assess the shear bond strength data. For every test, a significance level of $p<0.05$ was used. R statistical analysis was utilized to do the statistical analysis software version 4.4.0 for Windows.

1-Color change (ΔE):

Table 1, initially GSH had the highest color change after material application and then by time SDF group had highest color change which is expected followed by KI group and finally lowest color change in GSH group. This indicates that GSH had the highest masking effect by time.

Table 1: Inter, intragroup comparisons and summary statistics for color change (ΔE).

Interval	Color change (Mean±SD) (ΔE)			p-value
	SDF	SDF+KI	SDF+GTU	
After SDF	2.37±0.14^{Cb}	5.92±0.30^{Ba}	6.74±0.21^{Aa}	<0.001*
After 7days	2.10±0.36^{Ab}	1.25±0.16^{Bc}	1.03±0.05^{Bc}	<0.001*
After 10 days	3.02±0.16^{Aa}	1.97±0.16^{Bb}	1.55±0.19^{Cb}	<0.001*
p-value	<0.001*	<0.001*	<0.001*	

Values with different upper and lowercase superscripts within the same horizontal row and vertical column, respectively, are significantly different *; significant ($p<0.05$).

2-Shear bond strength (MPa):

There was a statistically significant difference between different groups (p=0.026). The highest bond strength was found in SDF+GTU (4.29±1.04) (MPa), followed by SDF+KI (4.28±0.97) (MPa) with no statistical significance between previously mentioned groups, while the lowest bond strength was found at SDF (2.80±0.43) (MPa). Post hoc pairwise comparisons showed SDF to have significantly lower bond strength than other groups (p<0.001).

Table 2: Intergroup comparison and summary statistics for shear bond strength (MPa).

Shear bond strength (Mean±SD) (MPa)			p-value
SDF	SDF+KI	SDF+GTU	
2.80±0.43^{B}	4.28±0.97^{A}	4.29±1.04^{A}	**0.026***

Values with different superscripts within the same horizontal row are significantly different *; significant (p<0.05).

3-Failure mode:

Most of the samples in the SDF group had an adhesive failure, while most samples in other groups had mixed failures. However, the difference was not statistically significant (p=0.274).

Table 3: Intergroup comparison and summary statistics for failure mode.

Failure mode	n (%)			p-value
	SDF	SDF+KI	SDF+GTU	
Adhesive	6 (60.00%)	2 (20.00%)	2 (20.00%)	0.274ns
Cohesive	1 (10.00%)	1 (10.00%)	1 (10.00%)	
Mixed	3 (30.00%)	7 (70.00%)	7 (70.00%)	

ns; non-significant.

Discussion

Pediatric dentists face difficulties while treating young patients, and to provide the necessary care, they may even need to use more sophisticated behavior management techniques such as protective stabilization or general anesthesia. Whenever previous methods are not indicated for medical or financial reasons, there must be a substitute; for this reason, finding a practical cost-effective way to treat children's caries lesions is crucial. [14] One effective, simple, and reasonably priced cariostatic agent has been found to be SDF.

SDF seems to be a practical method for managing dental cavities. Especially for those patients who are totally resistant or at a very young age where conventional treatment may pose a risk to their well-being. Nonetheless, its drawback of black staining still makes acceptance from the child and parent difficult, and maybe sometimes impossible especially anteriorly as aesthetics is of major concern. [15]

Several commercial products contain 38% SDF. This study used ETA SDF due to its availability at the time of conducting the research. Riva Star is the only product on the market that contains SDF and KI and therefore was used for standardization. [16]

Black discoloration is caused by accumulation of silver precipitate after SDF application. [17] Many studies have used KI to conceal black staining of SDF and that's why this study used KI as well to compare it to the newly emerging GSH being tested for the same reason. GSH has not been tested enough and that's why this study was employed to test its ability to mask SDF discoloration. According to some theories, silver iodide, a solid chemical with a bright yellow color, can be created from the reaction of SDF with KI, which might decrease black staining due to the reaction between free silver ions from the SDF and the iodide ions from KI. [6] The KI solution was administered after the SDF to eliminate excess silver ions, creating silver iodide. This was done because, although SDF delivers antimicrobial silver ions, surplus

silver ions can still accumulate and precipitate and lead to more staining over time Sayed et al.2018. [7]

GSH was chosen for this study because it coats silver particles, reducing their aggregation and influencing the rate of release silver ion according to Taglietti et al. 2012. This may help to slow down the color change of applied SDF on tooth surface over time. [18]

The concentration used was according to Sayed et al. 2018, 20% GSH powder mixed with SDF solution until a clear liquid without any precipitates was reached then applied. [7]

For the color assessment, carious teeth without pulpal involvement were used to stimulate actual intraoral carious scenario. Some research papers were done on demineralized samples like Gupte Manal et al. 2021[9] and Deepthy Priya et al. 2022 [10], but it was impossible to ensure the same amount of mineral content and demineralization in each sample. Also, another challenging factor was the different hues of carious and teeth. So, this study selected teeth with carious and although different stages of carious have different color, each tooth was measured at multiple intervals and each sample was used as a control for itself by measuring color before material application. In addition, every interval was measured by the same single operator three times and the mean was calculated to ensure superior accuracy, similarly to Sayed Mahmoud in 2018. [7]

A spectrophotometer, which has higher accuracy and reproducibility than the naked eye and cameras, was used to quantitatively assess color change. Spectrophotometer can record and quantify the entire visible spectrum for three-dimensional color, and therefore it was employed in this investigation. Another important factor for using a spectrophotometer is that SDF liquid is sensitive to light, [19] so a specialized device is needed to ensure the exact degree of discoloration. It was feasible to use International Commission on Illumination (CIELAB) color space to compute the color difference and measure the color before beginning as stated by Lim et al. 2010. [20] The limitation is that the spectrophotometer can't be used intra-orally.

According to the current study color change for intergroup comparisons was as follows, the highest color change after material application was found in GSH group followed by KI group and finally SDF group. GSH has a hemostatic effect which might have controlled the rate of silver ion release, [18] consequently prolonged the release of silver ions and therefore caused the highest color change. Also, it was assumed that the percentage of GSH was not sufficient to entirely coat all the excess silver particles to efficiently reduce the color change, compared to KI and SDF groups. [21,7]

Then at the 7-day interval and 10-day intervals for intergroup comparisons the highest color change was for SDF group followed by KI group and the lowest color change was in the GSH group. This can be attributed to SDF reaching its maximum release of silver ions and consequently the reaction between the particles from KI group and GSH group. These results are concurrent with Sayed et al. as the 7-day and 10-day interval had the same ascending order for color change indicating the effectiveness of both KI and GSH in masking SDF discoloration. [7]

While Kaurna et al.2023 discovered that GSH was comparably effective as KI when observed during the 6th month follow-up. It should be noted here that the current study was an *in vitro* study with only 10 days of observation while the later study was an *in-vivo* split mouth with 6 months of follow up and, and this may be a limitation that further follow- up time is needed. [24]

On the contrary to the current study Kamble et al. 2021, supposes that KI is superior to GSH in masking over a period, it can be attributed to complete caries excavation and cavity preparation. [10] Concurrently Gupte et al. 2021 states that GSH had no significant effect on staining while KI decreased staining significantly which can be due to the fact that the study was testing enamel and not dentine like the current study. [9] Also, another factor may be due to the longer follow-up interval than the current study.

For Intragroup comparisons in the current study the highest color change for SDF group was after the 10-day interval followed by immediate material application and the lowest color change at the 7-day interval. Logically since SDF effect increases with time and so the discoloration becomes more prominent by time which makes the 10-day interval with the highest color change. Black staining of carious dentine is clinically visible as soon as two minutes post-SDF administration, according to Patel et al. 2018.[25]

As for the KI group and GSH group for intragroup comparisons the highest color change was immediately after material application followed by the 10-day interval and then the 7-day interval. The color change is highest immediately after application due to the accumulation of free silver ions on dentine surface; however, over time, the reaction may reach a point of equilibrium where further discoloration slows down or stabilizes. Additionally, natural processes like salivary flow and oral hygiene practices may help to remove excess SDF and silver ions, reducing the visible discoloration.[27] The discoloration is lowest after 7 days because by then, some of the excess SDF and silver ions have probably been washed away by saliva or reacted fully with KI and GSH. Yet, the discoloration is still noticeable after ten days because of the continuous release of silver ions, and subsequently dentin surface may darken even more.

According to the current study after testing shear bond strength we discovered that the highest bond strength was found between GSH group followed by KI group and finally SDF with the lowest bond strength. This agrees with Priya et al. 2022, who stated that significantly stronger bonds were demonstrated by Group 3 (GSH) than by Groups 2 (SDF-KI) and 1 (SDF). [23] GSH is an antioxidant with the ability to stabilize and cross-link collagen, which may have strengthened the bonds within Group 3 (SDF + GSH). [22]

Caries effected dentine should be maintained according to the MID concept. But since the adhesive strength to CAD is lower than that of sound dentine, adhesion to this substrate is challenging. According to the current study, with the proper manufacturer's instructions MID concept can be carried out successfully without the need to remove caries. [26]

After conducting the shear bond strength failure mode was conducted to determine the type of failure. For SDF group mode of failure was mainly adhesive failure, while the other groups were mainly mixed failure, nonetheless the difference was not statistically significant. According to Zhao et al. showed that cohesive failure was less common between groups, which consisted of SDF, SDF+KI, and water. [12] For SDF group mostly adhesive failure was seen and for SDF+KI group mainly mixed failure was seen like the present study.

Limitations of the study

- An invitro study
- Shear testing was measured only once after 7 days needs more follow up time
- Spectrophotometer can't be used intraorally

Conclusion

- SDF application to tooth surface causes black staining which is a known disadvantage.
- Several materials are employed to overcome this disadvantage including KI and GSH.
- Both KI and GSH have proven to mask SDF produced discoloration, but according to the current study GSH has superior results.
- Comparable shear bond strength for GSH and KI groups after applying Glass Ionomer. While the SDF group had significantly lower bond strength.
- No statistical significance between groups in relation to mode of failure.

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This research wasn't funded through any source

Data Availability

Authors confirm that all data supporting the findings of the study are available within the article.

Ethical Deceleration

This research protocol was exempted prior to the conduction of the study by the Ethical Committee of Faculty of Dentistry, Ain Shams University FDASU-RECEM022114**.**

Competing Interest

Authors declare no conflict of interest

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