

**Influence of silver diamine fluoride, potassium iodide and glutathione treatment on the shear bond strength of glass ionomer cements to artificial caries affected dentine: An in vitro study**

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**Abstract**

**Objective:** To investigate the effect of silver diamine fluoride, Potassium iodide and Glutathione treatment on the shear bond strength of glass ionomer cements to artificial caries-affected dentin.

**Materials and methods:** 60 Dentin slices with 2mm thickness were prepared from freshly extracted sound human molars. The surfaces of the dentin slices were polished and immersed in a demineralizing solution for 7 days at 25<sup>0</sup>C to mimic caries-affected dentin. The samples were randomly allocated for treatment (n = 20)

SDF (control), SDF followed by application of a potassium iodide solution, SDF mixed with 20% GSH. All specimens were then immersed in artificial saliva for 24 hours followed by which the dentin slices will be bonded with GICs. The shear bond strength was assessed using a universal testing machine. The data was analyzed using a one-way ANOVA test.

**Results:** The SBS values (mean SD) after treatment with SDF, SDF + KI, SDF + GSH were 28.16<sub>±</sub>2.28 MPa, 29.24<sub>±</sub>2.05 MPa and 28.71<sub>±</sub>1.74 MPa respectively (P= 0.00001 (<0.001)).

**Conclusion:** The application of KI solution after SDF treatment and Glutathione mixed with SDF does not negatively affect adhesion of GICs to artificial caries-affected dentin.

**Keywords:** Glass ionomer cement, Glutathione, Potassium iodide, Shear bond strength, silver diamine fluoride.

### Introduction

Dental caries is a bacterial, multifactorial, dynamic disease prevalent all over the world which is caused by an imbalance between demineralization and remineralization processes of tooth structure.<sup>1</sup> The conventional protocol for the management of a carious lesion includes complete mechanical removal of the infected, demineralized tooth structure and placement of a restoration.<sup>2</sup> In recent years, the development of restorative materials and advancement in our conception of the caries process have enabled the clinicians to practice minimally invasive dentistry.<sup>3</sup>

Silver diamine fluoride [ $\text{Ag}(\text{NH}_3)_2\text{F}$ ] (SDF) is a topical fluoride solution that has been used to halt dental caries in a concentration of 38% (44,800ppm fluoride) throughout the world since the early 1970s.<sup>4</sup> SDF is considered as an efficient, cost effective, and non-invasive material that can be used on deciduous and permanent teeth. It has been demonstrated to have a unique anti-bacterial effect and can inhibit demineralization and increase the surface micro-hardness of the tooth structure.<sup>5</sup> SDF is proven to be more effective in caries prevention compared to fluoride varnish.<sup>6</sup> Annual and biannual applications of SDF can significantly reduce the development of new lesions, offering high effectiveness in protection against caries at low cost.<sup>7</sup>

Nevertheless, SDF has a major adverse effect that it stains the caries lesion black because of the reaction of

SDF products with tooth tissues.<sup>5</sup> SDF has not been widely accepted by patients with aesthetic concerns. Application of potassium iodide (KI) after SDF results in a creamy white precipitate of silver iodide (AgI), which is considered as one of the methods that can overcome the staining problem.<sup>8</sup> However, recently it was noticed that the color improvement with KI is only temporary and the darkening of tooth surfaces still occurs.<sup>9,10</sup> In addition, the use of SDF and KI together reduces free silver ions<sup>8</sup>, which may decrease the merits of SDF dramatically, most likely over a long period of time. An alternative method has been developed which may decrease staining from SDF and preserve the silver within the solution and on the substrate surface by mixing glutathione with SDF.<sup>11</sup> Glutathione (GSH) is a tri-peptide biomolecule and considered the best candidate with silver as it contains a thiol group (-SH) which has a high affinity for adsorption onto metal surfaces.<sup>12</sup>

Restorations are generally needed for cavitated lesions to allow for an easily cleansable surface that may reduce the potential for secondary caries initiation. Although laboratory studies<sup>13,14</sup> report that application of SDF is compatible with GIC restorations, there is insufficient evidence concerning the adhesive properties of GIC restorations when they are bonded to caries-affected dentin surfaces previously treated with SDF followed by application of KI and Glutathione. Therefore, the aim of this in vitro study was to investigate the effect of SDF, KI and Glutathione treatment and the shear bond strength (SBS) of GICs to artificial caries-affected dentin. A null hypothesis tested was SDF+KI and SDF mixed with glutathione does not affect adhesion of GIC restorations to caries affected dentin.

## Materials and Methods

**Sample preparation:** Sixty non-carious human third molars were collected with patient's written consent. The research protocol was reviewed and approved by the local Institutional Review Board of the MIDSR dental college, Latur. The teeth were stored in a 0.1% thymol solution at 4 °C prior to section. Sixty dentin slices with 2-mm thickness were prepared from 60 sound third molars using a low-speed diamond disk. All dentin slices were embedded using a dental cold-cure acrylic (DPI-RR cold cure). The surfaces of the dentin slices were polished with micro-fine 2000-grit sanding paper under running water. All samples were immersed in a demineralising solution (Acetic acid) for 7 days at 25 °C. They were then randomly allocated to three groups (n = 20 per group). For group SDF + KI, the demineralised surfaces were treated with SDF + KI (Lugol's iodine solution, Biobalance). A 38% SDF solution (Dengen caries arrest, Dengen Dental) topically applied to the demineralised surfaces, with immediately applying a saturated KI solution to treatment site until creamy white turned clear. Treated surfaces were then adequately washed with copious amounts of distilled water for 30 seconds. For the control group, the 38% SDF solution was applied to the demineralised tooth surfaces and agitated using a micro-brush for 1 min, left for 2 min and rinsed with copious amount of distilled water for 30 seconds. For group the SDF with GSH group the demineralised surfaces received application of mixture of SDF and 20% GSH (Scutonix Lifesciences) until the solution turned clear. After 30 minutes, all samples were immersed in the artificial saliva at 25 °C for 24 hours. A Teflon mould with 4-mm height and 3-mm diameter was placed on the each demineralised dentin surface. All dentin samples (n = 20 per group) were then bonded with RMGIC (GC, Gold Lable Light Cured Universal

Restorative Material, GC Corporation, Tokyo, Japan) which was mixed as per manufacturer's instructions. The mixture was applied in the Teflon mould to form a cylindrical button by using a cement carrier and a cement condenser to avoid voids and cured using a LED light polymerization device (Smart Lite Focus, Dentsply Sirona, USA). After bonding, the moulds were removed from each sample and samples were stored in 100% humidity at 37 °C for 24 hours after removal from the mould to allow complete setting of GICs. The SBS test was performed with a universal testing machine that had a flat-edge loading head (ElectroPuls 3000; Instron, Norwood, MA, USA). A shear force was applied perpendicularly to the GIC cylindrical button at a distance of 1 mm from the dentin surface to the loading head. The loading head moved at a fixed rate of 1mm/minute. The load necessary to debond GICs was recorded in Newtons. The bond strength was expressed in mega-Pascals (MPa) by dividing the load at failure by the bonded surface area in square mm.

## Statistical analysis

Data was analysed by using SPSS 24.0 version IBM USA. Comparison of mean and SD between all groups was done by using one way ANOVA test. When ANOVA comes significant, then Post Hoc Tukey's HSD test was carried out to assess whether the mean difference between a pair of group is significant or not. p value of <0.05 was considered as statistically significant whereas a p value <0.001 was considered as highly significant.

## Results

The mean bond strengths of GICs to dentin are shown in Table 1 (n=20 per group). The SBS (mean  $\pm$  SD) for groups SDF, SDF + KI and SDF with GSH were  $28.16 \pm 0.61$  MPa,  $29.24 \pm 0.65$  MPa and  $28.71 \pm 0.47$  MPa, respectively (P = 0.00001). The second group (SDF+KI)

showed increased bond strength compared to other groups. Post Hoc Tukey's HSD test to see whether the mean difference between individual group is significant or not and the results indicated that the difference in the mean is significant at 0.05 level (Table 2).

### Discussion

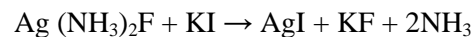
The current study was conducted in a controlled laboratory condition in which dentin was artificially demineralised to mimic caries-affected dentin. It is worth noting that natural caries-affected dentin, compared with demineralised dentin lesions, has a more complex microstructure. There may also be permeability differences between natural caries-affected dentin and artificial caries-affected dentin.<sup>15</sup> Two different substrates may therefore offer different conditions that will most likely lead to different adhesive properties. Furthermore, controlled laboratory conditions are different from the real oral environment. It is unclear whether the mechanical properties of GICs would be affected in a long-term exposure in the oral environment. Thus, caution should be taken when these findings are extrapolated to the clinical situation.

The traditional approach of managing cavitated carious lesions is to drill and fill, which refers to mechanically removing the soft and bacteria-infected dentin before filling the cavity with a proper restorative material. It is still reasonable to conduct the excavation because infected dentin is highly demineralised and physiologically not remineralisable.<sup>3</sup> Nevertheless, it has been demonstrated that bacterial count and activity were diminished over time if infected dentin in a cavitated caries lesion was restored with a well-sealed resin restoration.<sup>3</sup> However, the need for caries excavation seems still to be controversial because it has been reported that the fracture strength of composite resin fillings may be compromised by the underlying soft,

infected dentin.<sup>16</sup> On the contrary, caries-affected dentin structurally reserves enough collagen fibres to be remineralised and has a relatively low bacterial count. Thus, caries removal is not needed prior to restorative filling for caries-affected dentin or for arrested lesions.

Silver diamine fluoride has been identified as a bactericidal chemical that can reduce the adherence and growth of cariogenic bacteria.<sup>17</sup> Moreover, it can be used to prevent the formation of secondary dentin caries around GIC restorations.<sup>18</sup> Thus, SDF can be a promising biological approach in the practice of minimally invasive dentistry against conventional restorative methods. SDF is a colorless solution that contains a high concentration of fluoride (44,880 ppm) and silver (25.5%). Upon application of SDF, it reacts with tooth structure to release a large quantity of free silver ions ( $\text{Ag}^+$ ). When these ions are reduced, they aggregate and precipitate resulting in the formation of dark stains on the tooth surface, which has caused esthetic concerns among practitioners and patients alike.<sup>19</sup> Potassium iodide was introduced in an attempt to solve the color change problem associated with SDF. Upon application of a saturated solution of potassium iodide immediately after SDF application, silver iodide crystals are formed and precipitate on the surface as a creamy white precipitate.<sup>20</sup>

The reaction between KI and SDF is depicted as:



Glutathione (GSH) is a tri-peptide biomolecule and considered the best candidate with silver as it contains a thiol group (-SH) which has a high affinity for adsorption onto metal surfaces.<sup>21</sup> GSH forms a coat around silver particles, decreasing the aggregation of silver particles as well as controlling the rate of silver ion release (Homeostasis)<sup>22</sup>, which may play a role in decreasing the rate of color changes of an SDF-coated

tooth surface over time. In this study, we mixed SDF with 20% GSH by weight. Percentage of GSH cannot be increased to more than 20% as it gets difficult to dissolve the GSH in SDF.<sup>11</sup> The percentage of “20%” GSH was sufficient for enamel to minimize color changes, but for dentin it seems necessary to use an increased concentration of GSH to more effectively reduce color changes.<sup>11</sup>

Glass ionomer cements are regarded as one of the best options for fluoride-releasing restorative materials, which have been considered to be superior to compomers and giomers from the aspects of continuous fluoride release and recharge.<sup>23</sup> Nevertheless, the fluoride-releasing and anti-microbial effects of GICs are usually limited and insufficient. Hence, pretreating dentin surfaces with SDF or SDF + KI before GIC restoration has been proposed by some researchers<sup>13,19</sup> to enhance antimicrobial and remineralising ability of GICs.

The results indicated significant difference in SBS between all the three groups. The clinical implications of these findings are that if KI and GSH is applied after the application of SDF to arrest or prevent dentin caries in a tooth, the bond strength of GICs to the caries-affected dentin will not be affected. The results of this study demonstrated that pretreatment with SDF, SDF + KI and SDF with Glutathione did not adversely affect adhesion of the restoration to dentin which is consistent with the previous findings of other laboratory studies. Nevertheless, another study reported that there was an improvement in adhesion properties of fissure sealants applied after treating a tooth surface with SDF. The differences in the out-comes may result from different techniques or different characteristics of tooth substrates. The ability of GSH to decrease the color changes of enamel and dentin without affecting the bond strength of

GICs may encourage the use of SDF in a greater variety of clinical situations with better esthetic outcomes than SDF alone.

Groups	N	Mean bond strength	p
SDF	20	28.16±0.61	0.00001 (<0.001)
SDF +KI	20	29.24±0.65	
GSH	20	28.71±0.47	

Table 1: showing mean bond strengths of all three groups.

Groups	SDF +KI	GSH
SDF	-1.07	-0.54
SDF +KI		0.53

Table 2: showing mean difference between individual group (Indicates that the difference in the mean is significant at 0.05 level)

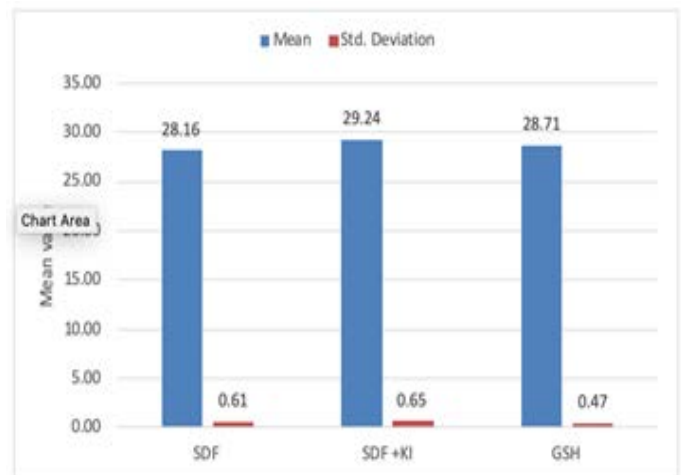


Figure 1: Mean SBS between GIC and demineralised dentin of the three groups.

### Conclusion

With the limitations of this laboratory study, the conclusion drawn was that the SDF, SDF + KI and SDF + GSH treatment does not negatively affect bonding of GICs to artificial caries-affected dentin.

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