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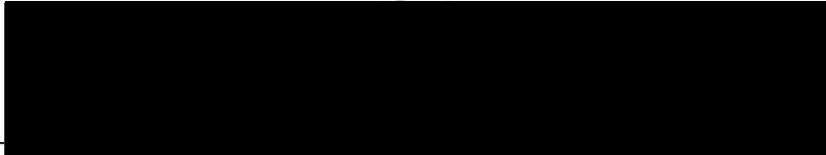
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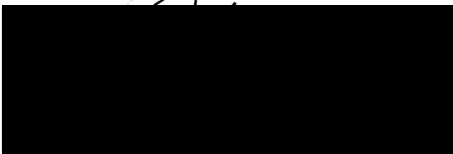
Effect of Silver Diamine Fluoride Treatment on Enamel/Dentin Bonding

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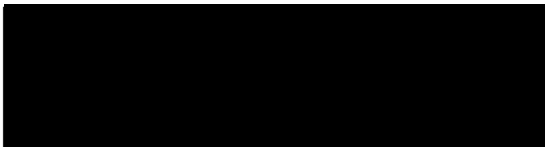


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Abstract

Objective: Silver Diamine Fluoride (SDF) is increasingly used to treat dental caries, but a preponderance of literature regarding its effects on adhesive dentistry is not observed. The purpose of this study is to evaluate the effect of SDF treatment on adhesion to enamel and dentin.

Methods: 100 human caries free human third molars were sectioned at the cemento-enamel junction (CEJ) to remove the roots followed by mounting in auto-cure acrylic. Enamel and dentin bonding surfaces were prepared following ISO 29022 instructions for grinding/polishing of specimens and stored in 0.2M phosphate buffered saline (PBS). Specimens were randomly assigned into 10 groups (n=20). Using a shear bonding jig, a nano filled resin composite cylinder was bonded to each respective surface using a self-etch two step adhesive. Enamel and dentin groups consisted of: no SDF surface application (Control), testing Immediately after one SDF surface application (E1I, D1I), testing immediately after second SDF surface application (E2I, D2I), testing at one-month after one SDF surface application (E1D, D1D), and testing at one month after two SDF applications (E2D, D2D). All materials were used following manufacturer's instructions. The samples were tested to failure on a universal testing machine at a rate of one millimeter per minute with the failure load recorded in Newtons with the mean calculated. Specimens were also evaluated for failure mode using microscopy (20X). Mean failure load was analyzed using Kruskal-Wallis/Dunn's post hoc at a 95 percent level of confidence ($\alpha=0.05$).

Results: Significant differences were found among the groups. For enamel specimens, E1I group had the highest bond strength, significantly above other groups. Followed by enamel control and E1D groups which were statistically higher than E2I and E2D groups. The dentin control and D1D were statistically higher than D1I, D2I and D2D groups. For enamel groups, the majority of failure modes were mixed between the adhesive and the composite junction; except for E1I which show increased failures in composite material. Failure modes for dentin groups were: D1I majority occurred between all three

layers (tooth, adhesive, and composite); D2D majority were adhesively; and Control/D2I/D1D had mixed between adhesive and composite junction surfaces.

Conclusions: Under the limitations of this study, enamel bond strength was significantly increased when tested immediately after one SDF application. Multiple SDF applications demonstrated a decrease in bond strength. A delayed bond with 1 application of SDF in both dentin and enamel samples showed a statistically similar bond as with the controls.

Manuscript

Introduction:

Silver Diamine Fluoride (SDF) was recently approved by the Federal Food and Drug Administration (FDA) as a medical device for the treatment of dental caries. SDF mode of action is based on the synergistic combination of fluoride and silver, as remineralization is enhanced by fluoride while the silver bactericidal action controls bacteria levels.^{1,2} Except for silver-allergic patients, SDF is reported to have relatively large safety margin but is remarkable with the black discoloration of carious tissue, which may present esthetic concerns. Potassium iodide (KI) has been suggested as a follow-on treatment that is said to alleviate discoloration by forming a precipitate, however some concerns have been voiced whether this precipitate formation may interfere with SDF effectiveness.³ Cavitated lesions that have been treated with SDF would benefit from restoration to establish cleansable contours to assist in oral hygiene. Furthermore, restorations would be required when SDF is utilized as an indirect pulp therapy modality.⁴ The effect of conventional fluoride application on adhesion using various methods has been reported.⁵⁻¹⁵ While some investigations have reported the effect of SDF to adhesion,¹⁶⁻¹⁹ current studies evaluating the effect of SDF on both enamel and dentin adhesion after immediate and delayed SDF application are limited. The purpose of this study was to evaluate the effect of SDF on both dentin and enamel adhesion after multiple SDF application immediately after application and after one month.

The null hypothesis was that there would be no difference in adhesion between:

1. The control enamel group and the SDF treated enamel groups
2. The control dentin group and the SDF treated dentin groups

Materials/Methods:

One hundred recently extracted caries free human third molars were used in this study, which were extracted for routine clinical indications collected from local oral and maxillofacial clinics following the

protocol defined by the 81st Medical Group Institutional Review Board (IRB). The roots were removed by sectioning at the cemento-enamel junction using a water cooled, slow speed diamond saw (Buehler, Lake Forest, IL, USA). The resultant crowns were then additionally sectioned in half down the central groove along the mesio-distal plane to produce two specimens, one with an intact facial surface and the other consisting of an intact lingual surface. The resultant specimens were mounted to expose the intact facial or lingual surface in self-curing tray resin (Bosworth Fastray, Gibbstown, NJ, USA) and then randomly distributed into two adhesion testing groups, enamel and dentin, respectively. All specimens were mounted in and then stored in 0.2M PBS at 37 °C.

Enamel group: Enamel bonding interfaces were prepared for shear testing, based on ISO 29022²⁰, using a two-step sequential planning process under running water. First P120 paper ($125 \pm 1 \mu\text{m}$) was used to create a flat bonding area sufficient for placing a composite button with a diameter of 2.38 mm, followed by P400 paper ($35 \pm 1 \mu\text{m}$) until the surface was even and smooth when visually inspected.

Dentin group: Dentin bonding interfaces were prepared for shear testing, based on ISO 29022, using a two-step sequential planning process under running water. First P120 paper ($125 \pm 1 \mu\text{m}$) was used to create a flat bonding area within superficial dentin sufficient for placing a composite button with a diameter of 2.38 mm, followed by P400 paper ($35 \pm 1 \mu\text{m}$) until the surface was even and smooth when visually inspected. All specimens were stored in 0.2M PBS at 37°C until testing.

The prepared enamel & dentin specimens were then randomly distributed into five groups (n=20):

Enamel:

Group 1: No SDF surface treatment (Control)

Group 2: Immediate adhesion after one SDF surface application (E1I);

Group 3: Immediate adhesion after two SDF surface applications (E2I);

Group 4: Adhesion at one month after one SDF application (E1D); and

Group 5: Adhesion at one month after two SDF surface applications (E2D).

Dentin:

Group 1: No SDF surface treatment (Control)

Group 2: Immediate adhesion after one SDF surface application (D1I);

Group 3: Immediate adhesion after two SDF surface applications (D2I);

Group 4: Adhesion at one month after one SDF application (D1D); and

Group 5: Adhesion at one month after two SDF surface applications (D2D).

Enamel specimens were rinsed and dried with oil-free compressed air for 5-10 seconds. A 38% solution of silver diamine fluoride (Advantage Arrest, Elevate Oral Care, West Palm Beach, FL, USA) was dispensed into a plastic dappen dish and applied for 5 seconds with an Appliquator brush (Advantage Arrest, Elevate Oral Care, West Palm Beach, FL, USA) according to group assignment and either immediately restored or replaced into the PBS for one month adhesion testing. The enamel surface was acid etched with 37% phosphoric acid for 10 seconds, and then rinsed and dried with (Advantage Arrest, Elevate Oral Care, West Palm Beach, FL, USA) oil free compressed air. A self-etch, two step adhesive (Clearfil SE, Kuraray America, New York, NY, USA) was applied with all required visible light polymerization provided by a PolyWave® visible curing light (BluePhase G2, Ivoclar Vivadent, Amherst, NY, USA). The treated specimen

was then mounted in a microshear bonding jig (Ultradent, South Jordan, UT, USA) centered over the enamel surface. The specimen was restored with a nanofilled resin composite (Filtek Supreme Ultra (shade B2), 3M ESPE, St. Paul, MN, USA) with the resin composite syringe orifice into the cylindrical mounting jig fixture consisting of a 2.38 mm internal diameter and 2mm height. The resin was then photopolymerized for 20 seconds using a visible curing light (BluePhase G2, Ivoclar Vivadent) for 20 seconds with the specimen then removed carefully from the mounting jig apparatus. Excess composite was removed when necessary with a scalpel blade with the diameter ascertained with a digital caliper as close to the tooth surface as possible. Restored specimens were stored in 0.2M PBS for 24hrs at 37 C and then mounted in a universal testing machine (Ultratester, Ultradent, South Jordan, UT, USA) and tested in shear until failure at a crosshead speed at 1mm/min with the calculated failure stress recorded with mean values recorded Any specimens that failed prior to testing or during testing set up were determined as pretesting failures and were recorded as zero. Failed specimens were visually examined at 20X magnification with a digital microscope (Hirox 8400, Hirox USA, Hackensack, NJ, USA) for mode of failure (adhesive, cohesive-material, cohesive-tooth, mixed material, mixed tooth, mixed tooth/adhesive/material).

The mean data was subjected to the Shapiro-Wilk and Bartlett's test which identified a non-normal data distribution and variance inhomogeneity, respectively. The data was then analyzed with Kruskal-Wallis test and Dunn's post hoc at a 95 percent level of confidence ($\alpha = 0.05$). Statistical analysis was performed using SPSS 21 (IBM/SPSS, Chicago, IL, USA). Results:

The enamel adhesion mean results are presented in Table 1 and Figure 1.

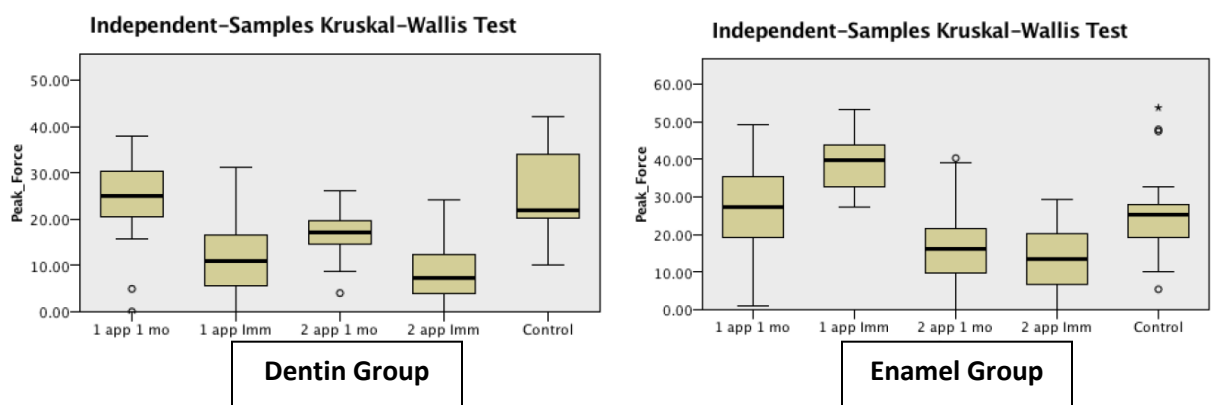
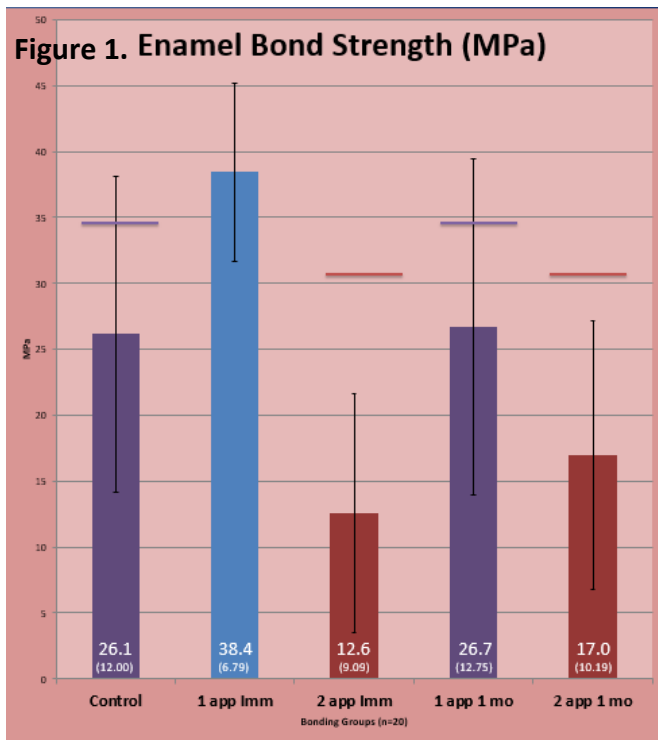


Table 1. Mean Enamel Failure Stress (MPa)

SDF Application	Time Restored	Stress (MPa)
None (control)	Immediate	26.1 (12.0) B
One	Immediate	38.4 (6.8) A
Two	Immediate	12.6 (9.1) C
One	1 Month	26.7 (12.7) B
Two	1 Month	17.0 (10.2) C

n = 20; pretesting failures were recorded as zero. Capital letters identify similar groups (Kruskal-Wallis/Dunn's; p = 0.05)



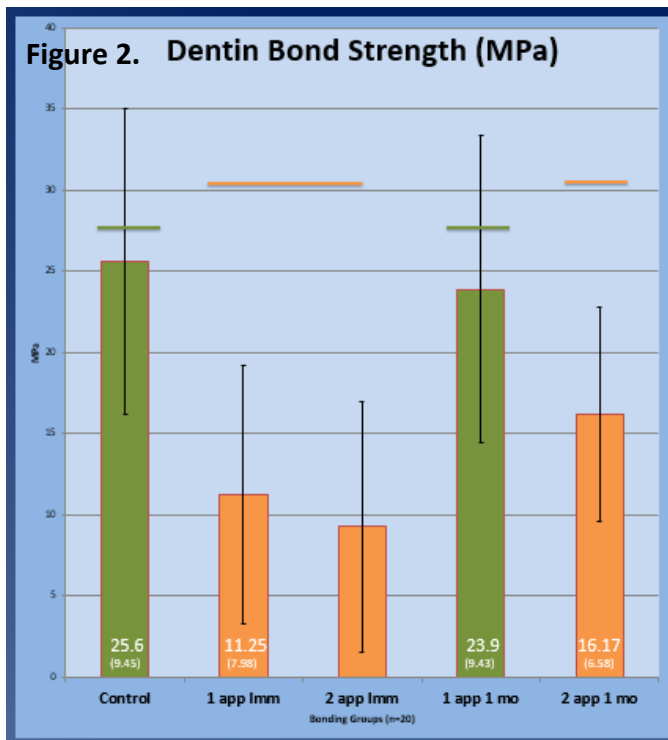
The group with one SDF application that was restored immediately demonstrated significantly greater enamel bond strength than the other groups. Two SDF applications demonstrated significantly lower enamel bond strengths with the control and one application restored at one month displaying intermediary results.

The dentin adhesion mean results can be seen in Table 2 and Figure 2.

Table 2. Mean Dentin Failure Stress (MPa)

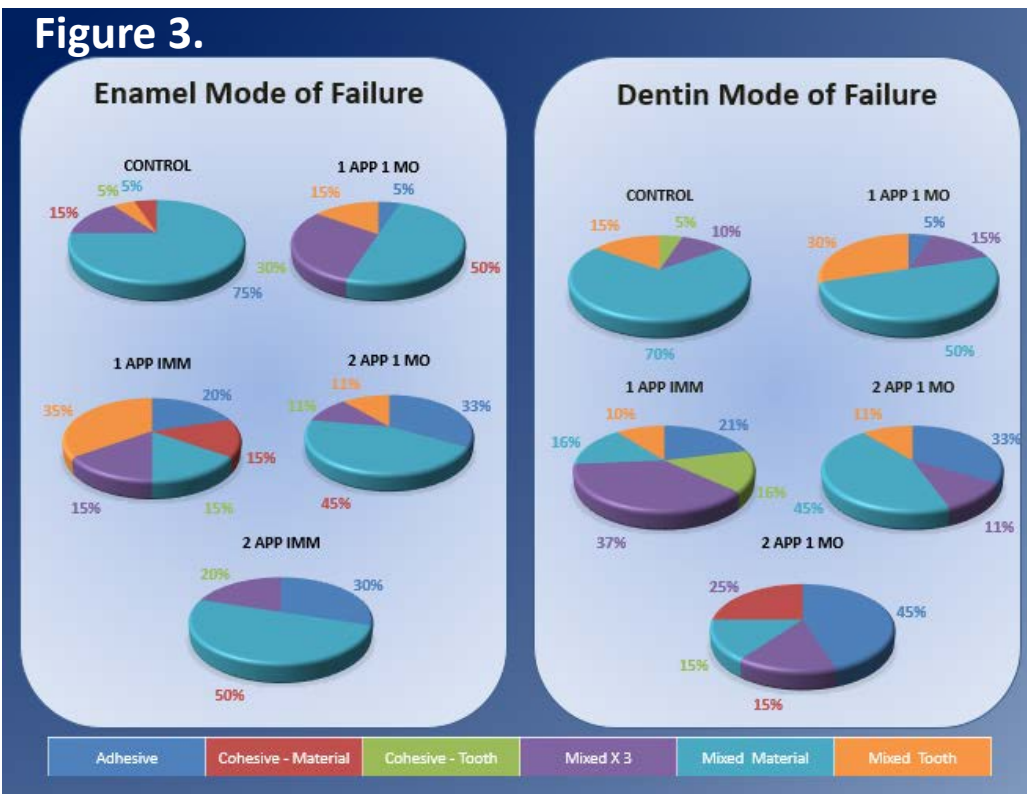
SDF Application	Time Restored	Stress (MPa)
None (control)	Immediate	26.5 (9.5) A
One	Immediate	11.3 (8.0) B
Two	Immediate	9.3 (7.7) B
One	1 Month	23.9 (9.4) A
Two	1 Month	7.3 (9.3) B

n = 20; pretesting failures were recorded as zero. Capital letters identify similar groups (Kruskal-Wallis/Dunn's; p = 0.05)



Within the dentin group, the highest bond strength was recorded in the Control group (25.6 MPa), followed closely by the 1 application and immediate bonding group (23.9 MPa). The remaining three groups had statistically weaker bond strengths.

The enamel and dentin failure modes can be seen in Figure 3.

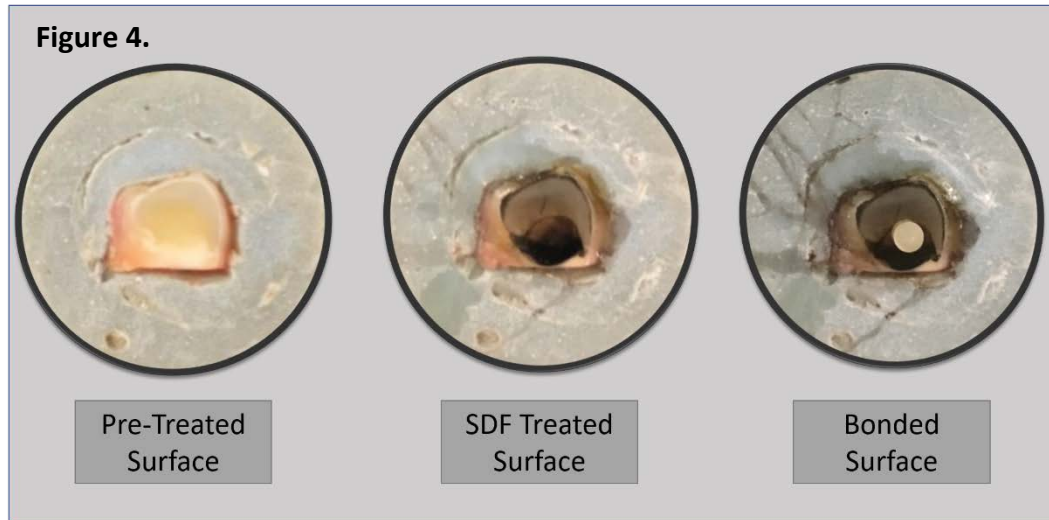


The enamel control group and the E1D group had statistically similar bond strengths and also appear to have similar failure modes. The other enamel groups demonstrate variations in failure mode and have dissimilar bond strengths from control.

The dentin control group and the D1D group had statistically similar bond strengths and also appear to have similar failure modes. The other dentin groups demonstrate variations in failure mode and have dissimilar bond strengths from control.

Brown-black color changes were observed for the SDF treated specimens. This color change was instant for samples that were treated with SDF and exposed to a photo curing light for immediate bonding,

Figure 4. A gradual color, less intense, change was also observed in specimens that were treated with SDF and then stored in solution.



Discussion:

Silver Diamine Fluoride has been reported to provide an effective means of arresting active caries in both the deciduous and the permanent dentition.^{1,2,4,21-29} Furthermore, SDF has been suggested to stabilize the collagen involved in the carious lesion as well as provide a beneficiary effect to collagen degradation mechanisms.³⁰⁻³²

SDF is proposed to present five positive features which include: non-invasive treatment, pain and infection control, simple application, low costs, and minimal training required.³³ While the preliminary studies of silver fluoride demonstrated an anti-caries effect, it was also recognized that silver fluoride can blacken caries lesions but not sound tooth surfaces. Therefore, newer in vitro experiments are examining silver fluoride followed by potassium iodide (Knight et al., 2006), which produces a white silver iodide reaction product.³⁴

The bulk of SDF literature has focused on its effectiveness on dental caries, while the question of its effect on adhesive bonding has not been thoroughly explored.¹⁷ SDF has been proposed to be able to penetrate enamel up to a depth of 25 microns, and approximately two to three times more fluoride is retained than that delivered by NaF-PO₄, NaF, or SnF₂.³⁵ The interaction of SDF with carious tooth structure does produce the commonly known Fluoroapatite, but silver phosphate is also produced, as well as the production of silver amino and nucleic acids from reacting with bacterial amino and nucleic acid thiol groups.¹ In vitro studies have begun evaluating the impact SDF might have on adhesive bonding but the results are not in agreement.

Knight and colleagues³⁶ evaluated the effect of SDF and KI with the use of Glass Ionomer (GI) and determined there to be limited to no effect with GI adhesion. Quock et al¹⁷ reported bonding compatibility for dentin pretreated with SDF for etch-and-rinse adhesives. Wu et al,¹⁸ and Selvarah et al¹⁹ concluded that SDF treated dentin might enhance adhesive resin bonding. Whereas, Koizumi et al³⁷ reported that all treated dentin surfaces showed a significant deterioration in bond strength and Quock et al¹⁷ also reported both compatibility and bond strength degradation of dentin pretreated with SDF depending on the concentration/formulation of the SDF. It is not uncommon for there to be contradictory results in the literature, especially when there are such differences in the protocols between studies. First, SDF is available in different concentrations from different manufactures and it can also be used in combination with KI. The instructions for application of SDF products vary between products and they lack specify; application time, dwell time, number of treatments. These studies also evaluated bond strength utilizing different brands and techniques of adhesive bonding agents.^{4-8, 16-19, 36-37} The variation in bonding protocols and results between studies points out the need for a bonding technique/protocol when treating patients who have had SDF treatments; previous SDF treatments and SDF pretreatment during adhesive bonding.

The null hypothesis for the both the enamel and dentin groups was rejected in our study. Interestingly, under the conditions of this study, the application of SDF was found to result in a significant increase in enamel bond strength as compared to control. However, the reason for this finding is not evident at this time.

It was shown in this study that multiple applications of SDF resulted in decrease in bond strength after immediately bonding and after delayed bonding. *Suzuki et al* showed that SDF can penetrate 25 microns into the enamel surface, and *Chu et al* showed that SDF can penetrate 50-200 microns into dentin. Quock et al ¹⁷ pointed out variations among SDF formulations and postulated that other chemicals within the solutions might have caused the bond strength reduction for self-etch adhesives and not etch-and-rinse adhesives.

As mentioned, the SDF bonding studies used various application times for applying SDF. Advantage Arrest SDF instructions state “Transfer material directly to the tooth surface with an applicator” and Riva Star instructions state “Using the silver brush provided, pierce through the foil of the silver capsule and carefully apply solution to treatment site only” and continues with “Immediately after” apply KI solution to the tooth. No time active application or dwell times are mentioned. The AAPD 2017 chairside guide for SDF application ³⁸ recommends dwell time of at least one minute after application of SDF. University of California San Francisco SDF application guidelines ³⁹ recommends application of SDF with up to one minute dwell time. Based on the available guidance, we determined to actively apply SDF for 5 second to each specimen and allow the SDF to dwell on the surface for approximately 1 minute prior to rinsing the specimens for 20 seconds with air/water syringe. Variability in application times and methods could also have an effect on adhesive bond strength of SDF treated teeth.

One unique finding in this study which needs further investigation was the color change observed during adhesive photopolymerization. During the adhesive visible light activation the SDF

treated area changed to a black color. The other samples that had the SDF applied did not turn immediately black, but through the transition time in the PBS, the samples slowly changed color. Ground/polished enamel and dentin appeared to be more susceptible to this discoloration as compared to unprepared enamel. However, this incidental finding was not designed to be evaluated in the original protocol and is only a subjective finding. The product information for Advantage Arrest states that only caries affect dentin is susceptible to staining from SDF. However, there does not seem to be a consensus in the literature on SDF discoloration effects to tooth structure.⁴⁰ If SDF does cause discoloration in tooth structure, the delivery system for SDF therapies might need to be reconsidered. Advantage Arrest, has extremely low viscosity as water is the carrier and main ingredient. A more viscous solution allowing for more control of application to teeth maybe needed. Other formulations of SFD were not available to us in the United States during the course of our research. This observed phenomena deserves additional scrutiny for the possible etiology.

The variability of tooth structure used in this our study is the first limitation to discuss. ISO 29022 directs the use of bovine incisors or human erupted permanent third molars. Human Third molars have considerable variations in size and contour.⁴¹ For smaller teeth with more rounded contours of buccal and lingual surfaces, greater reduction of enamel and dentin were required to achieve a flat bonding interface of 2.38 mm as compared to larger teeth with flatter contours. This can result in having a variation in the quality of the dentin for the bonding interface. Secondly, the specimens were shear bond tested only at 24 hours. This study did not examine bond strength after an aging or enzymatic challenge. Thirdly, this was an in vitro study. Although the teeth were stored in PBS at physiological temperature, this model is not a substitute for the conditions of an in vivo study.

Conclusions:

This study evaluated the bonding effects of SDF on both enamel and dentin surfaces. Under the conditions of this study and with the specific adhesive used it was found that SDF application to non-cariou enamel surfaces resulted in a significant increase in shear bond strength as compared to untreated control. Also, multiple SDF to both non-cariou dentin and enamel surfaces resulted in significant reduction in bond strength.

- 1.) Immediate application of SDF prior to bonding may enhance the bond strength on enamel surfaces.
- 2.) SDF decreases bond strength when applied to dentin surfaces except with delayed bonding after 1 application.
- 3.) Multiple applications of SDF decreased bond strength on both enamel and dentin surfaces.

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