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The effect of silver diamine fluoride on bond strength of self-etch adhesives to demineralized dentin (An In-vitro study)

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Abstract

Objectives: Silver diamine fluoride (SDF) is used to arrest and prevent dentin caries. However, no solid conclusion has been reached yet regarding bond strength of composite to SDF-treated dentin. The aim of this study was to evaluate the effect of SDF on microtensile bond strength of universal adhesives in self-etch mode to sound and demineralized dentin.

Materials and Methods: We used extracted 30 permanent molars and immersed half of them in a demineralizing solution to induce artificial dentin caries. We divided all the specimens into 4 groups: 1. Sound dentin without SDF, 2. Sound dentin with SDF, 3. Demineralized dentin without SDF, 4. Demineralized dentin with SDF. After SDF treatment, we applied 10-MDP containing universal adhesive followed by 4 mm nano-hybrid composite build-up. After restoration, we sectioned the specimens and measured microtensile bond strength (µTBS). We investigated the mode of failure and the resin-dentin interface after treatment. We analysed the data statistically using ANOVA, followed by Tukey's Post Hoc test.

Results: The microtensile bond strength of sound and demineralized dentin to composite using universal adhesives in self-etch mode dropped significantly after SDF application. The least bond strength values were evident in the 'demineralized dentin with SDF' group. The most common type of failure in 'sound dentin without SDF application' group was cohesive, while the predominant failure mode in the other 3 groups was adhesive.

Conclusion: We concluded that the bond strength of resin composite to either sound or demineralized dentin is negatively affected by the application of SDF prior to the use of universal adhesives in self-etch mode.

Keywords: adhesives, dentin, microtensile bond strength, silver diamine fluoride.

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Introduction

Deep dentin lesions in asymptomatic teeth are the main reason for going to endodontic intervention and loosing teeth [(1)]. Minimal Intervention Dentistry (MID) supports the removal of only the outer infected carious layer, while leaving the inner layer of soft caries within the pulpal floor. [(2)].

Bonding to sound enamel and dentin is much more reliable than carious ones despite the presence of the functional monomer 10-MDP in contemporary adhesives, which can chemically bond to calcium in hydroxyapatite [(3)]. This is because of the complexity of the hybrid layer [(4)], the low elastic modulus [(3)], and the presence of matrix metalloproteinases (MMPs) and cysteine cathepsins [(5)]. However, it is possible to use preventive agents before placing a restoration [(6)]; to inhibit the caries progression rather than removing the diseased tissues or increasing the risk of pulp exposure [(7)].

A non-invasive, brush-on treatment for dental caries has been recently adopted by dentists [(8)], named silver diamine fluoride (SDF). SDF has been entitled "silver-fluoride bullet" due to rapidity of its action [(9)]. SDF was cleared in the United States by the Food and Drug Administration in 2014 as an agent to treat dentin hypersensitivity [(10)]. Due to its effectiveness in a lot of laboratory studies and clinical trials since 1960s, it has received FDA Breakthrough as an anti-caries agent [(8)]. As dentin caries is a biofilm-mediated oral disease which involves bacteria and dentin, SDF can arrest caries; in terms of its effect on bacteria and the organic and mineral content of dentin [(11)].

However, any material applied to enamel and dentin prior to restoration placement could interfere with the bond strength of the restoration to the tooth structure [(12)]. To ensure a successful resin composite restoration, the bond strength of composite to sound and demineralized dentin after SDF application needs further research [(13)]. Thus, our study was carried out to investigate the bond strength of composite to sound and demineralised dentin pretreated with SDF, using self-etch adhesives. The null hypothesis was that silver diamine fluoride application does not affect the bond strength of self-etch adhesives to sound and demineralised dentin.

Materials and Methods:

I. Materials:

Nano-hybrid packable resin composite, 10-MDP containing adhesive, silver diamine fluoride, demineralizing solution, deionized and distilled water were used in our study.

II. Methods:

II.1.a. Specimen grouping

We used a total of 30 extracted caries-free human molars in this study. The teeth were collected after obtaining patients' informed consent and our study was approved by the ethics committee of Ain Shams University. We disinfected the molars in 1% thymol, and then stored them in distilled water. We divided the specimens according to the dentin substrate into 2 groups; sound and demineralized dentin. Then, each group was further subdivided into a control group (distilled water) and an experimental group (SDF). Each control group included 7 teeth, while each experimental group included 8 teeth. (Figure 1)

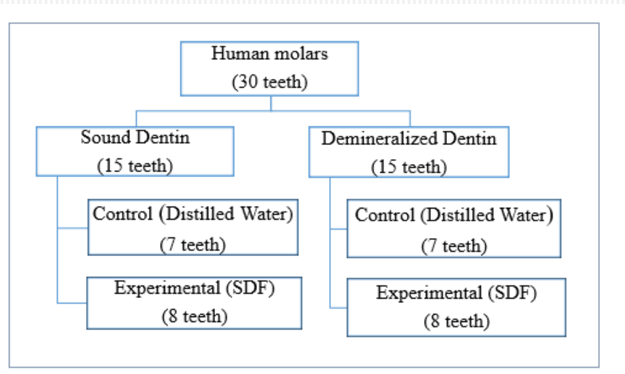

Figure (1): Flowchart of experimental design.

II.1.b. Specimen preparation

We placed double adhesive tapes on a glass slab where the specimens were attached to it with facing down. We then placed polyvinyl chloride rings (PVC) 16 mm in diameter over the specimens, poured fast set cold cure acrylic resin (Acrostone, Egypt) into PVC rings and left them to harden. We embedded the teeth half-way in acrylic resin, with only the crowns being exposed.

To expose occlusal flat dentin surface, we cut 2 mm of the crown horizontally from all teeth using a low-speed diamond saw (IsoMet® 4000, BUEHLER®) under water cooling. We used a digital caliper to measure the 2 mm occlusal thickness removed from the tooth. Then, we wet-polished the occlusal dentin surface by #300, #600, and #1000 silicon carbide paper for 30 seconds in a circular motion; to create a uniform, standardized smear layer.

II.2. Induction of artificial caries lesions:

We subjected half of the specimens to demineralizing solution in order to simulate caries-like lesions. First, we painted the teeth with an acid resistant nail varnish, leaving only the occlusal surface exposed, and then immersed them in a demineralising solution for 96 hours. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 M acetic acid. The pH was adjusted with 1 M KOH to 4.4. To confirm caries induction, we used polarized light microscope imaging with magnification power 10x and a digital camera (EOS 650D, Canon, Japan) which was mounted on a light microscope (BX60, Olympus, Japan). The average demineralization depth measured 375.394 µm into dentin.

II.3. Application protocol of SDF in the experimental groups

We applied silver diamine fluoride (Advantage Arrest®, Elevate Oral Care) in the experimental groups [14, 15]. Each specimen was dried thoroughly with triple syringe. One drop of SDF solution was dispensed into a disposable plastic dish. The material was then transferred using a micro applicator to the dentin surface, and actively rubbed for 10 seconds (Figure 2-A). The material was then left untouched on the specimen for 1 minute in order to be absorbed within the tooth. Excess material was removed by a cotton Q-tip. Using the triple syringe, the specimen was then rinsed with water for 15 seconds followed by air-dryness for 5 seconds prior to bonding (figure 2-B).

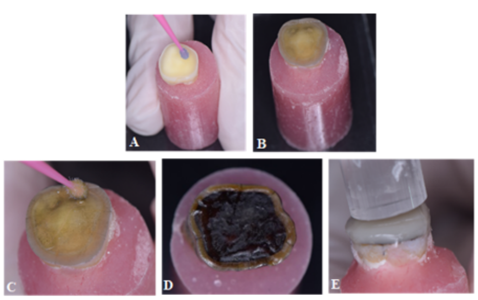

Figure 2:

A. SDF is applied on the tooth

B. The occlusal surface after rinsing SDF and complete dryness

C. Universal Adhesive application

D. The occlusal surface after light curing of the adhesive.

E. Resin composite build-up.

II.4. Bonding procedures and resin composite application

For the control and experimental groups, Single Bond Universal adhesive (3M™ ESPE) was applied in self-etch mode according to the manufacturer's instructions, air-dried, and then light-cured for 10 seconds using LED light-curing unit (Elipar™ DeepCure-S LED Curing Light. Light output: 1200mW/cm²) (Figure 2-D). We checked the light intensity using a radiometer (SDI LED radiometer) after each test group. We then placed 4 mm nano-hybrid resin composite restorative material incrementally (Filtek™ Z250 XT, 3M ESPE). Each 2 mm-increment was cured according to manufacturer's instructions, for 20 seconds using LED curing unit with four overlapping exposures from each surface. (Figure 2-E). All teeth were then stored in distilled water for 24 hours at room temperature before the testing procedures.

II.5. Microtensile testing

We sectioned the specimens in 'X' and 'Y' directions by a low-speed diamond saw (IsoMet® 4000, BUEHLER®) giving adhesive-dentin bonded sticks. Each stick consisted of restorative material, adhesive, and dentin. The cross-sectional area of each stick measured 1x1 mm² using a digital caliper. 40 beams were yielded from each restored specimen. The bonded surface area of each beam was measured using a digital caliper. The ends of the adhesive-dentin bonded beams were attached to a jig through cyanoacrylate adhesive and tested under tension using universal testing machine (Model 2519-104; Instron®, US). The force in Newtons (N) needed to displace the restoration was recorded. We calculated the microtensile bond strength (µTBS) by dividing the load at failure (N) by the cross sectional bonding area (1 mm^2). Readings were recorded in megapascal (MPa).

II.6. Failure mode analysis:

Following microtensile testing, we observed all the debonded specimens through stereomicroscope at 40x magnification to examine their fracture pattern. The failure modes were categorized according to the type and location into 4 groups: adhesive failure, cohesive failure in composite restorative material, cohesive failure in dentin, and mixed failure.

II.7. Evaluation of Resin-Dentin Interface:

Additional teeth (n=4) were used for environmental scanning electron microscope imaging (Quanta FEG-250 SEM) to evaluate the resin-dentin interface in each of the 4 groups. After dentin surface treatment of each tooth according to its group, 2 mm composite build up was applied. We cut the specimen longitudinally using a diamond saw under copious irrigation (IsoMet® 4000, BUEHLER®) to give thin sections of 2 mm thickness. In order to remove the smear layer, each specimen was etched with 37% phosphoric acid gel for 5 seconds and rinsed with water for 10 seconds. Ethyl alcohol was then applied for 3 times to ensure removal of remaining water. We left them in a dry closed container over-night. Then, they were placed on aluminum stubs, sputter-coated with gold (S150A sputter coater), and observed under scanning electron microscope with magnification 500x up to 4000x.

II.8. Statistical analysis:

One-way ANOVA followed by Tukey's Post-Hoc test was performed to compare the effect between the 4 groups using SPSS software Version 24.0 (IBM Corporation, New York, USA).

Results:

I. Microtensile bond strength testing:

'Sound dentin without SDF' showed the highest statistical significant mean microtensile bond strength value. Both groups, 'sound dentin with SDF' and 'demineralized dentin without SDF', showed lower statistical significance than 'sound dentin', and statistically significant higher microtensile bond strength values than 'demineralized dentin with SDF'. However, there was no statistical significance between 'sound dentin with SDF' and 'demineralized dentin without SDF'. 'Demineralized dentin with SDF' showed the least statistically significant microtensile bond strength value. (Table 1, Figure 3)

Table (1): Mean ± standard deviation (MPa) of microtensile bond strength values of all tested groups.

Bonded surface	Mean (MPa) ± S.D
Sound Dentin	38.55 ± 7.21^{A}
Sound Dentin + SDF	18.62 ± 5.41^{B}
Demineralized Dentin	17.89 ± 3.75^{B}
Demineralized Dentin + SDF	5.18 ± 1.08^{C}

*Superscripts with different capital letters are statistically significant at $P \leq 0.05$

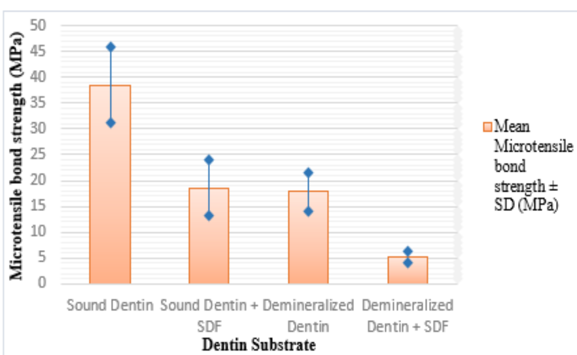

Figure (3): A bar graph representing the mean microtensile bond strength values ± S.D in MPa of all tested groups.

II. Mode of failure:

According to table (2) and figure (4), the failure patterns showed great variation between the control and SDF-treated specimens. The SDF-treated dentin specimens showed a marked increase in adhesive failure.

Table (2): Percentage (%) of failure patterns

Group	A/CC/CD/M
Sound Dentin	20/45/35/0
Sound Dentin + SDF	70/10/15/5
Demineralized Dentin	45/20/5/30
Demineralized Dentin + SDF	90/0/10/0

*A: adhesive, CC: cohesive in composite, CD: cohesive in dentin, M: mixed

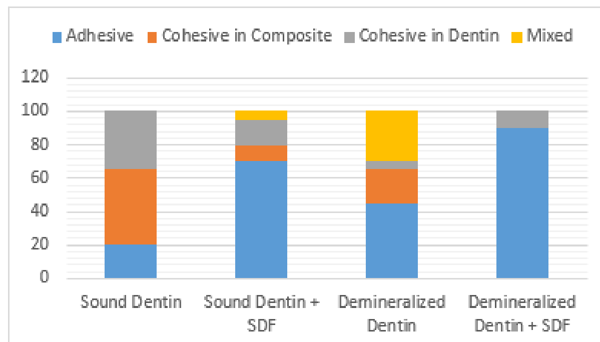

Figure (4): A bar graph representing failure modes in percentage (%)

III. Evaluation of resin-dentin interface:

We observed four specimens under scanning electron microscope, (one specimen for each group) to assess the resin-dentin interface.

1. Sound Dentin without SDF: The adhesive layer appears continuous with no gaps between dentin and resin composite. (figure 5-A)
2. Sound Dentin with SDF: SDF appears as white precipitate along the dentin surface and

within the dentinal tubules (red arrows). A gap is seen between SDF-treated dentin and the adhesive interface (blue arrows). (figure 5-B)

3. Demineralized Dentin without SDF: The image shows a gap formed between dentin and the adhesive layer indicating adhesive failure between demineralized dentin and the adhesive agent (blue arrows). (figure 5-C)
4. Demineralized Dentin with SDF: The image shows a gap between resin composite and the adhesive layer indicating adhesive failure between resin composite and the bonding agent (blue arrows). It also shows SDF precipitation on the surface and within dentinal tubules (red arrows). (figure 5-D)

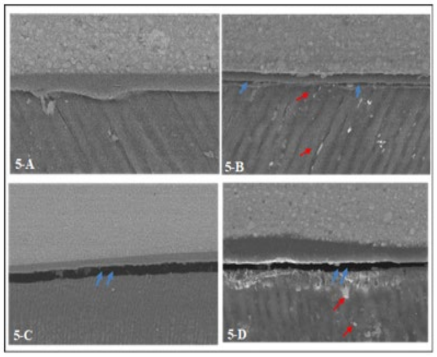

Figure 5: Scanning Electron Microscope images showing
A: Sound dentin without SDF application.
B: Sound dentin with SDF application
C: Demineralized dentin without SDF application
D: Demineralized dentin with SDF application

Discussion:

FDA cleared silver diamine fluoride as a cavity liner over soft dentin [8] since 2014 [16] and designated it as a breakthrough therapy [17]. However bonding to such affected dentin is one of the challenges in managing deep lesions. We selected Single Bond Universal (3M™ ESPE) in our study as it contains both 10-MDP and polyalkenoic acid copolymer (Vitrebond copolymer; 3M ESPE). Both react chemically with dentin hydroxyapatite which might improve the bonding performance [13, 18, 19].

We used the microtensile bond strength test to evaluate the bonding performance of universal adhesives to dentin after SDF application. It assessed the ultimate tensile strength and modulus of elasticity of SDF to sound and demineralized dentin. The µTBS proved to give more clinical significance than microshear tests as it can better simulate clinical performance [20].

In our study, sound dentin without SDF gave the highest statistically significant µTBS values. This could be explained by the presence of 10-MDP functional monomer which promotes proper demineralization depth followed by resin infiltration and a regularly-formed hybrid layer [21] (Figure 5-A).

We found that bonding of composite to demineralized dentin decreased to about half the sound dentin values (Figure 5-C). These unfavourable results might be a result of the porous structure of carious dentin, with disorganized collagen fibrils; [13] which caused deeper demineralization, improper adhesive penetration and thicker hybrid layer [2,4,22,23,24]. The lower mineral content of calcium and phosphate ions in the demineralized dentin could also affect their chemical interaction with the acidic functional monomers in the adhesive [4, 16]. Moreover, the presence of whitlockite mineral deposits within dentinal tubules cause the infiltrated resin tags to be short [2,21,24,25]. All these characteristics may have contributed to the low mechanical strength of demineralized dentin which in return, affects the bonding performance to universal adhesives [2, 22].

Studies reported that SDF bonding depends on its application protocol [20,26]. In our study, we rinsed SDF after application on the tooth structure by one minute to remove the precipitates of silver and fluoride ions that have deposited within the dentinal tubules, while still maintaining its actions [20]. Otherwise if SDF is not rinsed, its precipitates may affect resin impregnation into the dentinal tubules leading to bond deterioration [26].

We found that SDF application decreased bond strength to both sound and demineralised dentin to half of its control values (Table 1, Figure 3) leading us to reject the null hypothesis. These findings may be due to the deposition of silver and fluoride ions into the dentinal tubules which may not be removed even by rinsing. Consequently, the adhesive resin may fail to infiltrate into the dentinal tubules leading to formation of a thin, irregular hybrid layer (Figure 5-B, 5-D).

Our results are in agreement with Markham (2020), [27] Aye KO KO (2020) [28] LUTGEN (2018), [24] Koizumi (2016), [29] Kucukyilmaz (2016), [24] and SOENO (2001), [31] who stated that SDF application on sound and demineralized dentin reduces the bond strength to resin composite. The previous authors added that contamination of dentin with SDF might negatively affect the chemical bonding between 10-MDP and the calcium ions in dentin, preventing the formation of a proper adhesive seal at the interface [24,27,28,29,31]. The highly basic pH of SDF (around 10-10.5) can also disable the etching effect of the acidic functional monomers, thus mild universal adhesives could not remove the SDF-incorporated smear layer to allow for subsequent resin infiltration [27,30].

Our findings are contradicting with Wu (2016), [12] Siqueira (2020), [13] Selvaraj (2016), [15] Firouzmandi (2020), [22] and Quock (2012), [32] who stated that SDF did not affect bonding to sound and demineralized dentin. Their conclusion may be attributed to differences in the protocol used to clear SDF. Some authors rinsed SDF for 30 seconds [13, 32] which was double the rinsing time in our study (15 seconds), while others used etch and rinse adhesives [12, 22]. Both methods helped to wash away the SDF precipitate resulting in better resin infiltration and micromechanical interlocking.

The nature of the adhesive used in our study can also help explain our results. Being a mild self-etch adhesive might not allow for a higher degree of demineralization compared to the strongly acidic, 2-step self-etch adhesives (pH 0.8-1.2) used in other studies [15,32]. Consequently, the mild adhesive could not infiltrate enough into the dentinal tubules to give sufficient bonding. Future studies are needed to confirm the present hypothesis.

Other studies also reported contradictory results as Siqueira (2020), [13] who used different method of artificial caries induction and Abdullah (2018), [33] who measured microshear bond strength. The microshear test may result in considerable bending, variable and non-uniform loading conditions [34], which may explain why their results are different.

Evaluation of the failure mode for the tested specimens helped us determine the weakest area in the complex adhesive-dentin interface [15]. Cohesive failure in resin composite occurred in the control sound group (without SDF), which indicates strong bonding as the adhesive interface remains intact [32]. While SDF-treated dentin groups (sound and demineralized) and the demineralized control group (without SDF) showed adhesive failure, indicating poor quality of the formed hybrid layer [22]. (Table 2, Figure 4)

According to our results, it may be hypothesized that if resin composite is applied using universal adhesives in self-etch mode to sound and demineralized dentin treated with SDF, it will debond leading to failure of the restoration.

Conclusion:

Within the limitations of this study, We concluded that the bond strength of resin composite to either sound or demineralized dentin is negatively affected by the application of SDF prior to the use of universal adhesives in self-etch mode.

Clinical Recommendations:

1. It is not recommended to use universal adhesives (self-etch mode) over sound or demineralized dentin treated with silver diamine fluoride.
2. SDF may be used over carious spots of dentin within the cavity floor instead of a "whole cavity" remineralizing agent; otherwise adhesion would be compromised.
3. Clinicians may need to be provided with another presentation form to use SDF as spot application only. Therefore, dentin pretreatment with SDF still requires further future investigations in order to make use of all its advantages.

Conflicts of interest:

The authors received no financial support and declare no potential conflicts of interest with respect to authorship and/or publication of this article.

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