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Effect of Surface Pre-treatment with Silver Diamine Fluoride on Micromorphological Patterns and Microshear Bond Strength of Universal Adhesive to Dentin

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Objectives: This in vitro study aimed to investigate and compare the effect of surface pretreatment with Diamine Silver Fluoride (DSF) and silver fluoride (SF) on the microshear bond strength (μ SBS) of universal adhesives to dentin and micromorphological analysis of tooth restoration interface.

Methods: Sixty freshly extracted sound human molars were selected and randomly divided into three groups ($n=20$) according to the surface pretreatment applied as following: control group (no pretreatment applied), DSF group and SF group. Each group was further subdivided into immediate and aged subgroups ($n=10$). After mid-dentin exposure and pretreatment, universal adhesive and resin composite were applied and light-cured separately. μ SBS was tested using a universal testing machine. Micromorphological analysis of the dentin-resin interface was performed using Scanning Electron Microscope (SEM) for selected samples from each group. Two-way Anova was conducted and followed by Tukey's post-hoc multiple comparison tests were conducted. **Results:** Regarding μ SBS, two-way Anova test revealed statistically significant differences among the study subgroups ($p<0.05$). Tukey's post-hoc multiple comparison test revealed no significant differences between the three immediate subgroups ($p>0.05$), while within the aged subgroups, the control subgroup showed significantly higher μ SBS compared to DSF and SF subgroups ($p<0.05$). There were no significant differences between the immediate and aged subgroups ($p>0.05$) except for the control group that demonstrated statistically significant increase in the μ SBS of the aged subgroup compared to the immediate one ($p<0.05$).

Conclusion:

Surface pretreatment with either DSF or SF had adverse effect on the bonding ability or durability of the universal adhesive to dentin. **Keywords:** Diamine silver fluoride, silver fluoride, microshear bond strength, micromorphological analysis, universal adhesive.

Dental caries is one of the most common preventable diseases which is recognized as the primary cause of oral pain and tooth loss. It is a major public health oral disease that hinders the achievement and maintenance of oral health in all age groups.(1) Although technological advances and novel discoveries in dental materials are made each year, dental caries persists as a public health problem worldwide.(2)

Deep dentin lesions are the main reasons for going to endodontic intervention and losing teeth.(3) Traditional caries removal by conventional rotary pathway and placement of a restorative material does not address the ultimate cause of caries and is complicated by subjects with multiple advanced lesions.(4) 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 "selective caries removal in permanent teeth (SCRIPT)".(3)

A non-invasive, brush-on treatment for dental caries, named diamine silver fluoride (DSF) has been adopted in the last decade. Diamine silver fluoride has been entitled "silver fluoride bullet" due to rapidity of its action.(5)

Silver containing compounds such as silver nitrate, silver fluoride (not stabilized by amine groups), silver foils, and silver sutures have been used as antimicrobial agents for hundreds of years around the world to prevent and treat infections, but their popularity in the US has waned over time.(6)

Diamine silver fluoride was originally developed as a caries-arresting agent in Japan (7) later on, it becomes a compound of interest in the United States in recent years as research has shown it to be a cost-effective and simple product to use, resulting in favorable outcomes in the treatment of dental caries.

(6)

By limiting the progression of active lesions, ideally this compound would be used to treat carious lesions non-traumatically as an alternative to removing tooth structure.(8) While DSF's exact mode of action is uncertain, it has effect on caries arrest. Diamine silver fluoride reacts with hydroxyapatite to form calcium fluoride and silver phosphate which hardens the structure of existing lesions.(9) Furthermore, silver and fluoride ions together inhibit formation of carious lesions better than the sole use of silver nitrate or sodium fluoride.

A recent study suggested that fluoride ions released locally into the interface of composite restoration with bioactive bonding materials could

decrease the formation of caries, but the amount of fluoride available to deep tissue from this source is limited.(10) Additionally, DSF may be a beneficial anti cariogenic pretreatment compound for dental tissue prior to the placement of restorative material, preventing formation of recurrent caries.(11-13)

The unique caries arresting ability of DSF has been attributed to its effectiveness in reducing the load of cariogenic bacteria on surfaces of demineralized dentin and within dentinal tubules, which supports its use in treating active lesions.(14-16) A study by Beltran et al 2015, which examined the effects of various fluoride containing agents in preventing collagen breakdown and demineralization of dentin determined DSF to be the most effective, concluding that DSF may promote dentin health in caries-affected teeth.

Universal adhesives were introduced to the market in 2013 and gained popularity among dental professionals, owing to their unique properties, such as the potential to bond to different kinds of clinical dental substrates and fewer technical steps. They can bond to dental substrates, ceramic, composites and metal substrates. Therefore, they are also referred to as "multi-mode" adhesives.(22) Universal adhesives can generally be applied as one-step (self-etch) or two-step (etch and rinse) when phosphoric acid is used. Additionally, they contain, in their constitution, specific carboxylate and/or phosphate monomers that ionically bind to the calcium contained in hydroxyapatite.

Bonding of restorative materials to tooth substrate has improved significantly with the development of dental adhesives over the past several decades.(16) The presence of the functional monomer in contemporary adhesives allowed chemical bonding to calcium in hydroxyapatite.(17)

Bonding to sound dentin is much more reliable than carious ones because of the loss of minerals, high water content and weak nature structure.(19) However, it is possible to

use anti microbial before placing a restoration;to inhibit the car ies progression rather than removing the diseased tissues or increasing the risk of pulp exposure.(20,21) **Aim of the study:** This in vitro study aimt to investigat e and compare the effect of surface pretreatment with Diamine Silver Fluoride (DSF) and silver fluoride (SF) on the microshear bond strength (μ SBS) of univers al adhesives to dentin and micromorphological analysis of tooth restoration inter face immediately and after six months water storage.

Null Hypotheses

This study designed to test the null hypothesis that there is no adverse effect of Diamine Silver Fluoride surface pretreatment on micro shear bond strength o f universal adhesives to dentin and there is no adverse effect on micromorphol ogical analysis of tooth restoration interface.

1.Materials

The material utilized in the current study were: Diamine Silver Fluoride (Riva star, SDI, Australia), Silver Fluoride (Riva star aqua,SDI,Australia), one Nanoh ybrid resin composite (Luna, SDI,Australia) and one universal adhesive (Zipbond, SDI, Australia). The materials are presented in table 1

Product name	Manufacturer	Lot number	Composition
Riva star (Diamine Silver Fluoride)	SDI, Australia	1218958	Silver,Fluoride,Ammonia and Potassium Iodide
Riva star aqua (Silver Fluoride)	SDI, Australia	1221725	Silver,Fluoride,Water and Potassium Iodide.
Luna (Nanohybrid universal composite)	SDI, Australia	8452010	22.5%wt(39%vol.) multifunctional methacrylic ester. 77%wt(61%vol.)inorganic filler(40nm-1.5micron).
Zipbond (Universal adhesive)	SDI, Australia	8100502	Adhesive monomers including MDP,ethanol,water,fluoride

2.Methods

2.1.Sample size calculation

Sample size calculation was based on the mean shear bond strength among group in a previous study studying the effect of Diamine Silver Fluoride Surface Pre-treatment on the bond strength of universal adhesive to dentin.²³ Using G power program version 3.1.9.7 to calculate sample size based on effect size of 1.56, using 2-tailed test, a error=0.0 and power=80.0%, the total calculated sample size was 8 in each group that was elevated to 10.

2.2. Ethical considerations and teeth selection

The present study comprised 60 sound human molars that had been freshly extracted due to periodontal diseases. The teeth were extracted from patients ranging in age from 45 to 55 years old. The teeth were collected from Oral and Maxillofacial surgery Clinic at Faculty of Dentistry, Mansoura University after obtaining written informed consents from the patients to use their extracted teeth in the study. The protocol of the current study was approved by the Ethics Committee of the Faculty of Dentistry Mansoura University (A0208024CD). All teeth were cleaned from any hard or soft tissue remnants using a hand scaler (Nordent, Ivory #2-3, USA) and examined for exclusion of any crack or defect using a stereomicroscope at 20x magnification (Labomed magna, Model:6129001, Labo-America INC, Fremont CA, USA). The teeth were disinfected in 0.5% chloramine-T solution for 24 hours and stored in distilled water in an incubator (BIC, Egypt) until used. The storage procedure of teeth was conducted following the standard international and local infection control protocol.

2.3. Study design

For μ SBS test, the collected teeth were randomly divided into 3 equal groups (n=20) based on the dentin pretreatment performed as following: control group (no dentin pretreatment), DSF group (dentin pretreatment with DSF) and SF group (dentin pretreatment with SF). Each group was further subdivided into 2 subgroups (n=10) based on the time of testing either immediate or after 6 months of water storage. Only 5 specimens in each subgroup were subjected to micromorphological analysis test. The study design of the current study is illustrated in

figures1,

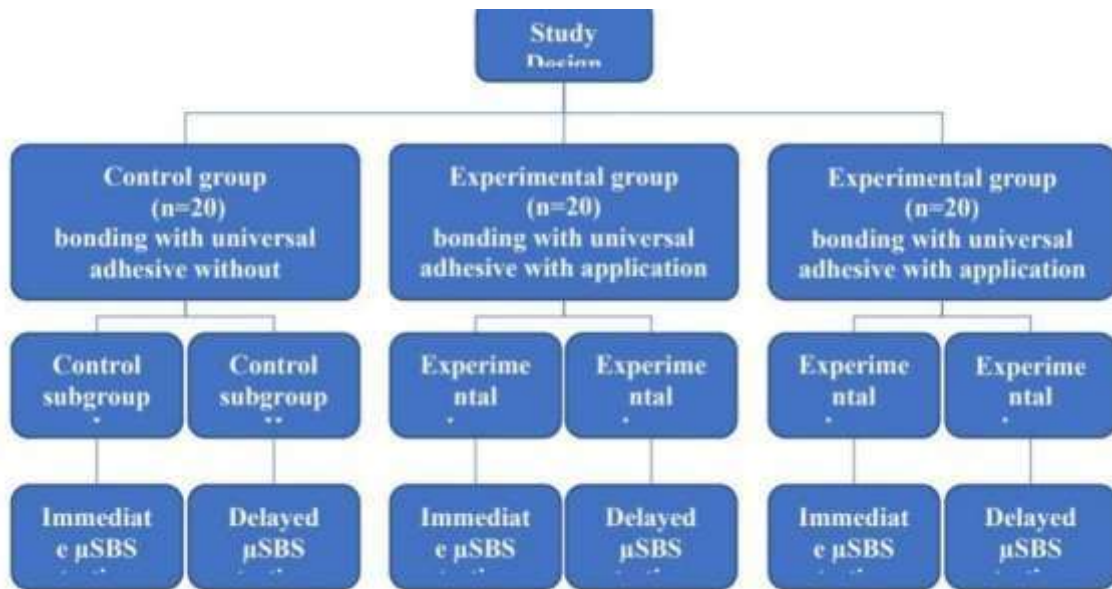
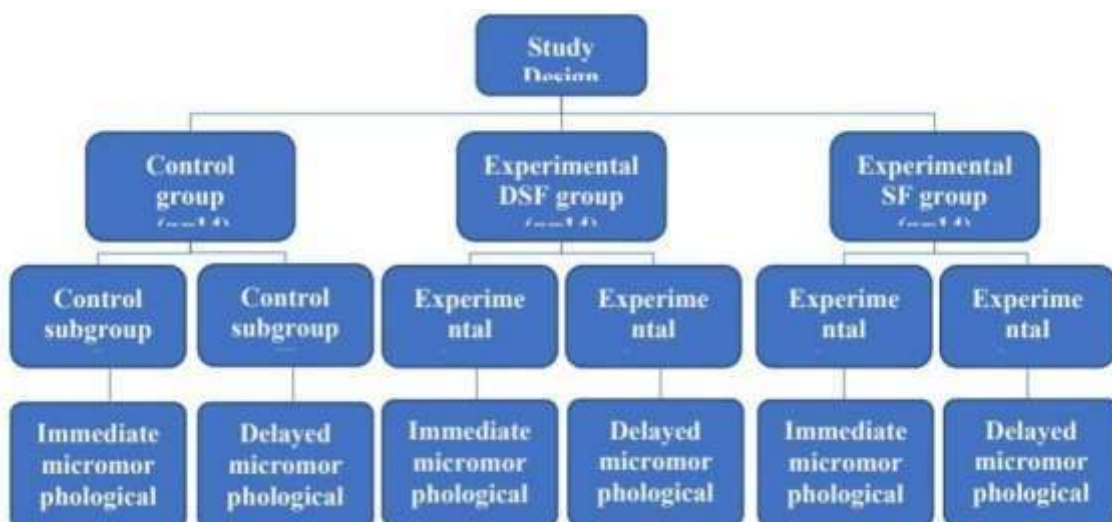


Figure1



2.4. Micro-shear bond strength test

2.4.1. Teeth mounting and specimen preparation

Each tooth was mounted vertically in a Polyvinyl Chloride ring (PVC, 1.4-2.5 cm) filled with auto-polymerizing acrylic resin (Acrostore, Egypt) with occlusal surface of the tooth facing upwards. After acrylic resin setting, the specimens were removed. Digital periapical radiographs were taken for all teeth to ensure almost equal coronal dentin thickness between all teeth with an accepted deviation of $\pm 5\%$. With the help of the digital scale software, the mid-coronal dentin of each specimen was marked on radiograph. The distance from a fixed cusp tip reference point to mid-coronal dentin point was measured. With the help of a premeasured reference (4-mm piece of percha), the actual distance from the selected cusp tip reference point to the mid-coronal dentin point was measured and marked on the buccal surface of each tooth.

The previously marked mid-coronal dentin was exposed by sectioning the tooth at the marked level by using a low-speed diamond saw (ISOMET 4000; Buehler, Lake Bluff, Illinois, USA) under water cooling system in order to get a flat dentin surface. To standardize the smear layer, the exposed dentin surfaces were polished for 60 seconds using 600-grit silicon carbide paper under continuous water irrigation.

2.4.2.Dentin surface pretreatment and restorative procedures

In the control subgroups, no pretreatment was performed on exposed dentinal surface. For the DF subgroups, the silver fluoride solution was applied to the exposed dentin surface using a microbrush (Fine,SDI,Australia) followed by immediate application of the potassium iodide Solution over the silver fluoride until the creamy white precipitate turned clear. The surface was blot dried to remove any solution material before any restorative procedures. For SF group, the Riva star aqua was applied in 2 steps as in the DSF subgroup.

After dentin Surface pre-treatment, the universal adhesive was applied through scrubbing the adhesive over the dentin surface for 10seconds. Then leaving it undisturbed for an additional 10 seconds.After that,the surface was gently air-dried until it appeared glossy without any wavy movements of the adhesive. Light_curing of the adhesive was performed using a LED light curing device (Demi Ultra, Kerr™,output 1100-1330 mW/cm², wavelength 250-470 nm) for 10 seconds.

After adhesive application and light curing, 3 transparent tygon tubes (1mm internal diameter and 2mm height) placed on the central area of dentin surface using addition silicon (polyvinylsiloxane) impression material in super light consistency (Ghenesyl, Super Light Body,Lascod,Florence, Italy) act as mold for fixed tygon, the tygon tubes were filled with resin composite material (luna, SDI, Australia) then covered with a clear celluloid strip followed by a transparent glass slide and light cured with the same LED curing unit for 20 seconds. The tip of the light guide was kept in intimate contact with the glass slide to ensure standardized curing distance. To remove the tygon tubes from around the restoration,two parallel cuts were carefully made longitudinally using a scalpel

(Paragon, Sheffield, England) to facilitate their gentle detachment from around the set material without stressing the adhesive interface.

The micro cylindrical specimens were checked with a stereomicroscope (SZ-PT, Olympus, Japan) for detection of interfacial defects. Finally, the specimens were stored in deionized water in an incubator 24 hours before μ SBS testing for immediate subgroups and 6 months for aged subgroup

2.4.3. Microshear bond strength testing

The mechanical μ SBS test was performed in a universal testing machine (Instron 3345, Canton, Massachusetts, USA). (Figure 8) Each specimen with the composite microcylindrical tubes was placed in the lower fixed compartment of the universal testing machine. A thin orthodontic wire (diameter 0.14mm) was looped around each microcylindrical tube as close as possible to its base. The wire was aligned with the loading axis of the upper movable compartment of the testing machine to ensure proper distribution of shear load. Shear force was applied to each specimen at a crosshead speed of 0.5 mm/min until failure occurred. The μ SBS values (expressed in MPa) were calculated from the maximum failure load (expressed in Newton) divided by the bonded surface area (mm²).

2.4.5 Failure mode analysis:

The failure mode was identified by examining the dentin surface of the debonded specimens under a stereomicroscope (SZ2-ILST, Olympus, Japan) at approximately 40x magnification. Representative samples from each failure pattern were sputter-coated with gold and examined by SEM (JSM-6510LVSEM, JEOL Ltd, Tokyo, Japan) at approximately 35x magnification for the verification of the fracture pattern.

classified as following:

- Adhesive (failure at interface).
- Cohesive (failure within the resin composite or with the tooth).
- Mixed (combination of adhesive and cohesive failure).

3.Micromorphological analysis:

Micromorphological analysis of the dentin/composite interface was conducted using a SEM. Five representative specimens were randomly selected from the second half of the teeth after sectioning, for each aging condition with DSF surface pre-treatment and without surface pre-treatment. These specimens were then undergoing specific cleaning procedures. These procedures involve ultrasonic cleaning for a duration of 10 minutes. Following the cleaning process, an acid-base challenge will be performed on the specimens. This challenge entails exposing the specimens to a 10% orthophosphoric acid solution for 5 seconds, followed by a 5% sodium hypochlorite solution for 5 minutes.²⁴ To prepare the specimens for analysis, a thin layer of gold was applied using a gold sputtering technique. The coated specimens were then be examined under the SEM at specific magnification levels of x50, x500, x1,000 and x2,000. The study design for Micromorphological analysis of all material sub-groups is represented.

4.Statistical Analysis

Statistical Software Package Program (SPSS, V.22, IBM Armonk, New York, United States) was used for the statistical analysis of the collected data. The data were tabulated and statistically evaluated using three-way analysis of variance (ANOVA) followed by Tukey honestly

significant difference post-hoc multiple comparison tests. The level of significance was set at $p < 0.05$ **1. Micro-shear bond strength test results**

The Shapiro-Wilk test results revealed a normal distribution pattern in the μ SBS data ($p > 0.05$). The two-way ANOVA test revealed significant differences in the μ SBS mean values among the study groups ($p < 0.05$). (Table 2) Tukey's post-hoc multiple Comparison test was performed to Compare between the subgroups. Within immediate subgroups, there were no significant differences between the three subgroups ($p > 0.05$). Among the delayed subgroups, the control subgroup showed significantly higher μ SBS mean value compared to the DSF and SF subgroups ($P < 0.05$). However, there were no significant differences between the delayed DSF and SF subgroups ($p > 0.05$). In addition, there were no significant differences in the μ SBS mean values between the immediate and delayed subgroups in the same group except for the control group where the delayed control subgroup presented significantly higher μ SBS mean value compared to immediate one ($P < 0.05$).

2. Micromorphological analysis

Micromorphological evaluation was performed using Scanning Electron Microscopy (SEM) to assess the structural integrity of the hybrid layer and resin tag formation at the dentin-adhesive interface following various surface pre-treatments. Specimens were analysed from each control subgroups, DSF subgroups, and SF subgroups under both immediate and aged conditions. Hybrid layer morphology in the control immediate subgroups was showed continuous, uniform, and well-

demarcated with an average thickness ranging between 24 μ m. This morphology is consistent with optimal adhesive infiltration and hybridization of the superficial collagen matrix.

After aging, SEM revealed visible degradation of the hybrid layer, characterized by micro-fractures and discontinuity, suggesting hydrolytic breakdown typical of resin-dentin interfaces subjected to long-term aqueous storage. Resin tags were prominent, elongated resin tags penetrating deeply into the dentinal tubules were evident in the immediate subgroup. These structures diminished in density and continuity in the aged subgroup, indicating partial demineralization and potential enzymatic degradation over time.

Hybrid layer in DSF immediate subgroups demonstrated a disrupted and non-homogeneous hybrid layer with a thickness often $<2\mu$ m or entirely absent in localized regions. The surface showed irregular deposition of granular or globular silver-rich precipitates. These deposits appeared to interfere with adhesive monomer penetration, preventing effective micromechanical interlocking and hybrid layer formation.

In the aged subgroup, the interface exhibited severe morphological deterioration with no distinguishable hybrid layer

and extensive collapse of the collagen network. This is likely due to the persistent silver ion residues and the cytotoxic effect on dentin matrix proteins, which impair the bonding substrate's integrity. Resin tag was minimal to absent in both immediate and aged subgroups. The obstructive nature of silver deposits and possible denaturation of tubular walls were hypothesized to hinder monomer diffusion and polymerization within tubules. DSF treated surfaces exhibited substantial silver precipitation at the dentin entrance, especially at intertubular regions. These zones lacked the characteristic funnel-shaped resin tags, suggesting a barrier effect of silver compounds against adhesive infiltration.

Hybrid layer in SF immediate subgroups showed a partially continuous hybrid layer with a more regular architecture compared to DSF. The average thickness was estimated at 2-3 μ m. Despite the presence of surface precipitates, they were less abundant and more diffusible, likely due to the aqueous-based vehicle and absence of ammonia stabilizers.

After aging, moderate degradation of the hybrid layer was observed, but with residual structural preservation in contrast to DSF. The results indicate that SF allows better long-term interface stability. Resin tags in SF subgroups were visibly shorter and fewer than in the control group but were better developed than in DSF specimens. In aged SF samples, remnant

tags were still detectable, albeit fragmented, indicating partial resistance to hydrolytic and enzymatic degradation. SF application produced finer silver deposits that were more easily rinsed from the dentin surface. Consequently, intertubular and peritubular infiltration by adhesive monomers was partially preserved, especially in the immediate condition. The methodology adopted in this study was carefully designed to evaluate the effect of silver-containing pretreatments—Silver Diamine Fluoride (SDF) and Silver Fluoride (SF)—on the microshear bond strength (μ SBS) of a universal adhesive to dentin. The use of freshly extracted, sound human molars provided a clinically relevant substrate that closely mimics natural tooth behavior. Compared to bovine or synthetic analogs, human dentin exhibits greater structural and chemical similarity to the clinical environment, including dentinal tubule density, water content, and organic matrix composition. Standardization was further enhanced by radiographically confirming dentin thickness, ensuring uniformity across all specimens. (23)

Flat mid-coronal dentin surfaces were created using a diamond disc under water cooling, followed by polishing with 600-grit silicon carbide paper to simulate the clinical smear layer. This preparation step was crucial, as surface roughness and smear layer characteristics significantly influence adhesive interaction with dentin. Embedding the specimens in self-cure acrylic within PVC rings ensured mechanical stability during bonding and testing, thus reducing the risk of operator-related variability. (24)

To simulate clinical application, SDF and SF pretreatments were applied strictly according to the manufacturer's instructions. The dual-step protocol for SDF (silver solution followed by potassium iodide) and the two-step SF application (blue and green

capsules) mirrored real-world usage. This approach strengthens the clinical applicability of the findings. However, it should be acknowledged that the high operator sensitivity of these materials-particularly in the application timing, rinsing, and drying protocols-could introduce variability in clinical outcomes.(25)

The use of microshear bond strength testing (μ SBS) provided an appropriate and sensitive method for evaluating dentin adhesion. This test allows for the assessment of small, localized areas of adhesion and can detect subtle differences between groups, particularly important when testing chemical pretreatments that may influence surface energy or microstructure. Unlike traditional macro tests, μ SBS minimizes the impact of substrate variability and avoids the introduction of microcracks that often occur during sectioning for microtensile testing.(26)

Tygon tubing molds were used to build consistent composite cylinders (1 mm in diameter and 1 mm in height), which helped standardize the bonded area. This uniformity was essential for precise calculation of bond strength values and minimized discrepancies due to inconsistent stress distribution. The use of a looped orthodontic wire for loading, instead of a chisel blade, further ensured uniform force application and avoided stress concentration at the loading site-an improvement supported by the literature for minimizing premature failures.(27)

Limitations of the study:

1. the study was conducted under controlled laboratory conditions, which do not fully replicate the complex biological environment of the oral cavity. Factors such as salivary enzymes, masticatory forces, pH fluctuations, microbial biofilm, and thermal changes were not accounted for.

2. Only one universal adhesive (Zipbond, SDI) and one nanohybrid resin composite (Luna, SDI) were evaluated. The outcomes may differ with alternative adhesive systems or restorative materials, limiting the generalizability of the findings.

3. The study exclusively investigated bonding to sound mid-coronal dentin. Caries-affected dentin, enamel, and deep dentin were not considered, although they are commonly encountered in clinical practice.

4. Aging was simulated using six-month water storage. However, intraoral conditions involve additional challenges such as thermal cycling, mechanical fatigue, and chemical challenges, which were not reproduced.

5. The application of silver-containing agents (DSF and SF) is highly technique-sensitive. Variations in application duration, rinsing protocols, and drying procedures may affect outcomes but were not systematically analyzed.

6. Micromorphological evaluation was performed on a relatively small number of specimens ($n=5$ per subgroup), which may not fully represent the structural variations across all samples.

7. Important clinical considerations such as esthetic outcomes (e.g., black staining from DSF) and biological responses (e.g., pulp irritation or biocompatibility) were not evaluated in this study.

Within the limitations of this *in vitro* study, the following conclusions can be drawn:

1. Surface pretreatment of dentin with Diamine Silver Fluoride (DSF) or Silver Fluoride (SF) negatively affected the microshear bond strength (μ SBS) of universal adhesives. This effect was evident in both immediate and aged conditions. 2. Aging further exacerbated the reduction in bond strength, especially in DSF-treated groups, indicating compromised long-term adhesion and resin-dentin interface stability.

3. SEM analysis revealed disrupted or absent hybrid layers, reduced resin tag formation, and silver precipitate deposition in DSF and SF groups. These morphological changes likely interfered with monomer infiltration and polymerization.

4. While DSF and SF are beneficial for caries prevention, their application prior to adhesive restorative procedures may compromise adhesive performance. Therefore, caution is advised when using silver-containing pretreatments in clinical settings that require strong and durable adhesion.

5. Additional long-term clinical studies are recommended to evaluate the impact of DSF and SF on different adhesive systems, various tooth substrates, and under simulated oral conditions to establish comprehensive clinical guidelines.

Recommendations

the following recommendations are proposed to guide future research and clinical application of silver-containing surface pre-treatments in conservative dentistry:

1.Exercise caution when using (DSF) Before adhesive restorations.

The present study demonstrated that pre-treating dentin with DSF significantly reduces (μ SBS) of universal adhesives, particularly after artificial aging. Therefore, DSF should not be routinely used prior to permanent adhesive restorations in load-bearing areas where long-term bond durability is essential.

2.Consider selective use of DSF in high caries-risk or special populations. Despite its negative impact on bond strength, DSF provides substantial antimicrobial and remineralizing benefits.

3.Avoid Silver Fluoride (SF) in Permanent Adhesive Restorations.

Silver Fluoride, though slightly less aggressive than DSF, still resulted in a notable reduction in bond strength post-aging. It is therefore not recommended for use in permanent adhesive procedures, especially when using resin composites.

4.Evaluate the compatibility of adhesive systems with silver-based agents. The effect of DSF or SF varies depending on the adhesive system, its monomer content (e.g., MDP), and the bonding strategy (etch-and-rinse vs. self-etch). It is advised to test different adhesive systems before routine clinical use, consider using an etch-and-rinse technique in cases

an intermediate liner, such as resin-modified glass ionomer cement, to enhance bonding

5. Encourage long-term clinical studies. While this study provided short-term in vitro insights, further randomized clinical trials and longitudinal research are needed to validate the findings under dynamic intraoral conditions include thermal cycling, masticatory forces, and saliva interactions. Assess clinical outcomes such as marginal adaptation, discoloration, and secondary caries

6. Develop Standardized, Evidence-Based Clinical Protocols for DSF Application to ensure optimal outcomes and minimize adverse effects, there is a need to establish clinical protocols detailing application sequence and duration, rinsing steps post-DSF application, timing before adhesive placement, optional use of potassium iodide (KI) to mitigate staining and enhance bond strength.

These recommendations aim to balance the antimicrobial advantages of silver-based agents with the mechanical demands of adhesive dentistry, while emphasizing the importance of clinical judgment, material selection, and continued research.

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