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RESEARCH

Effect of ammonia-free silver fluoride application on the resin–dentin interface subjected to an *in situ* cariogenic challenge

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Abstract

Objective This study aimed to evaluate whether an ammonia-free silver diamine fluoride solution can prevent adhesive interface degradation when subjected to an in situ cariogenic challenge (CC).

Materials and methods Fifty-six sound human molars were sectioned to obtain flat mid-coronal dentin surfaces, and half of the specimens received ammonia-free silver diamine fluoride pretreatment. Specimens were then restored using two universal adhesives (Single Bond Universal, SBU; and Zipbond, ZIP) applied with either the etch-and-rinse (ER) or self-etch (SE) strategy. Composite buildups were constructed, sectioned into resin–dentin bonded sticks, and allocated for immediate testing or after an in situ cariogenic challenge. Twenty volunteers wore (14 days) palatal devices containing eight resin–dentin sticks from different groups. CC were induced (20% sucrose solution; 4×/day). Microtensile bond strength (μTBS) was assessed, and failure modes were classified. Adhesive interfaces were analyzed by scanning electron microscopy with energy-dispersive X-ray spectroscopy (EDX-SEM). Data were analyzed using four-way repeated measures ANOVA and Tukey's post hoc test ($\alpha = 0.05$).

Results At immediate evaluation, no significant differences were observed between groups, while after the CC, μTBS values significantly decreased in groups without Riva Star Aqua. Ammonia-free silver diamine fluoride solution treatment maintained μTBS, showing higher μTBS compared to untreated groups ($p < 0.05$). EDX-SEM confirmed silver and calcium deposition within the hybrid layer and dentinal tubules after ammonia-free silver diamine fluoride treatment.

Conclusion Pretreatment with ammonia-free silver diamine fluoride solution preserved resin–dentin bond strength under cariogenic conditions.

Clinical significance ammonia-free silver diamine fluoride solution pretreatment may protect adhesive interfaces in high-caries-risk patients by enhancing hybrid layer stability and reducing biofilm-related degradation.

Keywords Silver diamine fluoride · Universal adhesives · In situ cariogenic challenge · Resin–dentin bond strength

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Introduction

The technology of adhesive systems has been successfully implemented in minimally invasive operative dentistry. However, concerns about the long-term durability and stability of the resin-dentin interface remain [1, 2]. The primary cause of failure at this interface is the degradation of the hybrid layer, particularly the breakdown of exposed collagen fibrils [3, 4]. Incomplete diffusion of resin monomers into acid-exposed dentin creates poorly infiltrated zones along the base of the hybrid layers, leaving collagen fibrils unprotected [5]. These exposed fibrils are highly susceptible to enzimatic degradation by matrix metalloproteinases (MMPs) and cysteine cathepsins [6, 7].

As a result, adhesive interface failure often manifests as leakage, which facilitates bacterial biofilm accumulation around the restoration and ultimately leads to secondary caries [8, 9]. Moreover, composite resin themselves can promote the proliferation of cariogenic bacteria, such as *Streptococcus mutans* (*S. mutans*), increasing the risk of resin hydrolysis and enzymatic biodegradation [10]. Esterases produced by *S. mutans* can cleave ester bonds within resin composites, further weakening the interface [11].

Consequently, recurrent caries remains one of the leading causes of restoration failure, often necessitating replacement [12, 13]. This not only imposes substantial costs on both individual and the healthcare system [14], but also underscores the need of develop antibacterial restorative materials capable of inhibiting bacterial growth at the adhesive interface, thereby reducing caries risk, improving restorations longevity of dental, and preserving tooth structure.

Silver diamine fluoride (SDF) solution, first introduced in the 1970 s to arrest dental caries [15–17], is a clear liquid that combines the antibacterial properties of silver with the remineralizing effects of fluoride, making it a promising therapeutic agent for managing carious lesions. SDF has also been recognized for its ability to reduce cariogenic bacterial growth and biofilm formation [18], occlude dentinal tubules [19], stimulate tertiary dentin formation [20], and inhibit dentin demineralization while promoting dentin remineralization [21, 22]. Nevertheless, the clinical use of silver diamine fluoride is often limited by the dark discoloration observed on dental tissues after its application [23].

To address these limitations, ammonia-free silver fluoride (Riva Star Aqua, SDI) has been developed. In contrast to SDF, these materials were designed to improve stability and handling properties [24]. Moreover, the application of potassium iodide (KI) as a subsequent clinical step following SDF treatment has been proposed as a strategy to reduce tooth discoloration [23]. Together, these approaches aim to maintain the antimicrobial and bioactive effects of silver-based agents [25] while minimizing their aesthetic drawbacks [23].

However, no consensus has yet been reached regarding the effect of SDF or ammonia-free silver fluoride (SF) on the adhesive properties of restorative materials [26, 27]. While some studies report that SDF or ammonia-free silver fluoride application does not compromise bonding performance, either immediately [27–31] or after long-term aging [32–34], other investigations have reported a detrimental effect on dentin adhesion [35–37]. Notably, when long-term bond durability is assessed, several studies have shown that SF solutions can actually reduce degradation of the resin-dentin interface over time, leading to improved stability compared to untreated controls [32, 36].

However, it remains unclear whether the properties of ammonia-free SF, when applied directly to the adhesive interface, can effectively prevent resin-dentin degradation when subjected to a cariogenic challenging. To the best of our knowledge, no in situ study has yet evaluated this possibility. Therefore, the present study aimed to investigate whether ammonia-free SF solution application can prevent degradation of the adhesive interface when subjected to an in situ cariogenic challenge, using different universal adhesives and bonding strategies (etch-and-rinse or self-etch). The tested null hypotheses were that ammonia-free SF pretreatment would not influence: (i) the immediate or post-cariogenic challenging bond strength to dentin; (ii) the performance of different adhesive systems; (iii); and the outcomes of different bonding strategy (etch-and-rinse or self-etch).

Materials and methods

Tooth selection and preparation

The present study was approved by the Ethics Committee in Research at the State University of Ponta Grossa (Ponta Grossa/PR, Brazil; number 2.631.289). After that, 56 caries-free human molars were disinfected in 0.5% chloramine and stored in distilled water changed weekly until use. The occlusal third of the crowns were removed with a diamond saw under water-cooling using a hard tissue precision cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) to obtain a flat mid-coronal dentine surface. Enamel around the margins was removed using a diamond bur (#3195; KG Sorensen, Barueri, SP, Brazil; Fig. 1A), and the dentin surface of blocks were wet-polished with 600-grit SiC papers for 1 min to create a standard smear layer prior to bonding procedures.

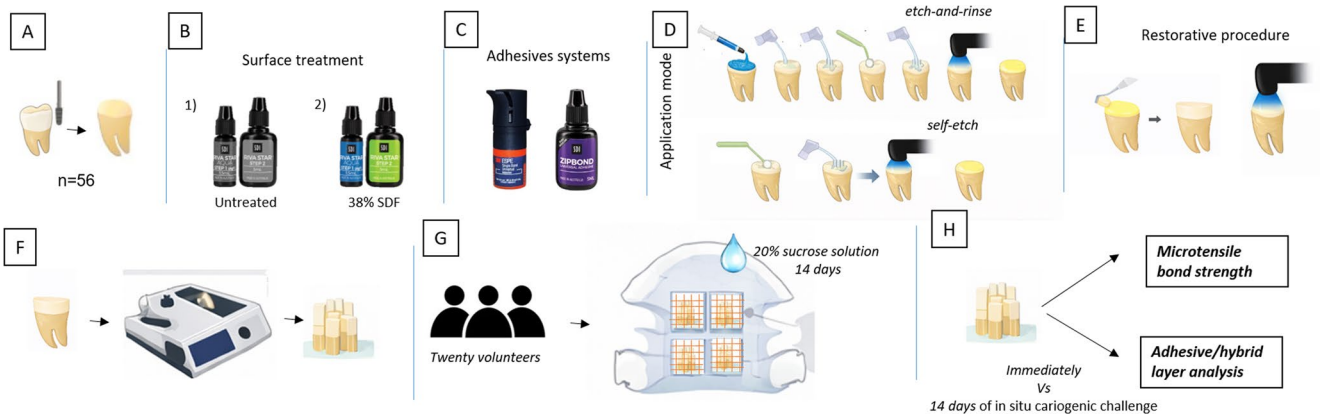

Fig. 1 Scheme of the study's methodology. **A** Teeth preparation, exposing enamel-free dentin surfaces; **B** Surface treatment, untreated and 38% ammonia-free silver fluoride applied according to Table 1; **C-D** Adhesives systems and application mode; Restorative procedures; **F**. Tooth sectioning in resin-dentin sticks; **G**. Palatal device with four sites, one for each experimental group. **H**, resin-dentin sticks fixed with wax and covered with a plastic mesh. Sucrose dripped upon each hemi-tooth for 14 days of cariogenic challenge. **H**. Resin-dentin sticks distribution to the evaluation methods

Sample size calculation

The sample size was calculated using the website www.sealedenvelope.com. For the sample size calculation, the microtensile bond strength values of Scotchbond Universal on carious dentin after the application of SDF were considered. According to the literature, the mean and standard deviation of the microtensile bond strength of Scotchbond Universal (Solventum, St Paul, MN, USA) are 25.4 ± 4.0 MPa [28, 32]. To detect a difference of 10 MPa [38] between the test groups, using a significance level of 5%, a power of 80%, and a two-sided test, the minimum sample size was 5 teeth per group. To account for potential losses, two teeth per group were added, totaling 7 teeth per experimental group.

Experimental design

The teeth were randomly divided into 8 experimental groups ($n = 7$) according to the following variables: (1) Application of SF solution at 2 levels (untreated and ammonia-free silver fluoride solution application [Riva Star Aqua, SDI; Bayswater, Victoria, Australia] Fig. 1B); (2) Adhesive system at 2 levels (Scotchbond Universal [SBU, Solventum, St Paul, MN, USA, also known as Single Bond Universal in some countries] and ZIP bond [ZIP, SDI, Bayswater, Victoria, Australia] Fig. 1C); (3) Adhesive strategy at 2 levels (Etch-and-Rinse [ER] and Self-Etch [SE]). For the evaluation time variable, specimens from the same tooth were collected at 2 levels (immediate and after in situ cariogenic challenge).

Bonding procedures

Ammonia-free SF and the universal adhesive systems were applied according to the manufacturer's recommendations (Table 1; Fig. 1D [39]). After the bonding procedure, a composite restoration (Opallis, FGM Dental Group, Joinville, SC, Brazil) was a build-up in 2–3 increments of 2 mm thickness (Fig. 1E), and each was light-cured for 20 s at 1 mm of distance, using an LED unit set at 1,400 mW/cm^2 (Valo, High Power Mode, Ultradent, South Jordan, UT, USA) for all specimens. A single trained operator performed all bonding procedures.

The teeth were then stored in distilled water at 37 °C for 24 h, after which they were sectioned into mesiodistal and buccal-lingual segments using a precision cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) to obtain resin-dentin bonded sticks with a cross-sectional area of approximately 0.8 mm² (Fig. 1F), as measured by a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). A total of 20–25 resin-dentin bonded sticks were obtained per tooth, including premature failures. All specimens were sterilized using an autoautoclave (Autoclave ALT Plus, Equipamentos Medico Odontológicos, Riberão Preto, SP, Brazil) at 121 °C and 1,2 Kgt/cm^2 of pressure [40]. Afterwards, half of the resin-dentin bonded sticks from the same tooth were tested immediately, and the other half was subjected to an in situ cariogenic challenge.

Cariogenic challenge in situ

To minimize operator-related variability during the cariogenic challenge, all experimental procedures were performed by a single trained and calibrated operator following a standardized protocol.

For this part of the study, 20 healthy adult volunteers aged between 20 and 30 years were selected. All volunteers had to meet the following inclusion criteria: good general health, normal salivary flow, and no antibiotic intake in the

Table 1 Adhesive systems, batch number, composition and, application mode*

Adhesives system (batch number) and composition**	Groups	Application mode	
		ER	SE
Scotchbond Universal (SBU); Solventum, St Paul, MN, USA (2227900246) 10-MDP, Dimethacrylate resins, HEMA, methacrylate- modified polyalkenoic acid copolymer, nanofiller, ethanol, water, initiators, silane; pH 2.7	without Riva Start Aqua (water)	1. Apply etchant for 15 s; 2. Rinse for 15 s; Air dry 2 s; 3. Apply adhesive as for the SE mode.	1. Apply the adhesive to the entire preparation with a microbrush; 2. Rub it in for 20 s; 3. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely; 4. Light-cure for 10 s.
	with Riva Start Aqua, (SDI Limited, Bayswater, Victoria, Australia; #1230235A) Step 1: Silver 32.6%, fluoride 5.7%, water Step 2: Potassium iodide (KI) 58.3%.	1. Apply etchant for 15 s; 2. Rinse for 15 s; 3. Dispense one drop of the solution (Step 1) on a dappen dish; 4. Carefully apply solution for 10 s to treatment site with a micro brush; 5. Immediately after, dispense two drops of KI solution (Step 2) onto fresh dappen dish; 6. Apply a generous amount of the solution to treatment site until the creamy white precipitate turns clear; 7. Wash thoroughly with water for at least 10 s; 8. Air-dry for 10 s; 9. Apply the adhesive as for the SE mode.	1. Dispense one drop of the solution (Step 1) on a dappen dish; 2. Carefully apply solution for 10 s to treatment site with a micro brush; 3. Immediately after, dispense two drops of KI solution (Step 2) onto fresh dappen dish; 4. Apply a generous amount of the solution to treatment site until the creamy white precipitate turns clear; 5. Wash thoroughly with water for at least 10 s; 6. Air-dry for 10 s; 7. Apply the adhesive to the entire preparation with a microbrush; 8. Rub it in for 20 s; 9. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely; 10. Light-cure for 10 s.
Zipbond Universal (ZIP); SDI, (220121) 10-MDP, ethanol, water, fluoride; pH 2.5	without Riva Start Aqua (water)	1. Apply etchant for 15 s; 2. Rinse for 15 s; Air dry 2 s; 3. Apply adhesive as for the SE mode.	1. Apply the adhesive to the entire preparation with a microbrush; 2. Rub it in for 10 s; 3. Wait another 10 s; 4. Evaporate excess solvent by air-drying for 5 s; 5. Light-cure for 10 s.
	With Riva Start Aqua, SDI Limited, Bayswater, Victoria, Australia, #1230235A) Step 1: Silver 32.6%, fluoride 5.7%, water Step 2: Potassium iodide (KI) 58.3%.	1. Apply etchant for 15 s; 2. Rinse for 15 s; 3. Dispense one drop of the solution (Step 1) on a dappen dish; 4. Carefully apply solution for 10 s to treatment site with a micro brush; 5. Immediately after, dispense two drops of KI solution (Step 2) onto fresh dappen dish; 6. Apply a generous amount of the solution to treatment site until the creamy white precipitate turns clear; 7. Wash thoroughly with water for at least 10 s; 8. Air-dry for 10 s; 9. Apply the adhesive as for the SE mode.	1. Dispense one drop of the solution (Step 1) on a dappen dish; 2. Carefully apply solution for 10 s to treatment site with a micro brush; 3. Immediately after, dispense two drops of KI solution (Step 2) onto fresh dappen dish; 4. Apply a generous amount of the solution to treatment site until the creamy white precipitate turns clear; 5. Wash thoroughly with water for at least 10 s; 6. Air-dry for 10 s; 7. Apply the adhesive to the entire preparation with a microbrush; 8. Rub it in for 10 s; 9. Wait another 10 s; 10. Evaporate excess solvent by air-drying for 5 s; 11. Light-cure for 10 s.

* The materials were applied according to the recommendation of their respective manufacturers

**10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate

two months prior to the experiment. Participants with systemic diseases, pregnancy or breastfeeding, use of fixed or removable orthodontic appliances, use of fluoride mouthwash or professional fluoride application in the last two months were excluded from the study.

After accepting to participant, palatal devices were individually prepared for each participant. For this, all volunteers were molded with alginate (Hydrogum 5, Zhermack, SpA, Rome, Italy) to obtain models for the fabrication of intraoral acrylic palatal devices. Four cavities (6.5 mm x 6.5 mm x 4 mm) were distributed on the left and right sides of each device.

In each cavity, 8 resin-dentin bonded sticks were positioned using a block randomization of 4, conducted through the website www.sealedenvelope.com. This ensured that each device contained specimens representing the variables of silver fluoride solution application and adhesive strategy. To promote biofilm accumulation and protection the specimens from mechanical disturbances, a plastic mesh was secured with acrylic resin, maintaining a 1.0 mm space from the specimen surface [41, 42].

For one week before starting the experimental phase, volunteers stopped brushing their teeth with fluoridated toothpaste and started using a non-fluoridated toothpaste (Formulare, São Luis, MA, Brazil) provided by the research team. To induce a cariogenic challenge in all specimens, volunteers were instructed to remove the device and drip a 20% sucrose solution (Formulare, São Luis, MA, Brazil) over the resin-dentin bonded sticks four times a day (8 AM, 11 AM, 3:30 PM, and 7 PM) for 14 days (Fig. 1G). Five minutes later, the device was reinserted into the oral cavity [41, 42]. Application time, solution volume, and handling procedures were strictly controlled by the same operator and kept identical across all experimental groups.

Participants were not subjected to any restrictions concerning the consumption of liquids. Volunteers were not subjected to dietary restrictions and were instructed to wear the intraoral device continuously for 14 days, removing it only during oral hygiene procedures and meals. Likewise, no restrictions were imposed regarding the consumption of liquids. The device was cleaned extra-orally by brushing, and the volunteers were instructed to brush carefully only over the resin of the device but completely avoiding the sticks or the biofilm forming within the plastic mesh protecting the sticks. On the 15th day of the intraoral phase, approximately 12 h after the final sucrose application, all volunteers returned to the clinic, and the intraoral appliances were removed by a trained operator under controlled clinical conditions. Immediately after removal, the devices were placed in sterile containers and transported to the laboratory for specimen retrieval. The resin–dentin bonded sticks were carefully removed using sterile instruments and gently rinsed with tap water to remove loosely adherent biofilm.

Microtensile bond strength test (μTBS)

Prior to microtensile bond strength test, all dentin beams were visually inspected under stereomicroscopy to identify pre-existing defects or cracks. Specimens presenting visible structural damage were excluded from the analysis. No additional dentin damage attributable to the application of the tested SF materials was observed prior to mechanical testing. Immediate or after 14 days of in situ cariogenic challenge, resin-dentin bonded sticks were affixed to a Geraldeli's jig using cyanoacrylate adhesive (Fig. 1H). Tension tests were performed on these sticks using a universal testing machine (Kratos Dinamometros, Cotia, SP, Brazil) at a speed of 0.5 mm/min until failure occurred. To determine the μTBS values (in MPa), the maximum load (in N) at the point of failure was divided by the cross-sectional bonding area (in mm^2). For statistical analysis, the mean μTBS of sticks from the same tooth was averaged.

To analyze the failure modes of the resin-dentin bonded sticks, a classification was employed. Failures were categorized as cohesive ([C] indicating failure exclusively within either the dentin or the resin composite) or adhesive/mixed ([A/M] indicating failure at the interface between the resin and dentin or failure at this interface with partial cohesive failure in the neighboring substrates). This classification process was carried out using a stereomicroscope at a magnification of 100× (Olympus SZ40, Tokyo, Japan). Instances of premature failures (PF) were also recorded.

Adhesive/hybrid layer analysis by energy dispersive X-ray spectroscopy (EDX-SEM)

Three resin-dentin bonded sticks from each experimental group and storage time, which had not been used in the μTBS test, were set aside for adhesive/hybrid layer analysis (Fig. 1H). Resin-dentin bonded sticks selected for this test were ultrasonically cleaned, air-dried, mounted on stubs, and polished with a wet #600, 1000-, 1500-, 2000- grit SiC paper. The resin-dentin bonded sticks were analyzed using a field emission scanning electron microscope (MIRA, TESCAN ORSAY HOLDING, Warren- dale, PA, USA) coupled with an energy-dispersive X-ray spectrometer (EDX). The bonding area was observed, and the analyses focused on the middle of the hybrid layer. Three photomicrographs of representative surface areas were obtained at 2.500× magnification and a semi- quantitative chemical microanalysis was performed via EDX-SEM.

Statistical analysis

The mean μTBS (MPa) values of resin-dentin bonded sticks from the same tooth at each storage time (immediate and in situ environment) were averaged for statistical purposes; thus, the experimental unit was the hemi-tooth. Prematurely failed specimens were included in the tooth mean for statistical analysis. The value attributed to prematurely failed specimens was 0 MPa, as per the Academy of Dental Materials guidance on microtensile bond strength testing [43].

Initially, the data were subjected to statistical analysis, starting with the Kolmogorov-Smirnov test to assess normality and the Bartlett test for equality of variances to examine the assumption of equal variances (data not reported). Since the data followed a normal distribution, the μTBS (MPa) values were analyzed using a 4-way repeated measures ANOVA, considering 'application of SF', 'adhesive system', 'adhesive strategy', and 'evaluation time', with the hemi-tooth as the repeated measure. Tukey's test was applied for post hoc comparisons. All analyses were performed with a 5% level of significance. Failure modes were statistically evaluated using the χ^2 test ($\alpha = 0.05$) (Jamovi, Statistical software, version 2.3). The chemical microanalysis by EDX-SEM was evaluated qualitatively.

Results

Microtensile bond strength (μTBS)

The percentage of premature failures and the fracture pattern distribution are presented in Table 2. Adhesive or adhesive/mixed failures were the most prevalent failure mode, accounting for 97.7% of all specimens, followed by cohesive failures (0.4%) and premature failures (1.9%). No significant differences were detected among the experimental groups regarding failure mode distribution ($p > 0.55$).

The four-way repeated-measures ANOVA revealed that three two-factor interactions were statistically significant: treatment × evaluation time, adhesive system × evaluation time, and treatment × adhesive system ($p = 0.002$, $p = 0.001$, and $p = 0.005$, respectively; Table 2). No significant effect was observed for the main factor adhesive strategy ($p = 0.82$).

At the immediate evaluation, μTBS values were not significantly affected by the application of ammonia-free SF or by the adhesive strategy for either adhesive system (Table 3, $p > 0.05$). However, a significant difference was observed between the adhesive systems, with SBU presenting higher μTBS values than ZIP at the immediate evaluation ($p < 0.05$; Table 3).

Table 2 Number of specimens (%) according to fracture mode and the premature failure of all experimental groups

Groups		SBU						ZIP					
		ER			SE			ER			SE		
		A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP
Immediate	**Without** Riva Start Aqua	78 (97.5)	2 (2.5)	0 (0)	80 (100)	0 (0)	0 (0)	79 (98.8)	0 (0)	1 (1.2)	77 (96.3)	0 (0)	3 (3.7)
	With Riva Start Aqua	80 (100)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)	78 (97.6)	1 (1.2)	1 (1.2)	78 (97.5)	2 (2.5)	0 (0)
In situ cariogenic chalenge	**Without** Riva Start Aqua	78 (97.5)	0 (0)	2 (2.5)	75 (94)	0 (0)	5 (6.2)	75 (93.8)	0 (0)	5 (6.2)	74 (92.5)	0 (0)	6 (7.5)
	with Riva Start Aqua	79 (98.8)	0 (0)	1 (1.2)	80 (100)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)

C cohesive fracture mode, *A/M* adhesive or mixed fracture mode, *PF* premature failure, *SBU* (Single Bond Universal), *ZIP* (Zipbond), *ER* (Etch-and-rinse), *SE* (self-etch)

Table 3 Means and standard deviations of resin-dentin bond strength values (MPa), as well as statistical analysis for all experimental groups (*)

Groups		SBU		ZIP	
		ER	SE	ER	SE
Immediate	**without** Riva Start Aqua	52.2 ± 3.8 A	52.7 ± 2.9 A	41.2 ± 2.5 C	40.1 ± 2.2 C
	with Riva Start Aqua	54.2 ± 3.3 A	53.1 ± 3.9 A	40.2 ± 2.6 C	40.3 ± 2.7 C
In situ cariogenic challenge	**without** Riva Start Aqua	46.4 ± 2.8 B	43.6 ± 2.2 B	30.8 ± 2.1 D	30.9 ± 2.5 D
	with Riva Start Aqua	50.6 ± 2.8 A	49.9 ± 2.4 A	37.7 ± 2.6 C	38.0 ± 2.5 C

(*) Different letters are means differences statistically significant between groups (Four-way ANOVA; Tukey test; $p<0.05$)

SBU (Single Bond Universal), *ZIP* (Zipbond), *ER* (Etch-and-rinse), *SE* (self-etch)

After the in situ cariogenic challenge, μTBS values were significantly higher in the groups treated with ammonia-free SF compared with untreated groups (Table 3, $p<0.002$), regardless of the adhesive system or adhesive strategy used. When comparing evaluation times, a significant reduction in μTBS values was observed after the in situ cariogenic challenge in untreated groups ($p=0.005$; Table 3). In contrast, no significant differences in μTBS values were found between immediate and post–in situ evaluations for the groups treated with ammonia-free SF, irrespective of adhesive system or adhesive strategy (Table 3, $p>0.05$).

Adhesive/hybrid layer analysis by energy dispersive X-ray spectroscopy (EDX-SEM)

Silver deposition was consistently observed at the resin-dentin adhesive interface following ammonia-free SF application, regardless of adhesive system, bonding strategy, or evaluation time (Fig. 2). Additionally, silver precipitation was noted within the resin-dentin interfaces and dentinal tubules. An increase in the calcium peak was also detected post ammonia-free SF treatment, correlating with the concentration applied (Fig. 2). Since both universal adhesives exhibited similar patterns of silver deposition and calcium peaks, representative images are shown for one universal adhesive (ZIP).

Discussion

In the present study, immediate measurements revealed no significant differences in μTBS values between groups with or without ammonia-free SF application, regardless of the universal adhesive or the bonding strategy employed. This result is particularly relevant for clinicians incorporating ammonia-free SF into caries management protocols, as it supports that this treatment does not negatively affect the bonding performance of universal adhesives to dentin.

After the in situ cariogenic challenge, ammonia-free SF -treated groups maintained their immediate μTBS values, untreated groups showed a significant reduction in μTBS, both compared to their own immediate values and to the ammonia-free SF -treated groups after in situ challenge. Accordingly, the first null hypothesis was rejected.

After only 14 days submitted to an in situ cariogenic challenge, a significant decrease in the μTBS values was observed in the untreated groups. This demonstrates that, despite its short duration, the in situ model can effectively simulate the aging of adhesive restorations under a cariogenic challenge. The model replicates the challenging conditions restorations face in the oral environment [44], exposing the adhesive interface to mechanical, thermal, and chemical factors, including bacterial products [45].

Specifically, when the adhesive interface is exposed to a biofilm continuously supplied with sucrose, it increases the cariogenicity and virulence of acidogenic and aciduric

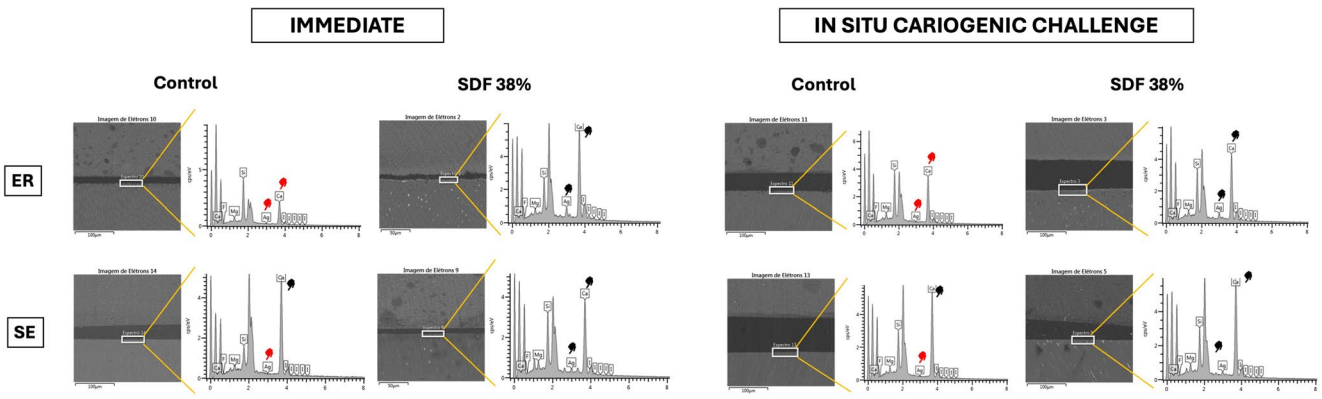

Fig. 2 Representative EDX images of resin-dentin adhesive interfaces after treatment with ammonia-free SF solution using Zip Bond Universal in both etch-and-rinse and self-etch strategies, evaluated immediate and in situ. Increased Ag and Ca peak intensities (black hands) confirm the presence of silver particles immediately after ammonia-free SF treatment, regardless of storage time or adhesive strategy, compared to control groups (red hands)

bacteria, promoting bacterial adhesion, mature biofilm formation, and extracellular polysaccharides synthesis [46]. Additionally, these bacteria exhibit esterase activities at levels sufficient to degrade polymeric materials [47], suggesting that oral biofilms can compromise the adhesive interface and contribute to the development of secondary caries. Consequently, the interface durability diminishes as dentin and the adjacent hybrid layer are degraded by the acidic by-products of the biofilm, leading to mineral loss. Bonding to dentin can deteriorate in situ through hydrolytic processes that attack the collagen matrix [48], polymer plasticization [49], and acid production by the biofilm [50].

On the other hand, no significant decrease in μTBS values was observed after the in situ cariogenic challenge when ammonia-free SF was applied. This finding can be explained by the chemical composition and reactions of the ammonia-free SF solution. Unlike other SDF solution, this new formulation contains silver and fluoride ions without ammonia [51–53].

Although detailed mechanistic evidence for ammonia-free SF formulations remains limited, the anticariogenic actions of silver and fluoride ions, well established for conventional SDF, are primarily attributed to their antimicrobial activity and capacity to promote remineralization. Therefore, it is reasonable to hypothesize that similar ion-driven mechanisms may occur after ammonia-free SF application [31, 34], although this extrapolation should be interpreted with caution. Following ammonia-free SF application, a remineralization process may begin as fluoride ions interact with hydroxyapatite to form calcium fluoride (CaF_2) and silver phosphate in a basic environment [54]. Calcium fluoride serves as a slow-release fluoride reservoir during cariogenic challenges, while hydrogen phosphate ions (HPO_4^{2-}) promote the conversion of calcium fluoride into more acid-resistant fluorapatite crystals deposited within dentinal tubules [55, 56].

In parallel, silver ions, known to be highly reactive and infiltrative in dentin, as demonstrated for conventional SDF formulations [15, 48], may interact with potassium iodide and hydroxyapatite, leading to the formation of stable and insoluble by-products such as silver iodide and tripotassium phosphate within the tooth structure [27, 57]. Although most mechanistic evidence derives from studies on conventional SDF, these insoluble by-products may benefit the hybrid layer in several ways: (i) silver ions have been shown to inhibit matrix metalloproteinases (MMP-2, MMP-8, and MMP-9) responsible for collagen fibril degradation [36, 58]; and (ii) increased mineral deposition and silver particle incorporation may mechanically reinforce the adhesive interface by filling voids and enhancing hybrid layer strength [27, 28, 59]. In particular, the presence of silver precipitation observed in the EDX-SEM analysis supports the occurrence of such interfacial deposition in the ammonia-free formulation evaluated (Fig. 2).

Additionally, silver-based fluoride treatments, including ammonia-free formulations, have been associated with reduced biofilm formation. Silver ions bind to sulfhydryl groups and bacterial proteins, disrupting enzymatic activity, inhibiting DNA replication [60], and inducing cell death [52, 54]. This may reduce bacterial colonization around the resin–dentin interface [61] this may reduce bacterial colonization around the resin–dentin interface [62, 63]. Finally, these processes may lead to the formation of a calcium- and fluoride-rich protective layer and a silver–protein layer on the hybrid layer surface [35, 64, 65], both resistant to acid dissolution and enzymatic degradation [35, 55, 57, 66, 67]. Although microbial or biofilm-related outcomes were not directly assessed in the present study, it is plausible that ammonia-free SF–treated specimens exhibited some of these effects, which may have contributed to the observed preservation of μTBS.

Several laboratory studies have demonstrated the effects of SF solutions on the adhesive interface, either immediately [28–30] or after long-term water storage [32, 33]. Collectively, these reports suggest that (i) SF solutions can biochemically inactivate proteolytic pathways responsible for hybrid layer degradation; (ii) silver species deposited within the adhesive interface may act as reservoirs for prolonged antimicrobial and MMP-inhibitory activity; and (iii) increased mineral deposition promoted by SF solutions can fill interfacial voids and enhance mechanical reinforcement. Considering that this study used ammonia-free SF solution, which differs from the traditional amine-containing formulations, the absence of amines did not significantly impact silver penetration when SF and conventional SDF were compared [68]. Therefore, similar effects on the adhesive interface were expected.

In contrast, a previous study [36] reported a significant reduction in dentin bond strength despite observing enzymatic inhibition and potential collagen preservation. This finding differs from our results, where no decrease in bond strength was observed after an in situ cariogenic challenge when ammonia-free SDF was applied. A plausible explanation lies in the experimental design: the former study used laboratory-based conditions, whereas our study employed a cariogenic in situ model that better mimics clinical conditions, such as biofilm presence and pH fluctuations [41, 69]. This distinction highlights the novelty of our approach, as, to the best of our knowledge, no previous in situ study has evaluated ammonia-free SF potential to both preserve the adhesive interface and counteract caries development at this vulnerable region.

On the other hand, although both universal adhesives preserved μTBS values after the in situ cariogenic

challenge when ammonia-free SF was applied, therefore supporting acceptance of the second null hypothesis, SBU showed higher values than ZIP. While both adhesives contain 10-methacryloyloxydecyl dihydrogen phosphate for chemical adhesion to hydroxyapatite [70], SBU additionally incorporates a methacrylate-modified polyalkenoic acid copolymer that may further enhance chemical adhesion [71]. Another factor is the solvent content: ZIP contains 30–35% solvent, whereas SBU contains only 10–15%. Complete evaporation of higher solvent concentrations is challenging, even with extended application times, potentially impairing ZIP's degree of conversion within the hybrid layer [72]. Given that both manufacturers recommend similar evaporation times, incomplete solvent removal in ZIP could explain its lower μTBS values compared to SBU. However, this hypothesis requires further investigation.

No significant differences in μTBS were found between the etch-and-rinse and self-etch strategies for either universal adhesive, regardless of pretreatment or storage period. This outcome supports the acceptance of the third null hypothesis, demonstrating that the protective effects observed were independent of the bonding strategy employed. Such flexibility in clinical application broadens the relevance of these findings, allowing practitioners to select their preferred bonding approach without compromising adhesive performance.

In addition to the favorable adhesive performance observed, concerns regarding the cytotoxicity of SF solutions should be interpreted in the context of clinical conditions. Although in vitro studies have reported cytotoxic effects under conditions of direct cell contact [73, 74], such experimental models do not account for the protective role of dentin. In deep carious lesions, the remaining dentin thickness acts as a diffusion barrier, limiting the transport of silver and fluoride ions toward the pulp and thereby reducing the potential for cytotoxic effects [67].

Furthermore, both in vitro and clinical evidence indicate that the biological response to SF–based agents is strongly influenced by formulation and concentration. Ammonia-free SF products have demonstrated acceptable biocompatibility at clinically relevant dilutions, despite exhibiting dose-dependent cytotoxicity under direct exposure [73, 75], Consistent with these findings, clinical studies have reported no adverse pulpal outcomes when silver diamine fluoride is applied to deep carious lesions without pulp exposure and in accordance with recommended clinical protocols [73, 75].

Nevertheless, this study has some limitations that should be acknowledged. First, the relatively short experimental period may not fully reflect the long-term stability of the adhesive interface under continuous cariogenic challenge. Moreover, restoration failure is a multifactorial process, and the present findings should be interpreted within the context of a controlled, short-term experimental model. Although Riva star Aqua demonstrated a protective effect within the evaluated timeframe, its long-term durability in the presence of marginal gaps and a dynamic, regenerating oral biofilm remains uncertain, thereby warranting further long-term and clinically oriented investigations.

Finally, bond strength and adhesive/hybrid layer analysis by energy dispersive X-ray spectroscopy were the only parameters assessed. Therefore, complementary evaluations would provide a more comprehensive understanding of the effects of ammonia-free silver diamine fluoride on the adhesive interface. On the other hand, the study included an in situ part, which allows for a "real-life" effect of saliva and biofilm occurring on the bonded specimens, thereby increasing the clinical relevance of the findings.

Conclusion

The application of ammonia-free silver fluoride solution preserved the bond strength values to dentin when submitted to in situ cariogenic challenge. These results should be related to potential of remineralization (fluoride) and antimicrobial (silver) released by the resin-dentin interface after the ammonia-free silver fluoride solution application.

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Data availability Corresponding author can provide the data supporting the findings of this in vitro study upon request.

Declarations

Ethics approval This study was approved (2.631.289) by the Ethics and Research Committee of the State University of Ponta Grossa (PR, Brasil). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Competing interests The authors declare no competing interests.

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