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Comparisons of ammonia- and water-based silver-containing solutions on dentin bonding and enzymatic activity: 1-yr evaluation

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ABSTRACT

Objective: To evaluate the effects of an ammonia-based and a water-based silver-containing solutions on bonding performance and matrix-metalloproteinases (MMPs) activity of a universal adhesive to dentin after 1 year of artificial aging.

Methods: Mid-coronal dentin surfaces of 60 sound human molars were exposed and the following groups were formed according to the surface pre-treatment and etching mode of the universal adhesive (Zipbond Universal, SDI) (n = 10): G1) Zipbond in the self-etch mode (ZSE); G2) Riva Star (SDI) applied before ZSE; G3) Riva Star Aqua (SDI) applied before ZSE; G4) Zipbond in the etch-and-rinse mode (ZER); G5) Riva Star applied before ZER; G6) Riva Star Aqua applied before ZER. The specimens were sectioned and subjected to microtensile bond strength (μTBS) test at baseline (T_0) and after 12 months (T_{12}) of artificial storage. Scanning electron microscope (SEM) and energy dispersive spectroscopy analysis (EDS) were also conducted. Three additional molars per group were processed for the in situ zymography analysis at T_0 and T_{12}. Data were statistically analyzed ($p < 0.05$).

Results: Dentin pre-treatments and aging decreased bonding values, regardless of the etching mode ($p < 0.05$). No differences in μTBS were observed between the two silver-containing solutions, both at T_0 and T_{12}. Riva Star Aqua and etching significantly increased the MMPs activity, independent of the storage period ($p < 0.05$).

Significance: Dentin surface pre-treatment with silver-containing solutions negatively affects the bonding performances of resin composite restorations placed with a universal adhesive. However, the ammonia-based product Riva Star might show better stability in the long term, due to lower activation of MMPs.

1. Introduction

Despite major efforts made to prevent the incidence of dental caries, this tooth disease is still predominant worldwide, demanding a series of economical, treatment approach and quality of life considerations [1]. The use of antimicrobial materials has been suggested as drill-free approach to arrest dental caries, of which silver diamine fluoride (SDF) currently represents one of the most attractive solutions [2].

SDF solutions have been claimed to reduce cariogenic bacterial growth and biofilm formation [3], occlude dentinal tubules [4], enhance tertiary dentin formation [5], inhibit dentin demineralization and promote dentin remineralization [6,7], and could therefore be considered bioactive materials [8,9]. Nevertheless, the unesthetic dark staining of the tooth portion in contact with the SDF solution due to the reaction of residual silver ions with organic material has been considered the main drawback of this anticaries approach [10,11]. In this regard, the application of potassium iodide (KI) over an ammonia-based silver fluoride solution (Riva Star, SDI, Bayswater Vic, Australia) has been proposed to minimize the risk of staining [12–14].

The actual mechanism of SDF action is debated and still not well understood [15] and the effect of SDF+KI on dentin bond strength of direct restorative materials is still uncertain, especially regarding the latest introduced universal adhesive systems [16]. Furthermore, scientific evidence suggests that the volatility and high alkalinity (pH = 13) of

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ammonia-based silver-containing solutions [17,18] may impair the establishment of a reliable bond between the restorative material and the underlying dentin substrate [19–22], and undermine the health of periodontal tissue [18]. Peculiarly, almost all studies available to date have evaluated only the initial bond strength of restorative materials without focusing on the effects of silver-containing solutions on the durability of resin-dentin interfaces and preservation of the HL.

It is well established that resin-dentin bonds, created from contemporary adhesive systems, weaken over time due to HL degradation [23]. The latter is promoted by the hydrolysis of the hydrophilic resin polymers of the adhesive systems [24] together with the deterioration of collagen matrix within the HL by host-derived proteases, particularly MMPs and CCs [25]. Hence, an increasing number of therapeutic agents have been used during bonding procedures to preserve HL integrity [26]. Also SDF has exerted a protective activity against dentin collagen matrix degradation [27] with an inhibitory effect on endogenous matrix metalloproteinases (MMPs) [28] and cysteine cathepsins (CCs) [29], possibly contributing to the preservation of the hybrid layer (HL) over time.

Among the various silver-fluoride products available on the market, a novel water-based solution (AgF) has been recently patented (Riva Star Aqua, SDI). This aqueous solution of AgF is hypothesized to reduce the limitations associated with ammonia-based SDF products, including limited storage stability [30]. In this regard, this product is claimed by the manufacturer to be less irritating to soft tissues, to possess a milder odour, and exhibit enhanced storage stability. However, due to its recent introduction to the market, there appears to be no data evaluating its influence on bonding mechanisms and hydrolytic degradation process mediated by endogenous proteases.

Therefore, the aim of this in vitro study was to evaluate the effects of dentin surface pre-treatment with an ammonia-based (SDF) and a water-based (AgF) silver-containing products on the bonding performances of a universal adhesive and dentinal MMPs activity at baseline (T_0) and after 12 months (T_{12}) of aging in artificial saliva. Specifically, the null hypothesis tested were threefold: 1) dentin pre-treatment with the silver-containing solutions does not influence the microtensile bond strength of composite restorations placed with a universal adhesive; 2) no influence on the endogenous enzymatic activity could be observed after application of the silver-containing products; 3) artificial aging does not influence the microtensile bond strength and the endogenous enzymatic activity.

2. Materials and methods

2.1. Teeth collection

Freshly extracted non-carious sound human molars were obtained from anonymous individuals following their signed informed consent to their use for research purposes under the protocol (protocol N°: 71/2019/OSS/AUSLBO) approved by the Ethics Committee of the University of Bologna (Italy).

2.2. Microtensile bond strength (μTBS) test

Sixty teeth were selected to conduct μTBS testing. To this aim, tooth crowns were sectioned parallel to the occlusal surface with a low-speed diamond saw (Microremet, Remet, Casalecchio di Reno, Italy) under copious water cooling to expose enamel-free mid-coronal dentin surfaces. The absence of enamel remnants was verified under a stereomicroscope. The roots were removed to obtain a 4 mm-thick dentin crown section. A standardized smear layer was created on each dentin surface with #240-grit wet silicon-carbide (SiC) paper, performing movements in a figure-eight pattern for 30 s and briefly rinsing with water. The tooth surfaces were lightly air-dried with oil-free compressed air for 5 s being careful not to desiccate the specimens.

Dentin blocks were randomly and equally assigned to one of the following groups according to the dentin surface pre-treatment (SDF or AgF) and etching mode (etch-and-rinse, ER, or self-etch, SE) of the universal adhesive (Zipbond Universal, SDI) used for bonding procedures (n = 10):

- •G1) Zipbond Universal used in the SE mode (ZSE);
- •G2) Riva Star Step 1 (SDF) & Step 2 (KI) applied before ZSE;
- •G3) Riva Star Aqua Step 1 (AgF) & Step 2 (KI) applied before ZSE;
- •G4) Zipbond used in the ER mode (ZER);
- •G5) Riva Star Step 1 (SDF) & Step 2 (KI) applied before ZER;
- •G6) Riva Star Aqua Step 1 (AgF) & Step 2 (KI) applied before ZER.

All the materials were used according to the manufacturer's instructions, as illustrated in Table 1. The silver-containing solutions (SDF and AgF, respectively) were applied on dentin surface, followed by KI, before the universal adhesive application of the bonding procedures. In the ER groups, dentin was first etched for 10 s with 37% H_3PO_4 gel (Super etch, SDI), rinsed with water for 10 s and dried with an oil-free air-stream for 10 s. After application of SDF or AgF and KI, respectively, the dentin was rinsed thoroughly with water for 10 s then the universal adhesive was actively brushed on dentin for 10 s, blowed with an oil-free air flow (no less than 5 s) before light-curing for 10 s with a light-emitting diode curing device (LED, Curing Light 2500, 3 M ESPE, St Paul MN, USA; wavelength: 460–480 nm). A nano-hybrid and microfilled resin composite material (Luna, A2 shade, SDI) was used for the restorative procedures, by placing two 2-mm-thick layers on the previously treated dentin surface. Each increment was polymerized for 20 s with a LED curing light (Curing Light 2500, 3M ESPE) to obtain a 4-mm-thick composite build-up. The restored specimens were stored in water at 4 °C for 24 h.

After storage, the restored specimens were serially sectioned perpendicular to the adhesive interface into resin-dentin sticks with cross-sectional surface area of approximately 0.9 mm^2 by a low-speed diamond saw, under copious water cooling (Microremet, Remet), in accordance with the non-trimming technique of the μTBS test. Within each group, the sticks were randomly and equally divided into two parts, then placed in a laboratory oven at 37 °C and immersed in artificial saliva for either 24 h (T_0) or 12 months (T_{12}). Throughout the one-year storage period, the artificial saliva was refreshed every 2 weeks. The artificial saliva composition consisted of KCl (12.92 mmol/L), KSCN (1.95 mmol/L), $Na_2SO_4{\cdot}10\ H_2O$ (2.37 mmol/L), NH_4Cl (3.33 mmol/L), $CaCl_2{\cdot}2\ H_2O$ (1.55 mmol/L), $NaHCO_3$ (7.51 mmol/L), and $ZnCl_2$ (0.02 mmol/L) in HEPES buffer solution [31].

The exact dimensions of each stick were measured with a digital caliper (± 0.01 mm) before being subjected to the μTBS test. The ends of the sticks were affixed to the test block of a microtensile testing machine (Bisco Inc., Schaumburg, IL, USA) with cyanoacrylate glue. Each stick was stressed to failure under tension at a crosshead speed of 1 mm/min. The force required to fracture the specimens was recorded in Newton (N) and was converted into units of stress (MPa) using the following equation: bond strength (MPa) = force (N) / bonded surface area (mm^2). μTBS data were collected and statistically analyzed. Some sticks prematurely debonded during cutting and before testing and, therefore, were not included in the statistical analysis.

After testing, the fractured sticks were examined by a single blind observer under a stereomicroscope at 50x magnification to determine the type of failure, classified as follows: adhesive (A, along the dentin/adhesive interface), cohesive in dentin (CD, within the dentin surface), cohesive in composite (CC, within the resin composite) or mixed (M, adhesive and cohesive failures occurred simultaneously).

2.3. Scanning electron microscope (SEM) evaluation and energy dispersive spectroscopy analysis (EDS)

The fracture patterns of two fractured resin/dentin sticks per group, each exhibiting bond strength values closely aligned with the mean for their respective group, were further examined using a scanning electron microscope (SEM, Nova NanoSEM 450, Thermo Fisher Scientific,

Table 1
Names, compositions and application mode of the materials used in the study (information supplied by the manufacturer). In order to faithfully attend to the instructions for use, all the materials used in this study belonged to the same manufacturer (SDI, Bayswater Vic, Australia).

Material	Composition	Mode of use
Riva Star	Step 1: Silver, fluoride, ammonia Step 2: potassium iodide (KI)	STEP 1: Dispense one drop of the solution (SDF) on a dappen dish. Carefully apply solution for 10 s to treatment site only using a medium sized micro brush. STEP 2: Immediately after, dispense two drops of KI solution onto fresh dappen dish. Apply a generous amount of the solution to treatment site using a medium sized micro brush until the creamy white precipitate turns clear. Wash thoroughly with water for at least 10 s and air-dry for 10 s
Riva Star Aqua	Step 1: Silver, fluoride, water Step 2: potassium iodide (KI)	Follow instructions as for Riva Star.
Zipbond Universal	Adhesive monomers including ethacryloyloxydecyl dihydrogen phosphate (MDP), ethanol, water, fluoride	Dispense 1-2 drops into a mixing well. Scrub onto tooth tissues with a brush for 10 s. Wait 5 s. Blow with oil-free air until no movement of the adhesive (no less than 5 s). Light-cure for 10 s (460- 480 nm wavelength) with LED curing light.
Super etch syringe	37% phosphoric acid etchant	Dry the surface to be etched. Etch for 10 s, then rinse with water for 10 s and air dry for 10 s
Luna (A2 shade)	22.5% wt (39% vol.) multifunctional methacrylic ester, 77.5% wt (61% vol.) inorganic filler (40 nm – 1.5 μm)	Place composite in increments of 2 mm or less. Light-cure each increment for 20 s for light shade with a LED curing device.

Waltham, MA, USA). The sticks were first fixed in a 2.5% glutaraldehyde in 0.1% cacodylate buffer and then dehydrated by means of ascending ethanol solutions (50%, 70%, 80%, 90%, 95%, 100%) and HMDS [32]. The dentin and the composite sides of the fractured sticks were mounted on metal stubs and gold-palladium sputter-coated before evaluation at different magnifications (accelerating voltage of 10.00 kV and magnifications at 200x and 2.000x).

Additionally, EDS was performed on the entire dentin portion of the fractured specimens at T_0 and T_{12} to investigate the chemical profile, especially in terms of the presence or absence of silver (Ag) ions on the dentin surface in the experimental groups.

2.4. In situ zymography analysis

To investigate the effect of the two tested solutions on dentin MMPs activity, the in situ zymography was performed using additional freshly extracted non-carious human molars (n = 3). After removal of occlusal enamel, two 1-mm-thick slabs of middle/deep coronal dentin were obtained from each tooth and further divided into quarters by means of a low-speed diamond saw under water cooling (Microremet, Remet). Slices of each tooth were randomly assigned to the same 6 groups (n = 3) as previously presented for the μTBS test. This experimental design enabled all groups to be examined on the same dentinal substrate, limiting the effect of anatomical variations. A standardized smear layer was created on each dentin surface with #240-grit wet SiC paper. Prepared tooth surfaces were lightly air-dried with oil-free compressed air for 5 s taking care not to desiccate the specimens prior to adhesive protocols. Dentin surface pre-treatment and bonding procedures were performed as previously described for the μTBS testing. Successively, a 1-mm-thick build-up with a nano-hybrid and micro-filled resin composite (Luna, A2 Shade, SDI) was created and polymerized for 20 s with a LED curing light (Curing Light 2500, 3 M ESPE).

The procedure was performed following the protocol previously reported by Mazzoni *et al.* [33]. After storage in water at 4 °C for 24 h, the restored slices were sectioned longitudinally into 1-mm-thick specimens to expose the resin-dentin interfaces by means of the same low-speed water-cooled diamond saw. Within each group, the slabs were equally divided in 2 groups and stored in a laboratory oven at 37 °C in artificial saliva for 24 h (T_0) or 12 months (T_{12}). Again, during the 1-year storage, the artificial saliva was refreshed every 2 weeks.

After each storage time, the bonded resin-dentin sites were affixed to a microscope glass slide (2 specimens from each tooth per group) with cyanoacrylate glue, ground down and progressively polished with wet SiC papers with increasingly fine grit size (600-, 1.200-, 4.000-) to obtain approximately ~50 μm-thick sections.

To produce the substrate, 1.0 mg/mL of a stock solution containing self-quenched fluorescein-conjugated gelatin (E-12055; Molecular Probes, Eugene, OR, USA) was prepared by adding 1.0 mL deionized water to the vial containing the lyophilized gelatin. The substrate was stored at − 20 °C until use. The gelatin stock solution was diluted 10 times with dilution buffer (NaCl 150 mm, CaCl 25 mm, Tris–HCl 50 mm, pH 8.0). Then, 50-μL quantity of gelatin mixture was placed on top of each polished specimen, covered with a coverslip and incubated in a dark humidified chamber at 37 °C overnight. During incubation, the assemblies were prevented from direct contact with water and were protected from exposure to light.

After incubation, the specimens were observed under a multi-photon confocal laser scanning microscope (Leica SP8, Leica Microsystems GmbH, Wetzlar, Germany; excitation/emission wavelength: 488/530 nm). Several z-stack images (~ 15 μm thick, one image per each 1 μm into the depth of the sample) of the HL were made per each slice. The hydrolysis of the quenched fluorescein-conjugated gelatin substrate, indicative of endogenous gelatinolytic enzyme activity, was quantified as the integrated density of the fluorescence signals for each image using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The differences in the intensity of the fluorescence between the tested groups were used as a relative measurement of the differences in the enzymatic activity of the HL.

2.5. Statistical analysis

The data retrieved from the μTBS test and the in situ zymography analysis were normally (Shapiro-Wilk test) and homogeneously (Brown-Forsythe test) distributed. Accordingly, the three-Way Analysis of Variance (3-way ANOVA) and all pairwise Multiple Comparison Procedures (Holm-Sidak method) were conducted to investigate the effect of 3 variables and their interaction: "pre-treatment" (control, Riva Star or Riva Star Aqua), "etching mode" (ER and SE) and "aging" (T_0 and T_{12}) on bond strength and endogenous enzymatic activity. The author who performed the statistical analysis was unaware of the group belonging. For all tests, the level of statistical significance was set at $p < 0.05$ (SigmaPlot 12.0; Systat).

3. Results

3.1. Microtensile bond strength (μTBS) test

The mean values and standard error of the μTBS (expressed as MPa) at times T_0 and T_{12} are reported in Table 2. Three-factor ANOVA revealed that the variables "pre-treatment" and "aging" significantly influenced bond-strength values ($p < 0.001$), while no effects were reported by the variable "etching mode" ($p > 0.05$). The interactions between the variables "pre-treatment", "etching mode" and "aging" were also statistically significant ($p < 0.05$). Regardless the etching mode, the experimental groups showed a significant bond strength reduction compared with the control groups ($p < 0.001$) and there was no

Table 2
Means ± standard error of μTBS values (expressed as MPa). T_0 and T_{12} indicate specimens that were tested after storage at 37 °C for 24 h or 12 months in artificial saliva, respectively. Different superscript upper-case letters indicate differences ($p < 0.05$) within the columns. Different superscript lower-case letters indicate differences ($p < 0.05$) within the rows. Distribution of failure mode among tested groups is also reported in percentages in square brackets and classified as: CC: cohesive failure in resin composite; M: mixed; A: adhesive; CD: cohesive failure in dentin.

	T_0		T_{12}	
	SE	ER	SE	ER
Control	$36.6 \pm 2.2^{A,b}$ [11CC, 68 M, 21A]	$44.4 \pm 1.6^{A,a}$ [17CC, 40 M, 38A, 5CD]	$20.3 \pm 2.3^{A,c}$ [9CC, 63 M, 14A, 14CD]	$20.8 \pm 1.7^{A,c}$ [20CC, 63 M, 17A]
Riva Star	$27.7 \pm 2.1^{A,a}$ [8CC, 40 M, 40A, 12CD]	$34.6 \pm 1.6^{A,a}$ [8CC, 48 M, 37A, 7CD]	$9.9 \pm 4.2^{A,B,b}$ [50 M, 50A]	$11.1 \pm 1.6^{B,b}$ [8CC, 70 M, 22A]
Riva Star Aqua	$38.0 \pm 3.7^{A,a}$ [8CC, 51 M, 41A]	$32.4 \pm 1.9^{A,a}$ [15CC, 66 M, 17A, 2CD]	$7.1 \pm 1.3^{B,b}$ [69 M, 31A]	$9.7 \pm 1.7^{B,b}$ [5CC, 63 M, 32A]

significant difference in bonding values between Riva Star and Riva Star Aqua groups neither at T_0 nor T_{12} (Control > Riva Star = Riva Star Aqua). Aging in artificial saliva negatively affected the bond strength values in all groups ($T_0 > T_{12}$; $p < 0.001$). The failure mode analysis of the sticks after testing revealed a predominance of mixed fractures among all groups, irrespective of pre-treatment, etching or aging (Table 2).

3.2. Scanning electron microscope (SEM) evaluation and energy dispersive spectroscopy analysis (EDS)

Representative SEM images showing the failure patterns are displayed in Figs. 1 and 2. The findings of the SEM fractographic analysis are in accordance with the failure mode analysis, with mostly mixed fractures in the majority of the analyzed fractured sticks. When the universal adhesive was utilized in SE mode, the dentin surface appeared mostly covered by the resin composite bulk and the smear layer limiting the observation of the underlying dentin (Fig. 1). This was also present after 12 months of artificial storage (Fig. 2). At this timepoint, partial detachments of the adhesive layer were observed in the Riva Star SE and Riva Star Aqua SE groups. In the latter, this was particularly evident and the image revealed a wet-polished dentin surface displaying lines created during the formation of the smear layer with the abrasive disks (Fig. 2). The application of the universal adhesive in the ER mode resulted in increased smear layer removal in both aging points (Figs. 1–2). In the silver-containing solution pretreated groups, the dentinal tubules were visibly open and partially infiltrated by the resin, and this was more evident in the Riva Star group (Figs. 1–2).

The EDS spectrum acquisitions at T_0 and T_{12} are displayed in Figs. 3 and 4, respectly. EDS surface elemental analysis indicated that the majorly represented chemical elements in the control groups were carbon, calcium, oxygen, aluminium and silicon. The analysis confirmed the presence of silver atoms on the dentin surfaces of various speciments treated with Riva Star in Riva Star Aqua. In the experimental groups where no silver was detected, a presence of silicon atoms was noted, indicating that the dentin of the fractured interface was mostly covered with composite resin.

3.3. In situ zymography analysis

Three-factor ANOVA revealed that all the investigated variables significantly influenced the endogenous enzymatic activity ($p < 0.05$). Furthermore, all the interactions between the 3 variables ("pre-treatment", "etching mode" and "aging") were statistically significant ($p < 0.05$).

The mean results of gelatinolytic activity, expressed as the integrated density of the green fluorescence signal within the hybrid layer, are summarized in Fig. 4B-5B. Groups pre-treated with Riva Star Aqua generated a significantly higher gelatinolytic activity within the HL when compared with the other groups tested ($p < 0.05$), while there was no significant difference in endogenous enzymatic activity between control and Riva Star groups ($p > 0.05$). ER groups resulted in significantly more expressed gelatinolytic activity within the HL when compared with SE groups, both at T_0 and T_{12} ($p < 0.05$). Aging significantly influenced dentin gelatinolytic activity ($p \leq 0.001$). In all the tested groups, fluorescence density level was higher at T_0 compared with T_{12} ($p < 0.05$).

Representative confocal laser scanning microscopy images showing the level of endogenous enzymatic activity as the density of the green fluorescence signal are displayed in Fig. 4A-4B. Confocal laser scanning microscopic examination of in situ zymography specimens in all groups detected green fluorescence within the HL as well as underlying dentinal tubules. A more pronounced fluorescence level is noted in the ER groups, with a pattern of the signal penetrating deeper in the dentin tubules, while in the SE groups the signal is more linear and mainly concentrated in the hybrid layer.

4. Discussion

The primary aim of this study was to investigate whether the surface pre-treatment of sound dentin with an SDF+KI and an AgF+KI solution can influence the bonding performances and endogenous enzymatic activity of a universal adhesive. Our results demonstrated that the 2-step silver-containing solutions used as therapeutic dentin agents significantly reduced the μTBS compared to the control groups. Secondarily, the experimental groups using the patented water-based Riva Star Aqua showed significantly higher gelatinolytic activity within the HL when compared to the control and Riva Star groups. Therefore, the first null hypothesis of the study was rejected, while the second one was only partially accepted. Thirdly, this study sought to examine if artificial aging has an influence on the μTBS values and endogenous enzymatic activity. According to the obtained results, artificial aging affected bond strength values and significantly influenced dentin gelatinolytic activity. Hence, the third null hypothesis was rejected.

In recent years, the traditional concept of minimally invasive dentistry has been strengthened by the continuous improvements of adhesive restorative materials. Indeed, the development of increasingly-performant bioactive materials [8] has expanded the application of minimal dental intervention in order to preserve as much natural tooth structure as possible [34,35]. To this aim, a variety of agents with antimicrobial and anticaries properties have been proposed over time, including silver nitrate ($AgNO_3$) [36], sodium fluoride (NaF) [37] and chlorhexidine [38,39]. Among these agents, SDF has been demonstrated to exert a protective activity against the degradation promoted by MMPs and CCs [28,29].

The results extrapolated from the existing studies investigating SDF products are fairly discordant. Indeed, while in some studies 38% SDF solutions applied prior to the adhesive procedures did not negatively affect the bond strength of resin composite restorations [21,40,41], other authors have observed a significant decrease on bonding effectiveness after their application [20,42–44]. The same contradictions have been observed when 2-step SDF+KI solutions were used for the same purposes. Indeed, while some studies have supported the use of SDF+KI before bonding procedures [45], others led to opposite findings [19,22,46], making it difficult to define the actual influence of KI on the bonding mechanism and performances. Some possible reasons for the inconsistency among the results in the aforementioned studies could be the lack of a standard methodology for specimens' preparation [47], the different application protocols of adhesive systems and the type of SDF product employed.

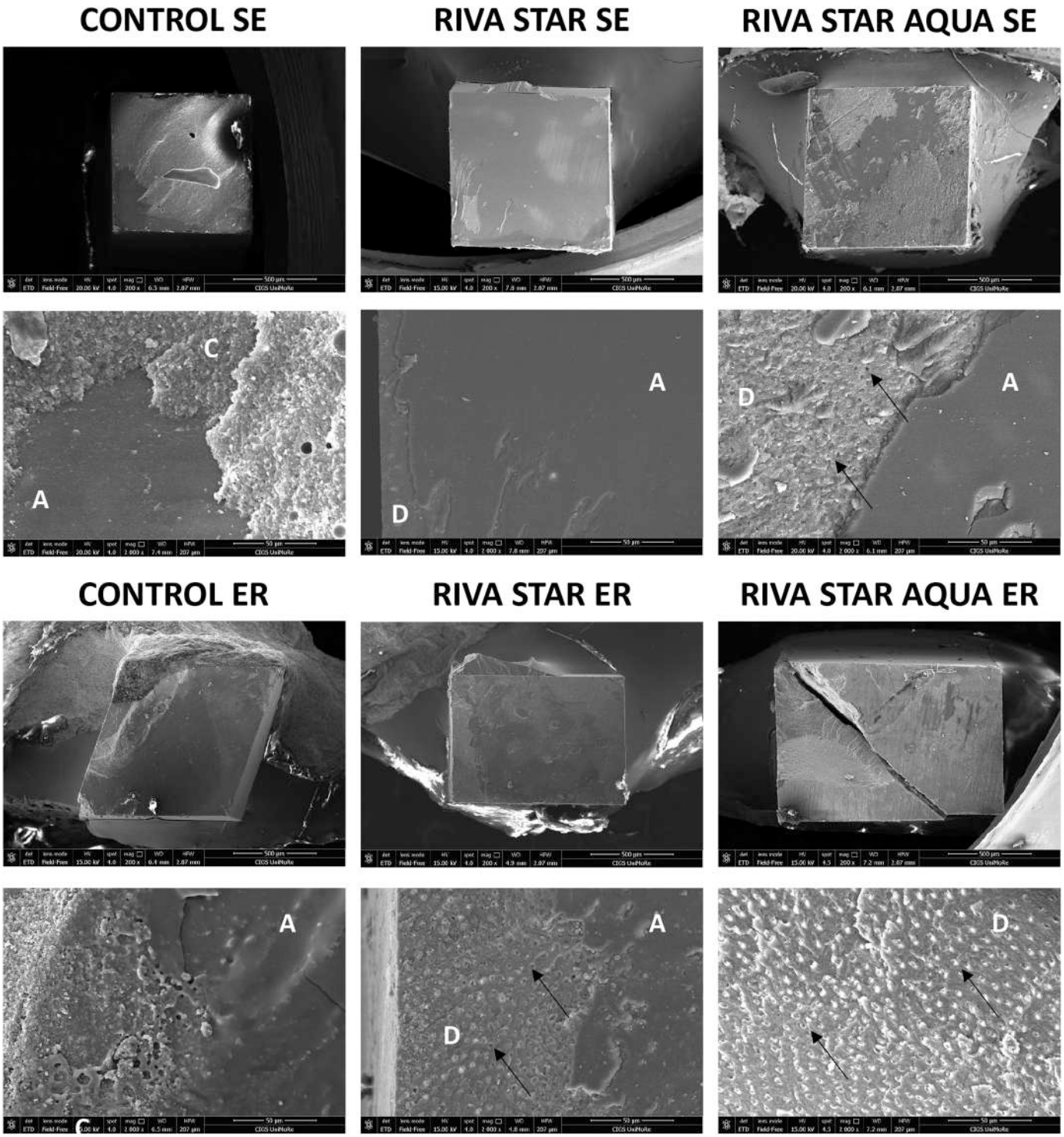

Fig. 1. Fractographic analysis after μTBS at T_0: SEM images of fractured microtensile sticks (200x magnification on the upper image and 2000x on the lower image for each group). D: dentin; A: adhesive; C: composite; black arrows: dentinal tubules. Observations were conducted on representative fractured specimens registering bonding values close to the mean of each group, as following indicated (values expressed in MPa): control SE 34.6; Riva Star SE 28.7; Riva Star Aqua SE 35.1; control ER 42.4; Riva Star ER 33.2; Riva Star Aqua ER 28.6.

In our study, lower bond strengths were observed in the groups where both silver-containing solutions were used before bonding procedures. Several factors may be responsible for the obtained results. As the effectiveness of adhesive systems relies on micromechanical retention and HL formation [48], the lower bonding values obtained can be cautiously attributed to adverse effects of the presence of silver salts covering the dentin surface and penetrating deep into dentinal tubules [27,49]. The presence of silver molecules inside the tubule orifices hampers the resin penetration into both peritubular and intertubular dentin, thus jeopardizing the establishment of a firm retention between resin and dentin [19,20]. Additionally, once SDF is applied on dentin, silver phosphate and calcium fluoride precipitates [20]. This should be taken into consideration when using a 10-MDP-containing universal adhesive (as the one used in the present investigation). Indeed, the

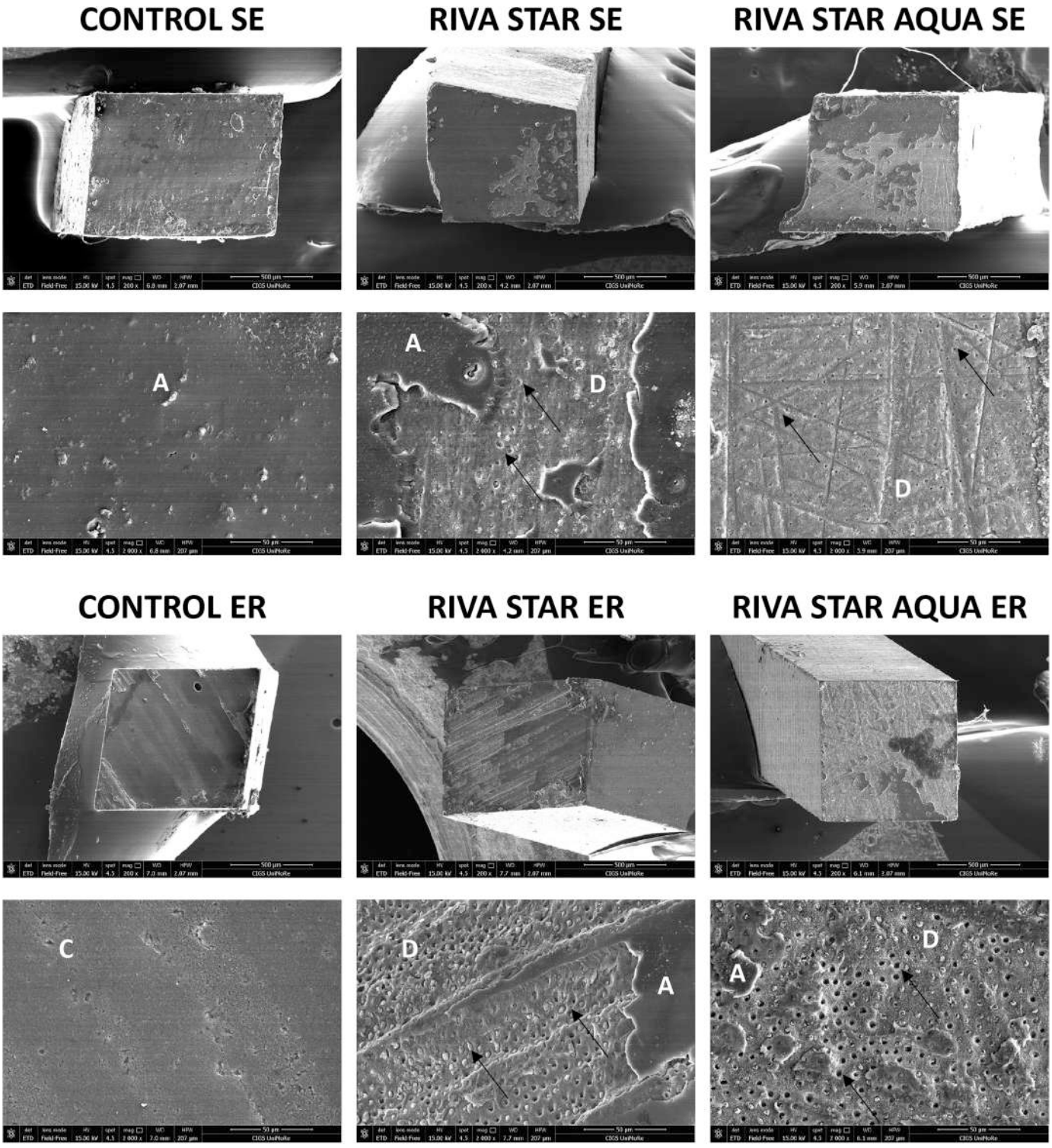

Fig. 2. Fractographic analysis after μTBS at T_{12}: SEM images of fractured microtensile sticks (200x magnification on the upper image and 2000x on the lower image for each group). D: dentin; A: adhesive; C: composite; black arrows: dentinal tubules. Observations were conducted on representative fractured specimens registering bonding values close to the mean of each group, as following indicated (values expressed in MPa): control SE 21.8; Riva Star SE 10.2; Riva Star Aqua SE 8.3; control ER 19.4; Riva Star ER 12.4; Riva Star Aqua ER 8.6.

presence of phosphate and calcium ions are the necessary standpoint for the 10-MDP functional monomer to establish a chemical bond with dentin by the formation of a nanolayering structure [19,41,50]. The reduced number of necessary ions for chemical interaction influences the activity of the functional monomer with catastrophic effect on the overall bonding ability to dentin. In addition, as previously proposed, the alkalinity of the silver-containing solutions tested (particularly high in Riva Star) can contrast the etching process, supposedly in a manner in which the more acidic the etching (reminiscent of ER modality), the more it is hindered by an alkaline environment, thus hampering the bonding mechanism [20,39]. Since no previous data exist on Riva Star Aqua, this issue remains to be further assessed.

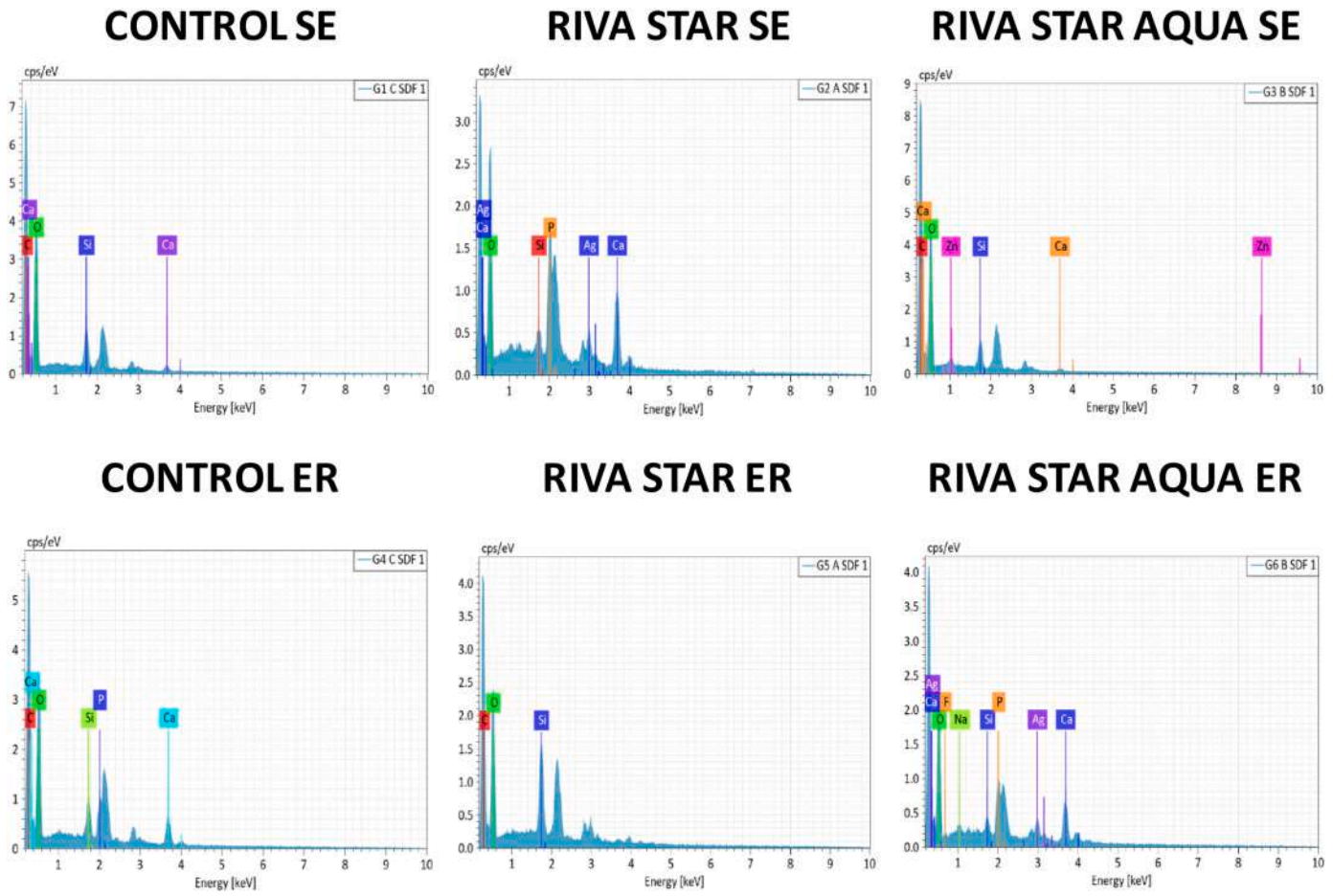

Fig. 3. EDS analysis of fractured microtensile sticks at T_0 (magnification 200x).

Interestingly, some authors have raised critical remarks on the application protocol of SDF+KI, being definied as highly technique-sensitive [20,51]. Indeed, some studies have suggested that rinsing off or not SDF+KI solution after a specific time may influence bonding effectiveness to sound dentin [20]. Accordingly, in our protocol water-rising of the ammonia-based and ammonia-free silver solutions before adhesive application has been performed. The water-rinsing step was intended to eliminate the superficial excess of silver precipitates, favouring the adhesion of composite restoration while preserving the therapeutic effect of SDF [20,22]. It can be presumed that if rinsing is not performed after applying SDF, the residual too alkaline pH and the amount of silver particles residues on the tooth surface could interfere with the etching mechanism and the resin penetration ability, consequently reducing the bond strength. This assumption could cautiously explain why no differences were observed between the ER and SE application modes [52].

Based on previous studies, it has been demonstrated that SDF can infiltrate the dentin; however, there is no consensus regarding the depth of penetration. One in vitro study reported that silver and fluoride ions can penetrate from 50 to 200 μm into carious dentin [53], while another *ex vivo* research on sound dentin suggested that silver can only permeate up to 40 μm [54]. It should be noted that these measurements were assessed using a microhardness test and an electron beam microprobe, respectively, which are not suitable for accurately measuring silver penetration. In a laboratory study using EDS line analysis, silver was not initially detected in the SDF-treated groups after 24 h and 2 weeks. However, after 1 year of storage, the analysis showed silver penetration up to 1000 μm. This could be attributed to the reduction of silver ions leading to the formation of mature silver crystals [49].

In the current study, the presence of Ag ions on the dentin surface was assessed using SEM/EDS analysis, which is a useful and cost-effective technique for evaluating surface elemental composition. The data was acquired at T_0 and T_{12} to determine any potential presence of Ag remnants both at baseline and after one year of storage in artificial saliva. In the surface elemental analysis, calcium and phosphate ions are representative of the mineral component of tooth substrate, carbon and oxygen indicate the demineralized dentin. Aluminium and silicon are widely used in resin composite materials, while the presence of niobium may be attributed to remnants of diamond saw used during specimen preparation. Regardless the aging, the control groups exhibited the expected presence of carbon, calcium, oxygen, aluminium and silicon. Regarding the results at T_0, in various experimental groups, the presence of Ag were observed. In Riva Star Aqua SE group, the analysis detected zinc atoms, which can be sometimes identified within the silver precipitation zone [55]. Regarding the results at T_{12}, in the Riva Star Aqua groups, the analysis confirmed the presence of Ag atoms on the dentin surfaces, while their presence was not detected in the Riva Star groups. In both T_0 and T_{12} analysis, the absence of Ag in the experimental groups can be attributed to the significant predominance of resin composite coverage on the surface (mixed failure modes), demonstrated by a high presence of silicon and non-representative distribution of atoms. Therefore, further analysis should be conducted to assess the presence and depth of Ag penetration after the use of two-step silver-containing solutions as therapeutic agents.

Apparently, it seems only few studies have attempted to evaluate the dentin μTBS over such long period of time. Contrary to most of the studies currently present in literature which evaluated SDF effectiveness on bonding procedures only in the short-term period, we aimed to

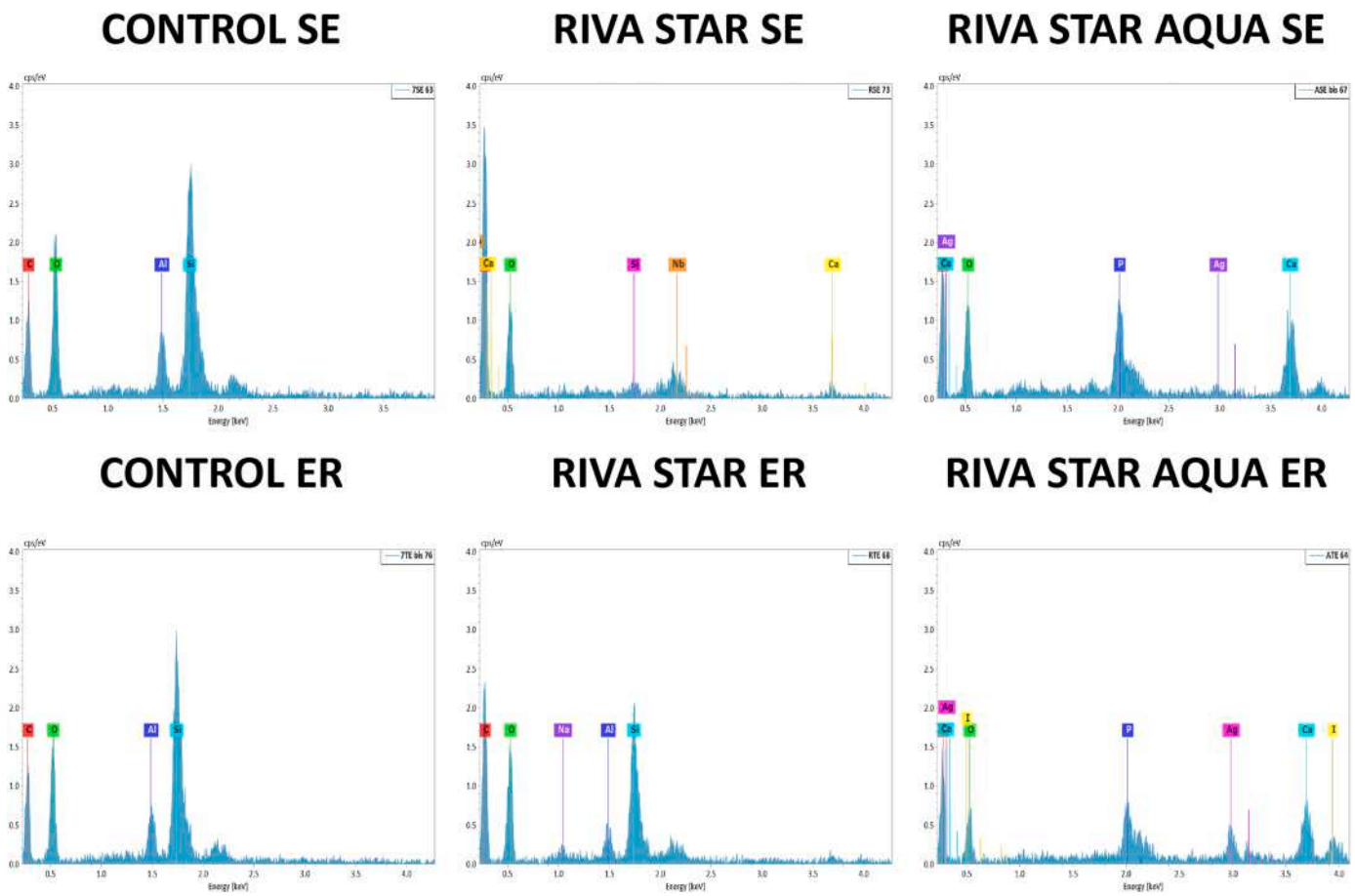

Fig. 4. EDS analysis of fractured microtensile sticks at T_{12} (magnification 200x).

expand the observation period (1 year of artificial storage), since only a long-term evaluation can give a more realistic perspective of the efficacy and ultramorphological characteristics of a therapeutic agent [31]. It would be warranted to encourage more studies with long-term evaluations.

The durability of the resin-dentin interface relies on the formation of a stable HL and endogenous dentin enzymatic activity undoubtedly contributes to the degradation of the collagen matrix [23]. Several studies have suggested the use of inhibitors of endogenous dentin proteases as an effort to improve the stability and durability of dentin bond strength over time [56,57]. Mei *et al.* have observed that SDF solutions at different concentrations exert an inhibitory effect on MMPs and CC [28,29]. Furthermore, the authors found that SDF can prevent dentin collagen degradation from bacterial collagenase challenge [27]. It can be assumed that the strong alkalinity of the ammonia-based Riva Star may limit the proteolytic activities of collagenases [58]. However, in the present study, groups pre-treated with the water-based Riva Star Aqua generated a significantly higher gelatinolytic activity, expressed as the density of the fluorescence signal within the HL, compared to the other groups (Riva Star Aqua > Control = Riva Star). MMPs are dentin endogenous proteases that require water to exert their activity. Accordingly, it could be hypothesized that the higher water content Riva Star Aqua compared to the ammonia-based SDF may have induced MMP-mediated degradation [59], or influenced the interaction of the adhesive resin with the dentin substrate. Again, as these are the first data present on this product, further studies are highly warranted to clarify the mechanism underlying these results.

In the current study, ER groups resulted in significantly higher endogenous enzymatic activity within the HL compared to SE groups, according to the scientific literature [60]. It is well accepted that an acidic environment, such as acid etching, activates pro-forms of MMPs and CC that are bound to dentinal matrix [31], allowing collagen hydrolysis and degradation of the HL [26]. Moreover, in non-carious dentin, ER approach completely removes the smear layer; therefore, during the storage period, the dentinal tubules might act as water-filled canals conducting water to the adhesive interface [19]. Water can cause hydrolysis of the denuded collagen at the base of the HL and adhesive resin, facilitating the bond degradation [19].

In our study, the endogenous enzymatic activity was higher at T_0 compared to T_{12}, regardless the pre-treatment. It is important to highlight that, during aging, MMPs and CC contribute to bond degradation by digesting the collagen matrices [25]. However, resin hydrolysis resulting from storage in artificial saliva also plays a key role in bond degradation and should not be neglected [57,61]. Although it could be expected that findings from in situ zymography correlate with the results of the μTBS test, the authors hypothesized that Riva Star and Riva Star Aqua may have other effects on bond strength such as impacting the surface tension of dentin [62]. It should be noted that the adhesive system has a low surface tension that enhances the spreading and hybridization of collagen fibrils [23]. Therefore, the significant difference in bond strength observed in the experimental groups might be attributed to an increase in dentin surface tension due to Riva Star and Riva Star Aqua pre-treatments. Furthermore, we cannot exclude the risk of residual water after using 2-step silver-containing solutions, especially the water-based Riva Star Aqua. The remaining water can hinder polymerization and render the resin more susceptible to hydrolytic degradation due to plasticization and swelling [63].

The findings of the current study have significant clinical implications. Since the tested products are already available on the dental market, clinicians should be informed about the potential effect of Riva

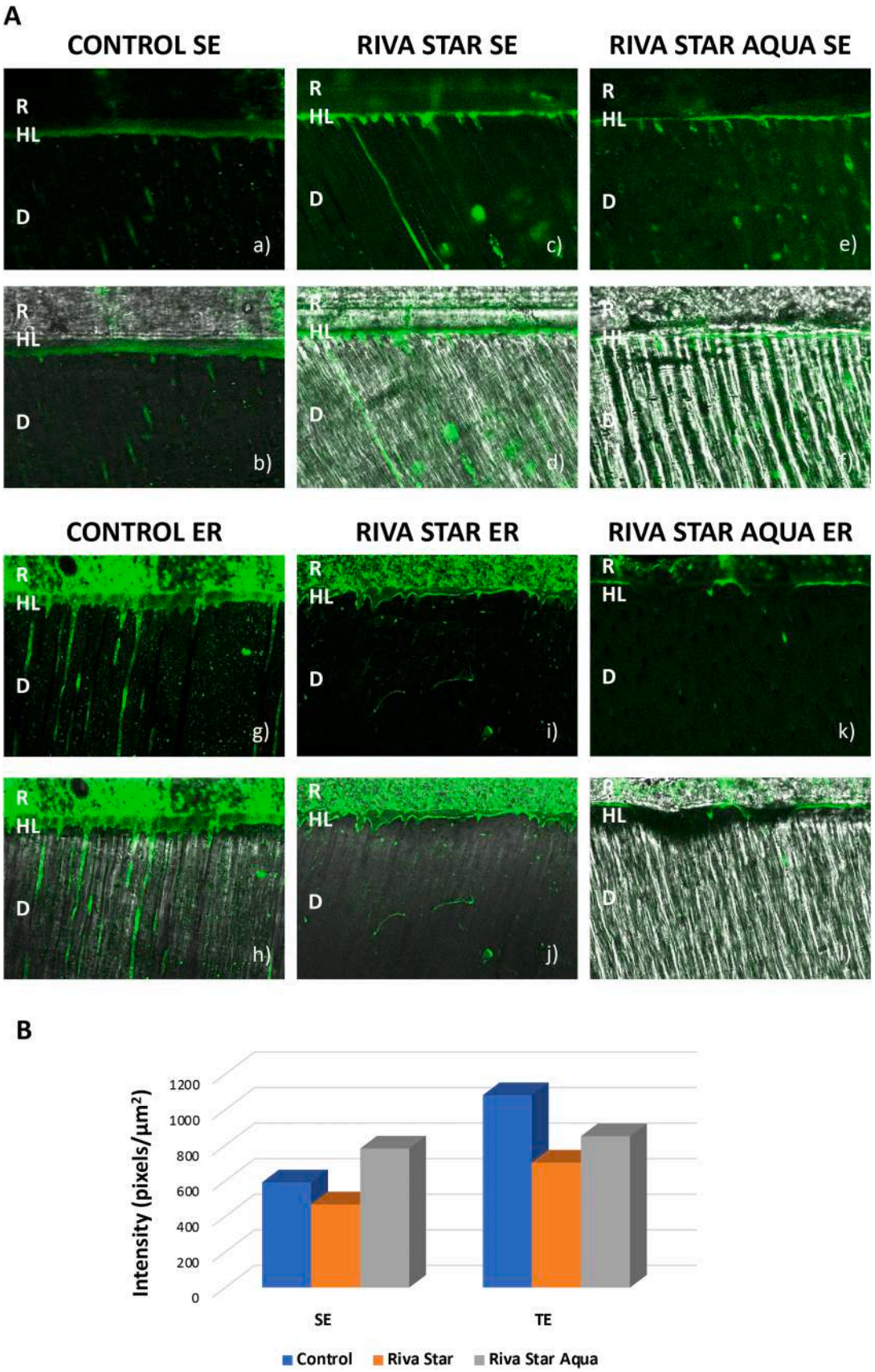

Fig. 5. *In situ* zymography results obtained after 24 h of storage at 37 °C (T_0). **A)**: Representative examples of the resin-dentin interfaces incubated with fluorescent gelatin. Images acquired in the green channel (a, c, e, g, h, j). Composite images obtained by merging the differential interference contrast image and those obtained in the green channel (b, d, f, h, j, l). D: dentin; HL: hybrid layer; R: resin composite. **B)**: Bar graph representing gelatinolytic activity, expressed as the intensity of green fluorescence (pixels/μm^2) within the HL.

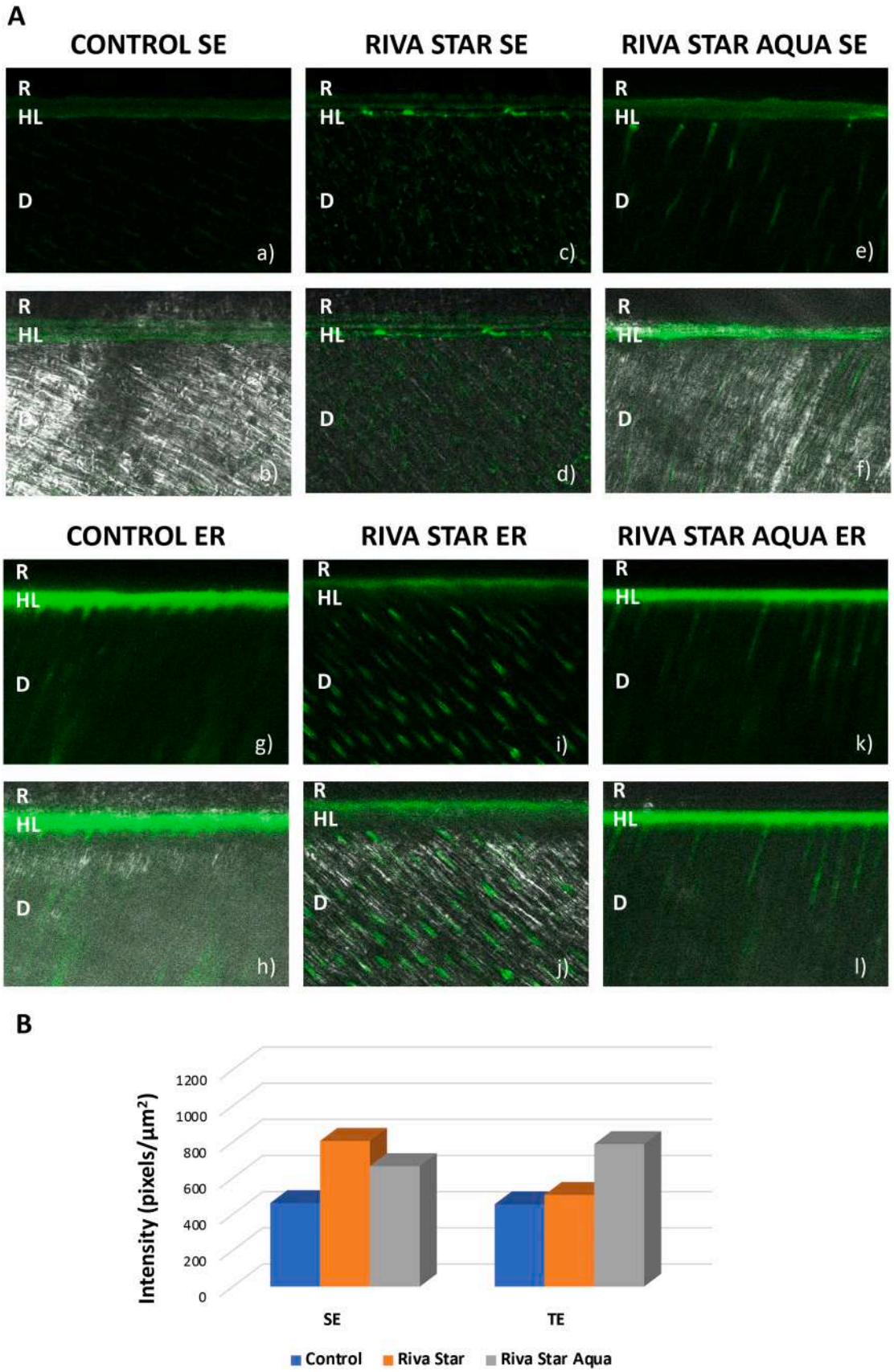

Fig. 6. *In situ* zymography results obtained after 1 year of aging in artificial saliva at 37 °C (T_{12}). **A**): **A**): Representative examples of the resin-dentin interfaces incubated with fluorescent gelatin. Images acquired in the green channel (a, c, e, g, i, k). Composite images obtained by merging the differential interference contrast image and those obtained in the green channel (b, d, f, h, j, l). D: dentin; HL: hybrid layer; R: resin composite. **B**): Bar graph representing gelatinolytic activity, expressed as the intensity of green fluorescence (pixels/μm^2) within the HL.

Star and Riva Star Aqua when used before bonding procedures for resin composite restorations. If the tooth surface has been treated with 2-step silver-containing solutions, the lifespan of composite restorations may be shortened since premature debonding is likely to occur.

5. Conclusion

Within the limitations of this laboratory study, it may be concluded that both the ammonia-based and the water-based 2-step silver-containing solutions reduced the bonding performances of resin composite restorations placed with a 10-MDP containing universal adhesive to dentin. However, the ammonia-based product Riva Star might show better stability in the long term, due to lower activation of MMPs. Further studies are warranted to evaluate the same protocol on carious affected dentin.

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Authors contribution

Conceptualization: [Claudia Mazzitelli, Tatjana Maravic]; Methodology: [Claudia Mazzitelli, Tatjana Maravic, Uros Josic, Edoardo Mancuso]; Formal analysis and investigation: [Carlo D'Alessandro, Federica Florenzano, Vittorio Checchi, Luigi Generali, Diego D'Urso, Annamaria Forte]; Writing – original draft preparation: [Claudia Mazzitelli, Tatjana Maravic, Carlo D'Alessandro]; Writing – review and editing: [Uros Josic, Lorenzo Breschi, Annalisa Mazzoni]; Supervision: [Claudia Mazzitelli, Tatjana Maravic, Lorenzo Breschi, Annalisa Mazzoni].

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References

[1] Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, et al. Dental caries. Nat Rev Dis Prim 2017;3:1–16. https://doi.org/10.1038/nrdp.2017.30.
[2] Rosenblatt A, Stamford TCM, Niederman R. Silver diamine fluoride: a caries "silver-fluoride bullet. J Dent Res 2009;88:116–25. https://doi.org/10.1177/0022034508329406.
[3] Mei ML, Li Q, Chu C-H, Lo EC-M, Samaranayake LP. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. Ann Clin Microbiol Antimicrob 2013;12:4. https://doi.org/10.1186/1476-0711-12-4.
[4] Kiesow A, Menzel M, Lippert F, Tanzer JM, Milgrom P. Dentin tubule occlusion by a 38% silver diamine fluoride gel: an in vitro investigation. BDJ Open 2022;8:1–5. https://doi.org/10.1038/s41405-022-00095-8.
[5] Rossi G, Squassi A, Mandalunis P, Kaplan A. Effect of silver diamine fluoride (SDF) on the dentin-pulp complex. Ex vivo histological analysis on human primary teeth and rat molars. Acta Odontol Lat 2017:30.
[6] Mei ML, Ito L, Cao Y, Lo ECM, Li QL, Chu CH. An ex vivo study of arrested primary teeth caries with silver diamine fluoride therapy. J Dent 2014;42:395–402. https://doi.org/10.1016/j.jdent.2013.12.007.
[7] Mei ML, Nudelman F, Marzec B, Walker JM, Lo ECM, Walls AW, et al. Formation of Fluorohydroxyapatite with Silver Diamine Fluoride. J Dent Res 2017;96:1122–8. https://doi.org/10.1177/0022034517709738.
[8] Zhang OL, Niu JY, Yin IX, Yu OY, Mei ML, Chu CH. Bioactive materials for caries management: a literature review. Dent J (Basel) 2023;11:59. https://doi.org/10.3390/dj11030059.
[9] Pires PM, Rosa T da C, Ribeiro-Lages MB, Duarte ML, Cople Maia L, Neves A de A, et al. Bioactive restorative materials applied over coronal dentine-a bibliometric and critical review. Bioeng 2023;10:731. https://doi.org/10.3390/bioengineering10060731.
[10] Patel J, Anthonappa RP, King NigelM. Evaluation of the staining potential of silver diamine fluoride: in vitro. Int J Paediatr Dent 2018;28:514–22. https://doi.org/10.1111/ipd.12401.
[11] Alsagob E, Sawan N, Aladhyan S, Alsalem N, Alshami A, Albluwi S. Silver diamine fluoride with delayed restoration reduces tooth discoloration. Saudi J Biol Sci 2022;29:1434–8. https://doi.org/10.1016/j.sjbs.2021.11.030.
[12] Roberts A, Bradley J, Merkley S, Pachal T, Gopal J, Sharma D. Does potassium iodide application following silver diamine fluoride reduce staining of tooth? A systematic review. Aust Dent J 2020;65:109–17. https://doi.org/10.1111/adj.12743.
[13] Haiat A, Ngo HC, Samaranayake LP, Fakhruddin KS. The effect of the combined use of silver diamine fluoride and potassium iodide in disrupting the plaque biofilm microbiome and alleviating tooth discoloration: A systematic review. PLoS One 2021;16:e0252734. https://doi.org/10.1371/journal.pone.0252734.
[14] Asghar M, Omar RA, Yahya R, Yap AU, Shaikh MS. Approaches to minimize tooth staining associated with silver diamine fluoride: A systematic review. J Esthet Restor Dent 2023;35:322–32. https://doi.org/10.1111/jerd.13013.
[15] Zhao IS, Gao SS, Hiraishi N, Burrow MF, Duangthip D, Mei ML, et al. Mechanisms of silver diamine fluoride on arresting caries: a literature review. Int Dent J 2018;68:67–76. https://doi.org/10.1111/idj.12320.
[16] Fröhlich TT, Botton G, Rocha R, de O. Bonding of glass-ionomer cement and adhesives to silver diamine fluoride-treated dentin: an updated systematic review and meta-analysis. J Adhes Dent 2022;24:29–38. https://doi.org/10.3290/j.jad.b2701679.
[17] Zheng FM, Yan IG, Duangthip D, Gao SS, Lo ECM, Chu CH. Silver diamine fluoride therapy for dental care. Jpn Dent Sci Rev 2022;58:249–57. https://doi.org/10.1016/j.jdsr.2022.08.001.
[18] Seifo N, Robertson M, MacLean J, Blain K, Grosse S, Milne R, et al. The use of silver diamine fluoride (SDF) in dental practice. Br Dent J 2020;228:75–81. https://doi.org/10.1038/s41415-020-1203-9.
[19] Koizumi H, Hamama HH, Burrow MF. Effect of a silver diamine fluoride and potassium iodide-based desensitizing and cavity cleaning agent on bond strength to dentine. Int J Adhes Adhes 2016;68:54–61. https://doi.org/10.1016/j.ijadhadh.2016.02.008.
[20] Lutgen P, Chan D, Sadr A. Effects of silver diammine fluoride on bond strength of adhesives to sound dentin. Dent Mater J 2018;37:1003–9. https://doi.org/10.4012/dmj.2017-401.
[21] Danaeifar N, Nejat AH, Cehreli Z, Ballard RW, Johnson JT. The Effect of Silver Diamine Fluoride on Bond Strength of Three Types of Bulk-Fill Restorative Materials to Dentin. Pedia Dent 2022;44:290–5.
[22] Farahat F, Davari A, Karami H. Investigation of the effect of simultaneous use of silver diamine fluoride and potassium iodide on the shear bond strength of total etch and universal adhesive systems to dentin. Dent Res J 2022;19:6. https://doi.org/10.4103/1735-3327.336691.
[23] Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater 2018;34:78–96. https://doi.org/10.1016/j.dental.2017.11.005.
[24] Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, et al. Adhesive/Dentin interface: the weak link in the composite restoration. Ann Biomed Eng 2010;38:1989–2003. https://doi.org/10.1007/s10439-010-9969-6.
[25] Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer-A review. Dent Mater 2013;29:999–1011. https://doi.org/10.1016/j.dental.2013.07.016.
[26] Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, et al. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability–A literature review. Dent Mater 2016;32:e41–53. https://doi.org/10.1016/j.dental.2015.11.007.
[27] Mei ML, Ito L, Cao Y, Li QL, Lo ECM, Chu CH. Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation. J Dent 2013;41:809–17. https://doi.org/10.1016/j.jdent.2013.06.009.
[28] Mei ML, Li QL, Chu CH, Yiu CKY, Lo ECM. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. Dent Mater 2012;28:903–8. https://doi.org/10.1016/j.dental.2012.04.011.
[29] Mei ML, Ito L, Cao Y, Li QL, Chu CH, Lo ECM. The inhibitory effects of silver diamine fluorides on cysteine cathepsins. J Dent 2014;42:329–35. https://doi.org/10.1016/j.jdent.2013.11.018.
[30] Yan IG, Zheng FM, Yin IX, Sun IG, Lo ECM, Chu CH. Stability of silver and fluoride contents in silver diamine fluoride solutions. Int Dent J 2023;73:840–6. https://doi.org/10.1016/j.identj.2023.05.001.
[31] Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83:216–21. https://doi.org/10.1177/154405910408300306.
[32] Josic U, Maravic T, Bossù M, Cadenaro M, Comba A, Ierardo G, et al. Morphological Characterization of Deciduous Enamel and Dentin in Patients Affected by Osteogenesis Imperfecta. Appl Sci 2020;10:7835. https://doi.org/10.3390/app10217835.
[33] Mazzoni A, Nascimento FD, Carrilho M, Tersariol I, Papa V, Tjäderhane L, et al. MMP activity in the hybrid layer detected with in situ zymography. J Dent Res 2012;91:467–72. https://doi.org/10.1177/0022034512439210.
[34] Pires PM, Neves ADA, Makeeva IM, Schwendicke F, Faus-Matoses V, Yoshihara K, et al. Contemporary restorative ion-releasing materials: current status, interfacial properties and operative approaches. Br Dent J 2020;229:450–8. https://doi.org/10.1038/s41415-020-2169-3.
[35] Cascales ÁF, Moscardó AP, Toledano M, Banerjee A, Sauro S. An in-vitro investigation of the bond strength of experimental ion-releasing dental adhesives to caries-affected dentine after 1 year of water storage. J Dent 2022;119:104075. https://doi.org/10.1016/j.jdent.2022.104075.

[36] Peng JJ-Y, Botelho MG, Matinlinna JP. Silver compounds used in dentistry for caries management: a review. J Dent 2012;40:531–41. https://doi.org/10.1016/j.jdent.2012.03.009.
[37] Weyant RJ, Tracy SL, Anselmo T (Tracy), Beltrán-Aguilar ED, Donly KJ, Frese WA, et al. Topical fluoride for caries prevention. J Am Dent Assoc 2013;144:1279–91. https://doi.org/10.14219/jada.archive.2013.0057.
[38] Walsh T, Oliveira-Neto JM, Moore D. Chlorhexidine treatment for the prevention of dental caries in children and adolescents. Cochrane Database Syst Rev 2015. https://doi.org/10.1002/14651858.CD008457.pub2.
[39] Abuljadayel R, Aljadani N, Almutairi H, Turkistani A. Effect of antibacterial agents on dentin bond strength of bioactive restorative materials. Polymers 2023;15:2612. https://doi.org/10.3390/polym15122612.
[40] Quock RL, Barros JA, Yang SW, Patel SA. Effect of silver diamine fluoride on microtensile bond strength to dentin. Oper Dent 2012;37:610–6. https://doi.org/10.2341/11-344-L.
[41] Firouzmandi M, Mohaghegh M, Jafarpisheh M. Effect of silver diamine fluoride on the bond durability of normal and carious dentin. J Clin Exp Dent 2020;12:e468–73. https://doi.org/10.4317/jced.56303.
[42] Kucukyilmaz E, Savas S, Akcay M, Bolukbasi B. Effect of silver diamine fluoride and ammonium hexafluorosilicate applications with and without Er:YAG laser irradiation on the microtensile bond strength in sound and caries-affected dentin. Lasers Surg Med 2016;48:62–9. https://doi.org/10.1002/lsm.22439.
[43] Markham MD, Tsujimoto A, Barkmeier WW, Jurado CA, Fischer NG, Watanabe H, et al. Influence of 38% silver diamine fluoride application on bond stability to enamel and dentin using universal adhesives in self-etch mode. Eur J Oral Sci 2020;128:354–60. https://doi.org/10.1111/eos.12701.
[44] Ko AK, Matsui N, Nakamoto A, Ikeda M, Nikaido T, Burrow MF, et al. Effect of silver diammine fluoride application on dentin bonding performance. Dent Mater J 2020;39:407–14. https://doi.org/10.4012/dmj.2019-057.
[45] Selvaraj K, Sampath V, Sujatha V, Mahalaxmi S. Evaluation of microshear bond strength and nanoleakage of etch-and-rinse and self-etch adhesives to dentin pretreated with silver diamine fluoride/potassium iodide: An in vitro study. Indian J Dent Res 2016;27:421–5. https://doi.org/10.4103/0970-9290.191893.
[46] Van Duker M, Hayashi J, Chan DC, Tagami J, Sadr A. Effect of silver diamine fluoride and potassium iodide on bonding to demineralized dentin. Am J Dent 2019;32:143–6.
[47] De Munck J, Mine A, Poitevin A, Van Ende A, Cardoso MV, Van Landuyt KL, et al. Meta-analytical review of parameters involved in dentin bonding. J Dent Res 2012;91:351–7. https://doi.org/10.1177/0022034511431251.
[48] Cardoso MV, de Almeida Neves A, Mine A, Coutinho E, Van Landuyt K, De Munck J, et al. Current aspects on bonding effectiveness and stability in adhesive dentistry. Aust Dent J 2011;56(Suppl 1):31–44. https://doi.org/10.1111/j.1834-7819.2011.01294.x.
[49] Sayed M, Matsui N, Uo M, Nikaido T, Oikawa M, Burrow MF, et al. Morphological and elemental analysis of silver penetration into sound/demineralized dentin after SDF application. Dent Mater 2019;35:1718–27. https://doi.org/10.1016/j.dental.2019.08.111.
[50] Cadenaro M, Josic U, Maravić T, Mazzitelli C, Marchesi G, Mancuso E, et al. Progress in Dental Adhesive Materials. J Dent Res 2023;102:254–62. https://doi.org/10.1177/00220345221145673.
[51] Yan IG, Zheng FM, Gao SS, Duangthip D, Lo ECM, Chu CH. A review of the protocol of SDF therapy for arresting caries. Int Dent J 2022;72:579–88. https://doi.org/10.1016/j.identj.2022.06.006.
[52] Ballal NV, Gandhi P, Kashyap NN. Influence of particulate alkaline biomaterial remnants in dentin on the adhesion of two resin-based bonding systems. Microsc Res Tech 2021;84:1036–41. https://doi.org/10.1002/jemt.23664.
[53] Chu CH, Lo ECM. Microhardness of dentine in primary teeth after topical fluoride applications. J Dent 2008;36:387–91. https://doi.org/10.1016/j.jdent.2008.02.013.
[54] Willershausen I, Schulte D, Azaripour A, Weyer-Elberich V, Briseno B, Willershausen B. Penetration potential of a silver diamine fluoride solution on Dentin surfaces. An ex vivo study. Clin Lab 2015;61. https://doi.org/10.7754/Clin.Lab.2015.150401.
[55] Li Y, Liu Y, Psoter WJ, Nguyen OM, Bromage TG, Walters MA, et al. Assessment of the silver penetration and distribution in carious lesions of deciduous teeth treated with silver Diamine fluoride. Caries Res 2019;53:431–40. https://doi.org/10.1159/000496210.
[56] Carrilho MRO, Carvalho RM, de Goes MF, di Hipólito V, Geraldeli S, Tay FR, et al. Chlorhexidine preserves dentin bond in vitro. J Dent Res 2007;86:90–4. https://doi.org/10.1177/154405910708600115.
[57] Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116–35. https://doi.org/10.1016/j.dental.2012.08.004.
[58] Boushell LW, Kaku M, Mochida Y, Yamauchi M. Distribution and relative activity of matrix metalloproteinase-2 in human coronal dentin. Int J Oral Sci 2011;3:192–9. https://doi.org/10.4248/IJOS11070.
[59] Water-induced Degradation in Demineralized Dentin Matrix IADR Abstract Archives n.d. ⟨https://iadr.abstractarchives.com/abstract/19iags-3187194/water-induced-degradation-in-demineralized-dentin-matrix⟩ (accessed July 26, 2023).
[60] Mazzoni A, Tjäderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res 2015;94:241–51. https://doi.org/10.1177/0022034514562833.
[61] Josic U, Mazzitelli C, Maravic T, Comba A, Cadenaro M, Radovic I, et al. The effect of carbodiimide on push-out bond strength of fiber posts and endogenous enzymatic activity. BMC Oral Health 2023;23:399. https://doi.org/10.1186/s12903-023-03067-y.
[62] Singh S, Kundra R, George SD, Hanosh S, Neelakantan P, Thomas MS. Effect of Dentine conditioning with silver diamine fluoride on wettability of root canal sealers. Chin J Dent Res 2023;26:179–84. https://doi.org/10.3290/j.cjdr.b4330827.
[63] Carvalho RM, Manso AP, Geraldeli S, Tay FR, Pashley DH. Durability of bonds and clinical success of adhesive restorations. Dent Mater 2012;28:72–86. https://doi.org/10.1016/j.dental.2011.09.011.