Influence of dentin and enamel pretreatment with acidic sulfur compounds on adhesive performance

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Abstract: **OBJECTIVE:** This study tested the potential hampering effects of acidic sulfur compounds (ASC) containing hydroxybenzene sulfonic acid, hydroxymethoxybenzene sulfonic acid, and sulfuric acid, prior to self-etch and etch-and-rinse bonding procedures on enamel and dentin. According to the manufacturer, ASC should be applied after cavity preparation and prior to application of a primer in order to reduce the remaining biofilm in the preparation cavity. Despite promoted marketing, data on the investigated liquid are almost completely lacking. **MATERIAL AND METHODS:** One hundred and fifty-two extracted mandibular bovine incisors were embedded and polished to expose either enamel (E) or dentin (D). Then, specimens were randomly divided and conditioned as follows ($n = 12/\text{group}$): ASC and consecutive phosphoric acid application (E1/D1), ASC (E2/D2; E5/D5), phosphoric acid (E3/D3), and no conditioning (E4/D4; E6/D6). Groups were then treated with either Optibond FL[®] (etch-and-rinse; 1-4) or Clearfil SE Bond[®] (self-etch; 5-6). Hollow acrylic cylinders were bonded with a hybrid composite resin (Filtek Supreme XTE[®]) to the specimens, and the shear bond strength was measured (1 mm/min). In addition, failure types were assessed. Descriptive statistics and statistical analyses were performed with one-way ANOVA followed by the Scheffé post hoc test. **RESULTS:** For enamel, the highest shear bond strength values were obtained applying routine bonding procedures (23.5 ± 5.6 MPa for etch-and-rinse and 26.0 ± 6.0 MPa for self-etch, respectively). In contrast, dentin pretreatment with a combination of ASC and phosphoric acid led to the highest shear bond values (22.8 ± 4.1 MPa). **CONCLUSION:** This study shows that ASC prior to dental restoration placement cannot be recommended for etch-and-rinse procedures on enamel but is appropriate for dentin without interfering with routine bonding procedures. **CLINICAL RELEVANCE:** The application of acidic sulfur compounds prior to adhesive restoration placement should be restricted to dentin only as it may negatively influence shear bond strength on sound enamel.

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Influence of dentin and enamel pretreatment with acidic sulfur compounds on adhesive performance

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Abstract

Objective This study tested the potential hampering effects of acidic sulfur compounds (ASC) containing hydroxybenzene sulfonic acid, hydroxymethoxybenzene sulfonic acid, and sulfuric acid, prior to self-etch and etch-and-rinse bonding procedures on enamel and dentin. According to the manufacturer, ASC should be applied after cavity preparation and prior to application of a primer in order to reduce the remaining biofilm in the preparation cavity. Despite promoted marketing, data on the investigated liquid are almost completely lacking. **Material and methods** One hundred and fifty-two extracted mandibular bovine incisors were embedded and polished to expose either enamel (E) or dentin (D). Then, specimens were randomly divided and conditioned as follows ($n=12/\text{group}$): ASC and consecutive phosphoric acid application (E1/D1), ASC (E2/D2; E5/D5), phosphoric acid (E3/D3), and no conditioning (E4/D4; E6/D6). Groups were then treated with either Optibond FL® (etch-and-rinse; 1–4) or Clearfil SE Bond® (self-etch; 5–6). Hollow acrylic cylinders were bonded with a hybrid composite resin (Filtek Supreme XTE®) to the specimens, and the shear bond strength was measured (1 mm/min). In addition, failure types were assessed. Descriptive statistics and statistical analyses were performed with one-way ANOVA followed by the Scheffé post hoc test. **Results** For enamel, the highest shear bond strength values were obtained applying routine bonding procedures (23.5 ± 5.6 MPa for etch-and-rinse and 26.0 ± 6.0 MPa for self-etch,

respectively). In contrast, dentin pretreatment with a combination of ASC and phosphoric acid led to the highest shear bond values (22.8 ± 4.1 MPa).

Conclusion This study shows that ASC prior to dental restoration placement cannot be recommended for etch-and-rinse procedures on enamel but is appropriate for dentin without interfering with routine bonding procedures.

Clinical relevance The application of acidic sulfur compounds prior to adhesive restoration placement should be restricted to dentin only as it may negatively influence shear bond strength on sound enamel.

Keywords Shear bond strength · Acidic sulfur compounds · Composite resin · Bonding

Introduction

Secondary caries is regarded as the most common reason for the failure and, therefore, replacement of dental restorations [1, 2]. This may be caused by residual bacteria, which remain in the cavity after preparation [3, 4]. During caries excavation, clinicians must make their decision as to whether the remaining dentin is still infected or not based on criteria such as the color and texture of the dentin [5, 6]. Even in case of a quasi-complete removal of a dentinal lesion based on clinical judgment, bacteria can remain at the enamel–dentin junction, at the cavity walls, in the smear layer, or in the dentinal tubules on a histological level [4]. Data indicate that residual bacteria can find their way through the dentinal tubules and may reach the non-exposed pulp tissue [7]. The presence of persistent bacteria in dentin and their proximity to the pulp have been clearly associated with pulpal inflammation [8, 9]. To reach remaining bacteria and ensure complete bacterial removal, it seems evident that additional treatment of the cavity, supplementary to the physical removal of the carious dentin, could be advantageous before placing a restoration. An antimicrobial effect and

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a modification or dissolution of the smear layer are desired. The application of chlorhexidine or sodium hypochlorite to prepared dentin surfaces has been suggested for this purpose [10]; however, previous results have shown that these solutions are not capable of completely removing the smear layer present after cavity preparation [11, 12]. Furthermore, these solutions may be less than ideal in combination with adhesive techniques because of their hampering effect on bond strength [13–15]. To ensure bacterial elimination in dentinal tubules, the smear layer must be removed by a strong chelator or an acid [11]. Treating dentin and enamel with acid or acid-containing liquids is widely used for routine bonding procedures. The use of 32 to 37 % phosphoric acid has been suggested to successfully etch caries-affected dentin so as to remove the intratubular deposits and obtain high bond strength and well-infiltrated demineralized dentin with exposed collagen network [16–18].

A concentrated aqueous mixture of acidic sulfur compounds (ASC) containing hydroxybenzene sulfonic acid, hydroxymethoxybenzene sulfonic acid, and sulfuric acid (HybenX®, EPIEN Medical; St. Paul, USA) was recently introduced and marketed as a “Plaque Biofilm Remover” for several indications, including use prior to the placement of dental restorations. However, no studies on this topic are available yet. As this liquid contains acidic sulfur compounds, it can potentially be used as conditioner before restoration placement. Since studies have shown that a strong acid is required to adequately etch caries-affected dentin in order to produce high bond strengths and well-infiltrated demineralized dentin [16], the use of an ASC-containing liquid could potentially improve bonding quality or, at least, not interfere with the bonding procedures.

Given this assumption, this *in vitro* study aimed to test the influence of ASC on the shear bond strength of enamel and dentin when used in combination with etch-and-rinse and self-etch procedures. The null hypothesis was that application of this product would not interfere with adhesion and that the mean shear bond strength values would be similar with those of routine bonding procedures.

Methods and materials

Specimen preparation

One hundred and fifty-two extracted mandibular bovine central incisors were cleaned, stored in deionized water, and embedded with the labial surface facing downwards in a self-curing acrylic resin (ScandiQuick, Scandia, Hagen, Germany) in cylindrical molds with a diameter of 25 mm. After polymerization of the resin, teeth were ground flat under water-cooling with SiC paper of P240 grit, followed by P400 grit to expose either the enamel or middle dentin. As shown in Table 1, specimens were randomly allocated to

12 groups ($n=12/\text{group}$). Eight specimens were used for electron microscopy examination.

Bonding procedures

The conditioning and bonding materials used in this study are listed in Table 2. As allocated to the groups in Table 1, specimens were either not conditioned or treated with ASC and/or with phosphoric acid for 15 s each. Routine bonding procedures served as positive controls, with the groups E3/D3 for the etch-and-rinse techniques and the groups E6/D6 for the self-etch techniques, respectively. The groups E4/D4 without a conditioning procedure served as a negative control for the etch-and-rinse techniques. For the self-etch procedures, there was no negative control group. After conditioning, specimens were sprayed with water after each step if required and then gently air-dried. Afterwards, bonding was accomplished using step-by-step application of either a self-etching or an etch-and-rinse adhesive system according to the manufacturer's instructions (Table 2).

Shear bond strength testing

After the conditioning and adhesive procedures, a transparent, hollow acrylic cylinder with an inner diameter of 2.9 mm and an outer diameter of 3.1 mm was pressed onto the exposed tooth surfaces of the specimen by means of a special bonding device as recently described in detail by Schmidlin et al. [19]. The opening of the cylinder was filled with a hybrid composite resin (Filtek Supreme XTE®; for details, see Table 2) in two increments of about 1–2 mm in height, which was light-cured for 40 s (Elipar Freelight 2®, 3M ESPE®; 970 mW/cm²). Afterwards, specimens were carefully removed from the device. Thereafter, the specimens were stored in distilled water at 37 °C for 1 week. The shear bond strength of the specimens was investigated using a universal testing machine (Zwick® Z010, Ulm, Germany). The specimens were positioned in the sample holder with the treated specimen surface parallel to the loading piston at a distance of 200 µm. The loading piston had a chisel configuration, and the load was applied with a crosshead speed of 1 mm/min. Load at failure was recorded, and shear strength values were calculated according to the equation $\sigma = F/A$, where σ is the shear bond strength, F is the load at failure (in newton), and A represents the adhesive area (in square millimeter).

After debonding, the failure types were investigated with loupes at a $\times 3.3$ magnification (Sandy Grendel® TP-740; Aarburg, Switzerland). Failure types were classified as follows: (1) adhesive failure, when fractures were observed between the resin and the tooth; (2) cohesive failure in the composite, when fracture occurred within the resin composite; (3) cohesive failure in the tooth, when fracture was seen in the tooth substrate; and (4) mixed failure, when fractures were judged as representing adhesive and cohesive failure simultaneously.

Table 1 Treatment groups and treatment steps

Group			Enamel						Dentin					
			Etch-and-rinse				Self-etch		Etch-and-rinse				Self-etch	
			E1	E2	E3	E4	E5	E6	D1	D2	D3	D4	D5	D6
I	Conditioner	ASC	+	+	–	–	+	–	+	+	–	–	+	–
II		Phosphoric acid	+	–	+	–	–	–	+	–	+	–	–	–
III	Adhesive procedure	Optibond FL®	+	+	+	+	–	–	+	+	+	+	–	–
		Clearfil SE Bond®	–	–	–	–	+	+	–	–	–	–	+	+

Electron microscopy

To assess the surface morphology of the surfaces of the enamel and dentin specimens after the different conditioning modalities, eight specimens were prepared for scanning

electron microscopy (SEM) analysis. Enamel and dentin specimens were conditioned with ASC and/or phosphoric acid for 15 s each and sprayed with water for at least 15 s. When both ASC and phosphoric acid were used, the specimens were sprayed with water after each treatment step.

Table 2 Composition and application steps of the products used in this study

Type	Component, (manufacturer; lot)	Composition	Application protocol
Etch-and-rinse	HybenX® (EPIEN Medical; St. Paul, USA; P8)	Hydroxybenzene sulfonic acid, hydroxymethoxybenzene sulfonic acid, and sulfuric acid (pH<2)	1. Apply material to the prepared enamel/dentin surfaces for 15 s. 2. Spray the surface with water for at least 15 s. 3. Gently air-dry for approximately 5 s.
	Ultra-Etch® (Ultradent; UT, USA; A204)	35 % phosphoric acid	1. Apply material to the prepared enamel/dentin surfaces for 15 s. 2. Spray the surface with water for at least 15 s. 3. Gently air-dry for approximately 5 s.
	Optibond FL Prime® (Kerr; Bioggio, Switzerland; 3490336)	HEMA, GPDM, MMEP, ethanol, water, initiators	1. Apply Optibond FL Prime to the prepared enamel/dentin surfaces with a light scrubbing motion for 15 s. 2. Gently air-dry for approximately 5 s.
	Optibond FL Adhesive® (Kerr; Bioggio, Switzerland; 3486698)	bis-GMA, HEMA, GPDM, barium–aluminum, borsilicate glass, disodium hexa-fluoro-silicate, fumed silica	1. Using the same applicator brush, apply Optibond FL Adhesive with light brushing motion for 15 s to the prepared enamel/dentin surfaces. 2. Blow to margin or to thin if necessary using a light application of air. 3. Light cure for 20 s.
Self-etch	HybenX®	See above.	See above.
	Clearfil SE Bond Primer® (Kuraray; Tokyo, Japan; 00983A)	HEMA, hydrophilic dimethacrylate, 10-MDP, <i>N,N</i> -diethanol <i>p</i> -toluidine, CQ, water	1. Apply primer for 20 s. 2. Dry with mild airflow.
	Clerafil SE Bond® (Kuraray; Tokyo, Japan; 01460A)	Silanated silica, bis-GMA, HEMA, hydrophilic dimethacrylate, 10-MDP, toluidine, CQ	1. Apply bond. 2. Air flow gently. 3. Light cure for 10 s.
Composite resin	Filtek Supreme XTE® (3M ESPE; Seefeld, Germany; N192427)	Silica filler, zirconia filler, zirconia/silica cluster filler, bis-GMA, UDMA, TEGDMA, PEGDMA, bis-EMA, water	1. Fill the hybrid resin composite into the opening of the hollow cylinder (increment height, 1–2 mm). 2. Light cure for 40 s.

Unconditioned specimens served as the control. After the conditioning procedures, specimens were dried and mounted on aluminum stubs and sputter coated with gold, then examined using a scanning electron microscope (CS4, Cam Scan, Waterbeach, UK) operating at 1,000 kV with a working distance of 8.2–12.6 mm. SEM pictures were captured at a magnification of $\times 10,000$. As only one image of one specimen per conditioning modality was taken, the pictures have to be declared as a nonrepresentative visualization and simply give an idea of the different etching patterns.

Statistical methods

The shear bond strength data were coded and analyzed in PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA). The shear bond strength data were analyzed under the assumption of a normal distribution. Descriptive statistics such as the mean and standard deviation for each treatment group were computed separately. One-way ANOVA together with the Scheffé post hoc test was applied in order to investigate the differences in the shear bond strength between the treatment groups for enamel and dentin separately. In addition, fracture modes were investigated, and the relative frequencies of the fracture modes were computed with 95 % CI in each treatment group [20]. The results of the statistical analysis with p values <0.05 were considered to be statistically significant.

Results

As the primary outcome parameter of the study, the mean shear bond strength values of the enamel and dentin specimens are presented in Fig. 1. For enamel, the values ranged from 5.8 ± 3.9 to 23.5 ± 5.6 MPa for the etch-and-rinse procedures and from 22.4 ± 6.4 to 26.0 ± 6.0 MPa for the self-etch techniques. The highest shear bond strengths were obtained with routine bonding procedures (E3+E6), which served as the positive control. Group E4, which served as the negative control for etch-and-rinse techniques, showed the lowest values. The one-way ANOVA revealed a significant hampering effect of ASC on adhesion with the etch-and-rinse techniques (E1–E2) compared to the control group (E3). In the self-etch groups (E5–E6), the use of ASC prior to the adhesive procedure negatively influenced the shear bond strength values, although the differences were not significant.

On dentin specimens, the shear bond strength values varied from 11.6 ± 4.0 to 22.8 ± 4.1 MPa for the etch-and-rinse techniques and from 18.0 ± 4.6 to 22.5 ± 4.4 MPa for the self-etch procedures. The highest shear bond strength values were achieved by using ASC prior to routine procedures (D1+D5). If the highest obtained values are compared to their corresponding positive control group (E3+E6), the differences tested by ANOVA were not significant, either for

the etch-and-rinse or for self-etch procedures. Thus, the use of ASC before routine bonding procedures did not significantly reduce the shear bond strength values in dentin but tended to increase the respective values (D1+D5). The use of ASC as a sole dentin conditioner (D2) hampered the adhesive performance as compared to the routine etch-and-rinse conditioning procedure with phosphoric acid (D3) or the combination of ASC and phosphoric acid (D1).

The results of the fracture analysis are presented in Table 3. For enamel, in all groups where conditioning procedures were used (E1–E3 and E5–E6), mixed failures were predominantly demonstrated. The percentages ranged from 67 % (34; 91) in group E3 to 100 % (73; 100) in group E1. The percentages of adhesive failures varied from 0 % (0; 27) in group E1 to 67 % (34; 91) in group E4. Group E4 served as the negative control without conditioning procedures. Cohesive failures in enamel occurred only in group E5 (8 %, 0; 39), whereas no cohesive failures in composite were detected. In dentin, mixed failures occurred frequently, which ranged from 42 % (15; 73) in group D1 to 83 % (51; 98) in group D2. Overall, the highest percentage of cohesive failures in teeth could be observed in the dentin specimens, which were treated with ASC and phosphoric acid (D1). In dentin, no cohesive failures in composite were observed.

The SEM images of the enamel and dentin surfaces are shown in Fig. 2. The labeling of the images corresponds to the conditioning procedures of groups E1–E6 (enamel) and D1–D6 (dentin) as described in Table 1. The untreated specimen showed smear layer-coated surfaces with distinct polishing patterns on the dentin and enamel (corresponding to groups E4, E6 and D4, D6, respectively). On enamel, specimens revealed predominantly a type 2 etching pattern where the prism core material was preferentially left intact and the prism peripherals were removed (corresponding to groups E1, E2, E3, and E5) [21]. On dentin, etching with phosphoric acid alone (D3) or etching with ASC followed by etching with phosphoric acid (D1) led to dissolution of the smear layer and opening of the dentinal tubules. Preconditioning with ASC alone led to a partial dissolution of the smear layer and incomplete opening of the dentinal tubules (corresponding to groups D2 and D5).

Discussion

In the present study, different preconditioning patterns were evaluated in vitro to assess the effect of ASC as a conditioner when used in combination with routine etch-and-rinse and self-etch procedures. According to the manufacturer, ASC should be applied after cavity preparation and prior to the application of a primer [22]. They claim that, through its use, residual biofilm can be eliminated from the cavity and that the occurrence of bonding failures should be

Fig. 1 Mean shear bond strength values±standard deviation (megapascal) after the different conditioning and bonding procedures on enamel (E1–6) and dentin (D1–6). Different letters (A, B, C, D for

enamel specimens; a, b, c, d for dentin specimens) represent a significant post hoc test between the levels of the test group factor

reduced [22]. However, studies are not yet available to support either the first or the second claim. Despite promoted marketing, data on the investigated ASC-containing liquid are almost completely lacking. The only published study available is on the treatment of recurrent aphthous stomatitis [23]. The present investigation was the first test of the influence of this product on the shear bond strength of enamel and dentin when used in combination with an etch-and-rinse or a self-etch procedure, which would be a prerequisite for application in adhesive dentistry. We hypothesized that the application of the ASC-containing liquid

before conditioning would not influence the bonding procedure in terms of altered shear bond strength values.

One hypothesis of this study was that, in the case of sufficient shear bond strength values achieved by etching with ASC alone, the tested product could be used as a therapeutic etchant for etch-and-rinse adhesives. However, on enamel, data from the etch-and-rinse techniques indicate that ASC can neither replace phosphoric acid as an etchant nor improve adhesive performance when used as an adjunct for the tested adhesive procedure. In contrast, with regard to the self-etch system tested in this investigation, our null hypothesis could

Table 3 Relative frequencies of fracture modes with 95 % confidence intervals of different treatment modalities in enamel and dentin (percentage; 95 % confidence interval according to Ciba-Geigy [20] in parenthesis)

	Enamel					Dentin				
	Group	Adhesive failure	Cohesive failure in enamel	Cohesive failure in composite	Mixed failure	Group	Adhesive failure	Cohesive failure in dentin	Cohesive failure in composite	Mixed failure
Etch-and-rinse	E1	0 (0; 27)	0 (0; 27)	0 (0; 27)	100 (73; 100)	D1	8 (0; 39)	50 (21; 79)	0 (0; 27)	42 (15; 73)
	E2	17 (2; 48)	0 (0; 27)	0 (0; 27)	83 (51; 98)	D2	0 (0; 27)	17 (2; 48)	0 (0; 27)	83 (51; 98)
	E3	33 (9; 66)	0 (0; 27)	0 (0; 27)	67 (34; 91)	D3	8 (0; 39)	25 (5; 58)	0 (0; 27)	67 (34; 91)
	E4	67 (34; 91)	0 (0; 27)	0 (0; 27)	33 (9; 66)	D4	25 (5; 58)	0 (0; 27)	0 (0; 27)	75 (42; 95)
Self-etch	E5	17 (2; 48)	8 (0; 39)	0 (0; 27)	75 (42; 95)	D5	0 (0; 27)	33 (9; 66)	0 (0; 27)	67 (34; 91)
	E6	17 (2; 48)	0 (0; 27)	0 (0; 27)	83 (51; 98)	D6	0 (0; 27)	25 (5; 58)	0 (0; 27)	75 (42; 95)

Fig. 2 SEM images after different conditioning modalities. The labeling of the images corresponds to the conditioning procedures of the groups E1–E6 (enamel, *left*) and D1–D6 (dentin, *right*) as described in Table 1. *E1* and *D1* a combination of phosphoric acid and ASC; *E2*, *E5* and *D2*, *D5* ASC alone; *E3* and *D3* phosphoric acid; *E4*, *E6* and *D4*, *D6* untreated samples. Please note that these images are not representative, since only the image of one specimen per experimental group is shown

not be violated. Previously recorded data showed that pre-etching with phosphoric acid increases the bond strength for self-etch techniques and can be considered a safe and clinically reliable approach [24]. However pre-etching with ASC did not significantly affect the bond strength measurements of the self-etch groups in our investigation.

In contrast to the enamel groups, our hypothesis was not rejected for dentin groups. Even better than expected, the highest shear bond strength values were achieved when ASC was used before routine bonding procedures with the etch-and-rinse adhesive. An explanation for the higher shear bond results could be based on the greater etching depth, which may be clarified by observing the SEM images. Whereas the use of ASC as a single etchant led to only partial dissolution of

the smear layer, a more accentuated dissolution pattern could be observed when a combination of ASC and phosphoric acid was used. These results are in agreement with other studies that showed the highest shear bond strength values with a total-etch technique [25, 26]. When applying self-etching adhesives, it was shown that additional or extended etching on sound dentin did not improve the tensile bond strength [27]. In addition, previous studies have indicated that there is no correlation between bond strength and the thickness of the bonding infiltrated smear layer [28, 29]. Other studies have shown, however, a negative effect on bond strength values when dentin was pretreated with phosphoric acid [30, 31]. In their review, Scherrer et al. described an average shear bond strength of Optibond FL® to dentin of 23.1 ± 7.9 MPa

including eight publications [32]. Fifteen publications assessed Clearfil SE Bond® and a mean shear bond strength value of 23.2 ± 7.1 MPa was reported [32]. These pooled values were slightly higher as compared to the findings of the present study with mean values of 20.6 ± 8.8 and 18.0 ± 4.6 MPa, respectively. These differences may be explained, in part, by different methodological and analytical approaches, e.g., specimen preparation, shear bond strength measurements, etc. In their review, Scherrer et al. included only studies which assessed the shear bond strength on human dentin, whereas bovine dentine in our investigation was used. Moreover, different media than those used in our treatment protocol, such as formalin, thymol, chloramine, sodium azide, or saline solutions, were used to store the extracted human teeth [32].

By analyzing the fracture types, the data show predominantly mixed failures in enamel. Only group E5, when ASC was applied before the application of the self-etch adhesive, showed a few pure cohesive failures in enamel. Comparing the shear bond strength values and the fracture type analysis of the dentin groups, the amount of cohesive failures in dentin correlated with the shear bond strength results, which is in accordance with a review by Salz et al., who described a significant correlation between higher bond strength and the rate of cohesive failures [33]. In addition, they mentioned an increased incidence of cohesive failure in dentin during shear bond tests in recent years [33]. They assumed that this fact is an intrinsic side effect of shear bond testing and does not mean a high adhesive performance, which can withstand the shear bond strength [33]. Thus, the observed cohesive failures do not necessarily imply an improved adhesion.

In the present study, bovine teeth were used. Due to their size and disposability, they represent an ideal substrate for shear bond strength testing. Studies have shown no statistically significant morphological differences between coronal dentin of human permanent molars and bovine central incisors when comparing the number of tubules per square millimeter or their diameters [34]. There is some doubt whether conclusions made with studies on bovine teeth can be directly applied to human teeth and whether such data are valid in a clinical situation [35]. Another potentially influencing factor, which may hamper the overall clinical validity of this investigation, was the fact that no pulpal pressure was applied. Further, it must be considered that the specimens used for this study were neither caries infected nor caries affected. This fact is important because tubule occlusion due to caries-affected dentin is referred to as the major factor for decreased bond strength in carious dentin as it hampers adequate tag formation [36]. Other authors have also reported lower bond strength with the self-etch and etch-and-rinse procedures to caries-affected dentin in comparison to sound dentin, even for groups with additional or extended acid etching [27]. Other investigations have revealed good penetration of bonding agents in sound, artificially and naturally induced carious dentin with

different adhesive systems [37, 38]. Another limiting factor was that no thermomechanical aging was performed in this study. Furthermore, an experimental group where ASC was applied after the self-etch adhesive was not included into this investigation. In our opinion, the use of ASC after self-etch adhesive offers no advantage and is not a clinical concept. However, future studies may look at this aspect as well. Overall, adhesion testing is a highly technique-sensitive procedure [33]. Even minor variations in any of the numerous experimental steps can significantly influence the results [33]. Thus, for these reasons, it is important to acknowledge that the conditions of the present investigation differ from in vivo situations and that a general comparability with other studies is difficult. Nevertheless, as this was a comparative investigation, the findings of this study allow at least the detection of treatment differences when using one substrate under standardized conditions.

Conclusions

Within the limitations of this in vitro screening investigation, it can be concluded that the application of ASC prior to dental restoration placement cannot be recommended for etch-and-rinse procedures in enamel. In contrast, ASC may be beneficial on dentin as a potential antibacterial adjunctive for routine bonding procedures, so the use of ASC can be recommended. However, we presume that the selective application of ASC on dentin could be challenging, especially in small cavities. In addition, the potential antimicrobial effect of ASC as a potential biofilm remover is yet to be investigated in further studies.

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Conflict of interest The authors declare no conflicts of interest.

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