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Beware of contamination! Do hemostatic agents influence the microtensile bond strength of a universal adhesive to dentin?

Introduction: The aim of this study was to evaluate whether hemostatic agents containing aluminum chloride affect the microtensile bond strength of a universal adhesive to dentin.

Materials and Methods: The occlusal enamel of 50 human molars was removed to expose a flat dentin surface. A universal adhesive was applied either in self-etch (SE) or etch & rinse mode (ER), or a contamination with a "hemostatic agent" (Astringent; ARP or Racegel; RAC) was performed prior to the adhesive application. In the control group (control; C), no contamination in SE and ER mode was performed. A composite build-up was placed onto the adhesively pre-treated surface and specimens were cut to obtain 80 microsticks per group. Half of the specimens were stored for 24 hours before the microtensile bond strength test was performed, the other half was tested after thermocycling (TC, 5,000 cycles). The fractured surfaces were evaluated using a light-optical microscope in order to analyze the failure pattern.

Results: In the SE-mode, dentin contamination with both hemostatic agents prior to universal adhesive application led to a significant decrease in bond strength, compared to the controls before (SE_ARP: 5.67 ± 7.64 MPa; SE_RAC: 5.08 ± 6.04 MPa vs. SE_C: 24.91 ± 12.06 MPa) and after TC (SE_ARP_TC: 2.38 ± 4.43 MPa; SE_RAC_TC: 4.01 ± 4.42 MPa vs. SE_C_TC 24.27 ± 10.67 MPa). Moreover, the SE-mode with prior contamination showed significantly lower bond strength values to dentin before (SE_ARP 5.67 ± 7.64 MPa vs. ER_ARP 20.90 ± 10.91 MPa and SE_RAC 5.08 ± 6.04 MPa vs. ER_RAC 25.62 ± 9.41 MPa) and after TC (SE_ARP_TC 2.38 ± 4.43 MPa vs. ER_ARP_TC 20.91 ± 11.21 MPa and SE_RAC_TC 4.01 ± 4.42 MPa vs. ER_RAC_TC 18.94 ± 9.54 MPa) compared to the ER-mode. In the ER-mode, only contamination with ARP led to a significantly lower bond strength compared to uncontaminated dentin before TC. The fracture analysis showed significant more adhesive fractures in the SE-mode than in the ER-mode.

Conclusion: Considering the limitations of this in-vitro study, the universal adhesive showed higher dentin bond strength when used in ER-mode after contamination with an aluminum chloride-based hemostatic agent.

Keywords: universal adhesive; hemostatic agent; microtensile bond strength; dentin

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1. Introduction

Basically, three different techniques can be used for adhesive bonding to enamel and dentin: etch & rinse, selective enamel etch, and self-etch. Universal adhesives can be used with simultaneous enamel and dentin etching, with selective enamel etching, or without prior phosphoric acid etching. Enamel etching with phosphoric acid is still considered the gold standard, and it is recommended when using universal adhesives [22]. Bonding to dentin usually requires three steps: etching of the dentin surface, application of an amphiphilic primer, and finally coating with a hydrophobic adhesive [24]. Universal adhesives combine all three steps in a one-step procedure. Like conventional adhesives, they require proper isolation to provide a sufficient bond [5]. The gingiva may bleed during treatment, especially in deep subgingival areas, and it is imperative that the bleeding has to be stopped before adhesive application [26]. In contrast to supra- and slightly subgingival defects, a rubber dam cannot be used for contamination control in all deep subgingival cases. However, gingival bleeding can be stopped using chemical (hemostatic agents) and mechanical (retraction cords or rings) methods separately or in combination [27]. The chemicals used for bleeding control and gingival retraction are divided into two main groups according to their pharmacology: group 1 comprises adrenergic (vasoconstrictive) substances, group 2 comprises astringent substances based on chloride or sulfate [19]. Most commonly, ferrous sulfate in concentrations of up to 20 % and aluminum chloride in concentrations of up to 25 % are used to stop gingival bleeding [14, 27]. Both belong to group 2. Ferrous sulfate very quickly forms a metal-protein complex upon contact with blood, mechanically occluding the vessels, whereas aluminum chloride has an astringent effect [14].

When using hemostatic agents in the gingival area, contamination of the tooth surface is usually inevitable. This is particularly true for very deep class-V and class-II cavities extending to root dentin, or during "proximal box elevation technique". An overview of the most common cavity depths shows that 15 % of all proximal cavities in posterior teeth are deeper than 8 mm [9]. Besides, the fact that successful tooth preservation in elderly patients is associated with an increase in root caries poses new challenges to clinicians in terms of adhesively bonded restorations [10, 21]. The use of hemostatic agents can be helpful in these situations, but a risk of interaction with adhesion, especially when using self-etch adhesives, cannot be ruled out [3]. Since the use of universal adhesives is increasing in clinical practice due to their easy handling properties, hemostatic agents may also affect the bond strength of these products. The objective of this study was therefore to examine the bond strength of a universal adhesive to human dentin contaminated with hemostatic agents containing aluminum chloride.

The following null hypotheses were set:

1. The contamination of the dentin surface with hemostatic agents containing aluminum chloride does not influence the bond strength of a universal adhesive used in the self-etch mode.
2. The contamination of the dentin surface with hemostatic agents containing aluminum chloride does not influence the bond strength of a universal adhesive used in the etch & rinse mode.

2. Materials and methods

For this in-vitro study, 50 caries- and restoration-free permanent human molars were collected and cleaned from debris. The teeth were stored in chloramine-T solution (0.5 %) at 8°C until preparation for no longer than 6 months. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. The use of extracted human teeth for bond strength testing was approved by the responsible ethical committee of the Hannover Medical School (no. 2092–2013). The teeth were randomly divided into 6 groups (Tab. 1) with at least 8 teeth per group. The teeth were kept moist throughout the entire experimental procedure. In two groups, the number of teeth had to be enlarged to 9 teeth because of the size of the teeth (leading to less than 80 sticks without adjustment). The teeth were embedded in gypsum parallel to the tooth axis, then, the occlusal enamel was cut at a right angle to expose a flat dentin surface (IsoMet Low Speed Saw, Buehler, Esslingen am Neckar, Germany). In order to create a clinically relevant smear layer, all dentin surfaces were roughened using grinding paper (SiC Grinding Paper 600 Grit P, Buehler, Esslingen am Neckar, Germany), rinsed with water and carefully dried using a SPRAYVIT syringe (KaVo Dental GmbH, Biberach, Germany). Then, either the adhesive (Scotchbond Universal, 3M Oral Care, 3M Deutschland GmbH, Seefeld, Germany) was applied in self-etch or etch & rinse mode (control groups), or contamination with a hemostatic agent was performed prior to adhesive application (experimental groups, Tab. 1 and 2). Subsequently, a composite build-up (3M Z100 MP, 3M Oral Care, 3M Deutschland GmbH, Seefeld, Germany) was placed onto the adhesively pre-treated surface (6 mm in height, 4 layers, each 1.5 mm in thickness). Each layer was polymerized for 20 seconds with a light-emitting diode (LED) unit (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) at 1100 mW/cm^2 from the top surface using a standardized protocol. Before each light curing cycle, the power output of the LED unit was checked with a testing device (Bluephase Meter, Ivoclar Vivadent, Schaan, Liechtenstein). The specimens for the microtensile bond strength test were cut with a high speed saw to obtain microsticks with a bonded area of approx. 1.66 mm^2 (IsoMet High Speed Pro, Buehler, Esslingen am Neckar, Germany). Before testing, all sticks were measured carefully with a digital gauge. Half of the sticks (n = 40) were stored for 24 hours in distilled water at 37°C before the microtensile bond strength (μTBS) test was performed. The other half (n = 40) was tested after thermocycling (5,000 cycles, dwell time 30 sec, changeover time 10 sec, 5°/55°C). Afterwards, the specimens

Group	Number of specimens (sticks) for µTBS-testing in total	Number of specimens included into the statistical analyses	Number of zero bonds	Sticks excluded from statistical analyses	Surface pre-treatment and adhesive application
SE_C	40	36	–	4	Application of Scotchbond Universal in self-etch mode
SE _ARP	40	18	22	–	Application of Astringent Retraction Paste prior to application of Scotchbond Universal in self-etch mode.
SE_RAC	40	21	19	–	Application of Racegel prior to application of Scotchbond Universal in self-etch mode.
ER_C	40	39	–	1	Application of Scotchbond Universal in etch & rinse mode
ER_ARP	40	39	–	1	Application of Astringent Retraction Paste prior to application of Scotchbond Universal in etch & rinse mode
ER_RAC	40	39	1	–	Application Racegel prior to application of Scotchbond Universal in etch & rinse mode.
SE_C_TC	40	40	–	–	Application of Scotchbond Universal in self-etch mode + TC
SE_ARP_TC	40	13	27	–	Application of Astringent Retraction Paste prior to application of Scotchbond Universal in self-etch mode + TC
SE_RAC_TC	40	23	17	–	Application of Racegel prior to application of Scotchbond Universal in self-etch mode + TC.
ER_C_TC	40	39	–	1	Application of Scotchbond Universal in etch & rinse mode + TC
ER _ARP_TC	40	37	–	3	Application of Astringent Retraction Paste prior to application of Scotchbond Universal in etch & rinse mode + TC.
ER_RAC _TC	40	35	3	2	Application of Racegel prior to application of Scotchbond Universal in etch & rinse mode + TC.

Table 1 Experimental and control groups, number of specimens (n) for µTBS-testing in total, specimens included into the statistical analyses, zero bonds, specimens excluded from statistical analyses, surface pre-treatment and adhesive application, SE: self-etch mode, ER: etch & rinse mode C: Control, ARP: Astringent Retraction Paste, RAC: Racegel, TC: thermocycling,

were mounted onto a special jig and loaded using a universal testing machine (MTD-500+, SD Mechatronik GmbH; Feldkirchen-Westerham, Germany) with a 500 N load cell travelling at a crosshead speed of 0.5 mm/min. Force values were measured in N. The bond strength in MPa was calculated by dividing the force in N by the surface of the respective microstick. Sticks which fractured during the cutting process or during thermocycling were included as zero bonds in the statistical analysis. Sticks which fractured far from the interface or during manipulation were excluded from the analyses. All fractured surfaces were evaluated using a

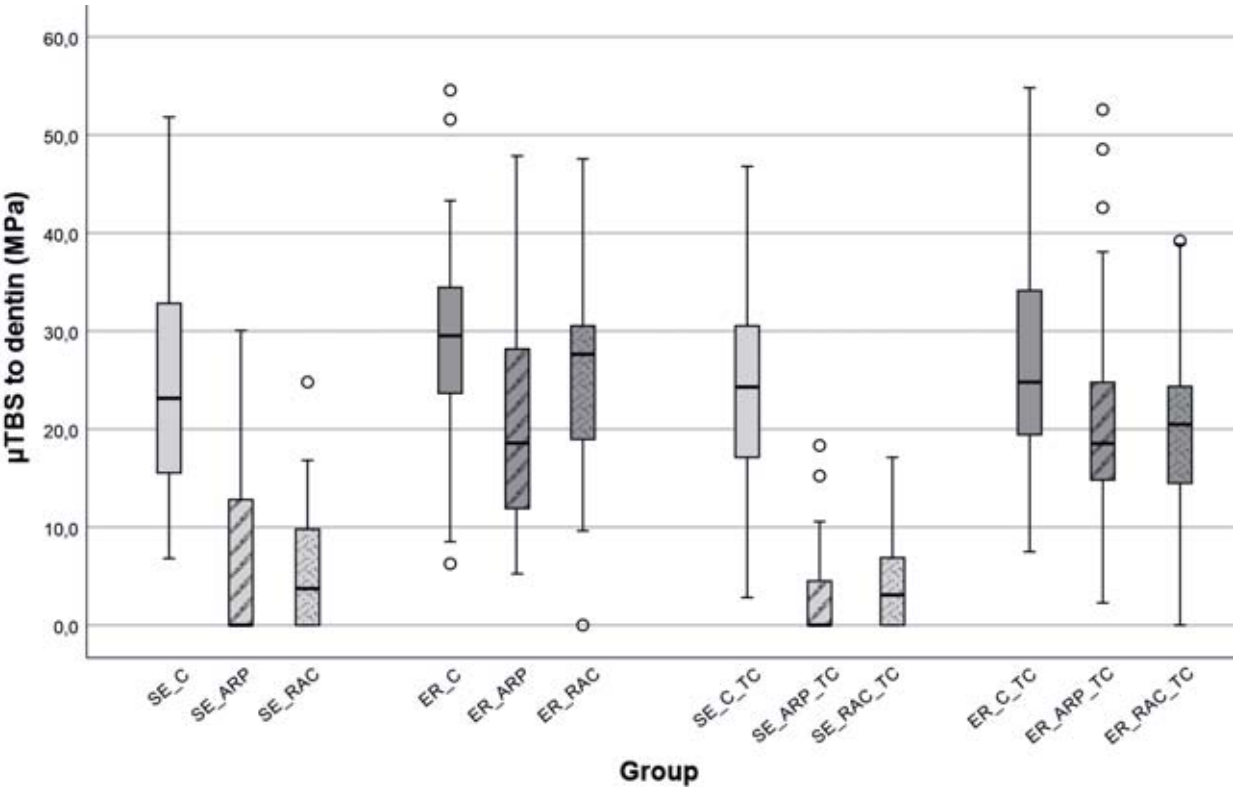

Figure 1 Mean microtensile bond strength in MPa with and without TC using the self-etch or the etch & rinse mode. The horizontal line within the box indicates the median values for each group. The circles represent outliers, SE: self-etch mode, ER: etch & rinse mode C: Control, ARP: Astringent Retraction Paste, RAC: Racegel, TC: thermocycling.

light-optical microscope (Stemi SV 6, Zeiss, Jena, Germany) in order to analyze the failure pattern (50x magnification). Three different types of failure were defined:

- adhesive fracture at the resin/dentin interface,
- cohesive fracture (fracture in dentin or composite),
- mixed fracture (combination of adhesive and cohesive fracture).

2.1. Statistical analysis

For the statistical analysis, the mean values and the standard deviations were calculated. The normal distribution of the data was tested using the Shapiro-Wilk-test. The level of significance was set at $p < 0.05$. Mean values of the different groups were compared by one-way ANOVA. In order to identify significant differences between each group, the Tukey-HSD was used. The results from the fracture analysis were statistically analyzed using cross tabs and the Chi-square test (SPSS 23.0 IBM Deutschland GmbH, Ehningen, Germany).

3. Results

3.1. Microtensile Bond Strength Test

The analysis of variance showed significant differences between the experimental groups ($p < 0.001$). The bond strength of the control groups, i.e. bonding to uncontaminated dentin in self-etch and etch & rinse mode, was not significantly different (SE_C: 24.91 ± 12.06 MPa vs. ER_C: 29.6 ± 10.27 MPa; $p = 0.554$). Thermocycling (TC) did not significantly influence the results when compared to the control before TC, either (SE_C_TC: 24.27 ± 10.67 MPa; $p = 1.000$; and ER_C_TC 27.27 ± 11.28 MPa; $p = 0.955$, Tab. 3, Fig. 1). In the self-etch mode, dentin contamination with both hemostatic agents prior to universal adhesive application led to a significant decrease in bond strengths, compared to the controls (SE_ARP: 5.67 ± 7.64 MPa; $p < 0.001$; and SE_RAC: 5.08 ± 6.04 MPa; $p < 0.001$). The same applies to the results obtained after thermocycling (SE_ARP_TC: 2.38 ± 4.43 MPa; $p < 0.001$; and SE_RAC_TC: 4.01 ± 4.42 MPa; $p < 0.001$, Tab. 3, Fig. 1). In the etch & rinse mode, only contamination with Astringent Retraction Paste (ARP) led to a significantly lower bond strength compared to uncontaminated dentin (ER_ARP: 20.90 ± 10.91 MPa vs. ER_C: 29.60 ± 10.27 MPa; $p = 0.002$). After thermocycling, however, experimental and control groups were no longer significantly differ-ent (ER_ARP_TC: 20.91 ± 11.21 MPa vs. ER_C_TC: 27.27 ± 11.28 MPa; $p = 0.115$). Dentin contamination with Racegel initially did not have any signifi cant influence on bond strength (ER_RAC: 25.62 ± 9.41 MPa vs. ER_C: 29.60 ± 10.27 MPa; $p = 0.752$). After TC, however, there was a significant difference between the groups (ER_RAC_TC: 18.94 ± 9.54 MPa vs. ER_C_TC: 27.27 ± 11.28 MPa; $p = 0.005$, Tab. 3, Fig. 1). A direct comparison of the two etching modes (self-etch vs. etch & rinse) after contamination with Astringent or Race-

Material	Batch-no	Composition	Manufacturer	Manufacturer´s instructions
DeTrey Conditioner 36	1803001142	Phosphoric acid, highly dispersed silicon dioxide, detergent, pigment, water	Dentsply DeTrey GmbH, Konstanz, Germany	Etching of dentin for 15 sec
Scotchbond Universal (SBU)	80912B	10-MDP, HEMA, Dimethacrylate, Vitrebond Copolymer, filler, ethanol, water, initiators	3M, 3M Oral Care, 3M Deutschland GmbH, Seefeld, Germany	**Self-Etch approach:** application of SBU and rubbing for 20 sec, air-thinning 5 sec, light curing for 10 sec **Etch & Rinse approach:** Etching of dentin for 15 sec (35 % phosphoric acid), rinsing with water (15 sec) and immediately air-drying. Application and curing: see self-etch approach.
Astringent Retraction Paste (ARP)	4382643	Aluminum chloride hexahydrate, mica-group minerals, water, kaolin, poly(dimethylsiloxane)	3M, 3M Oral Care, 3M Deutschland GmbH, Seefeld, Germany	Application of Astringent Retraction Paste on dentin surface, contact time to dentin 2 minutes, complete removal of the paste with air-water spray and suction.
Racegel (RAC)	B22819AE	Aluminum chloride hexahydrate, ethanol	Septodont GmbH, Niederkassel, Germany	Application of Racegel on dentin surface, contact time to dentin 2 minutes, complete removal of the paste with air-water spray and suction
Z100 MP Restorative – shade A3	N971244, N997767	Silane treated ceramic, TEGDMA, BISGMA, 2-benzotriazolyl-4-methyphenol	3M, 3M Oral Care, 3M Deutschland GmbH, Seefeld, Germany	Z100 restorative is intended to be cured by exposure to a halogen or LED light with a minimum intensity of 400 mW/cm2 in the 400–500 nm range. Cure each increment by exposing its entire surface to a high intensity visible light source. Hold the light guide tip as close to the restorative as possible during light exposure. Shade A3: Thickness 2.5 mm à 40 sec

Table 2 Material, batch-number, composition, manufacturer, manufacturer's instruction

gel showed significantly higher bond strengths after phosphoric acid etching, compared to self-etching (SE_ARP: 5.67 ± 7.64 MPa vs. ER_ARP: 20.90 ± 10.91 MPa; $p < 0.001$; and SE_RAC: 5.08 ± 6.04 MPa vs. ER_RAC: 25.62 ± 9.41 MPa; $p < 0.001$). The same applies to the results obtained after thermocycling (SE_ARP_TC: 2.38 ± 4.43 MPa vs. ER_ARP_TC 20.91 ± 11.21 MPa; $p < 0.001$; and SE_RAC_TC: 4.01 ± 4.42 MPa vs. ER_RAC_TC 18.94 ± 9.54 MPa; $p < 0.001$, Tab. 3, Fig. 1).

3.2. Fracture analysis

When the universal adhesive was applied in the self-etch mode without prior contamination, 77.78 % of the initial fractures were adhesive, 5.56 % cohesive, and 16.67 % mixed. After TC, there was an increase in mixed (42.5 %) and cohesive fractures (22.5 %) and a decrease in adhesive fractures (35 %) ($p = 0.001$). In the etch & rinse mode, no significant differences between the fracture types were observed, neither before TC (ER_C; adhesive 40 %, cohesive 20 %, mixed 40 %) nor after TC (ER_C_TC; adhesive 32.5 %, cohesive 25 %, mixed 42.5 %) ($p < 0.755$) (Fig. 2).

In the self-etch mode, dentin contamination with Astringent led to 100 % adhesive fractures, both before and after TC. Dentin contamination with Racegel showed similar results: before TC, 94.87 % of the fractures were adhesive and after TC, all samples (100 %) fractured adhesively 100 % ($p < 0.241$) (Fig. 2). In the etch & rinse mode, dentin contamination with Astringent showed 74.36 % adhesive, 2.56 % cohesive and 23.08 % mixed fractures before TC. After TC, adhesive fractures decreased to 56.76 %; 8.11 % of the fractures were cohesive, and 35.16 % mixed ($p < 0.228$). In the case of Racegel, there were initially 42.50 % adhesive, 2.50 % cohesive and 55 % mixed fractures. After TC, mixed fractures increased to 68.42 %, and adhesive fractures decreased to 26.32 % ($p < 0.296$) (Fig. 2).

	SE	ER	SE_TC	ER_TC
C	24.91 ± 12.06^{aA}	29.60 ± 10.27^{aA}	24.27 ± 10.67^{aA}	27.27 ± 11.28^{aA}
ARP	5.67 ± 7.64^{bA}	20.90 ± 10.91 ^{bB}	2.38 ± 4.43^{bA}	20.91 ± 11.21^{abB}
RAC	5.08 ± 6.04^{bA}	25.62 ± 9.41^{abB}	4.01 ± 4.42^{bA}	18.94 ± 9.54^{bB}

Table 3 Mean microtensile bond strength in MPa of the individual groups, second row: mean values of the control groups (Scotchbond Universal control; C) without prior contamination in self-etch and etch & rinse mode before and after TC, third and fourth row: mean values of the experimental groups (ARP and RAC) before and after TC. Values with different lowercase letters in vertical direction are statistically different. Values with different uppercase letters in horizontal direction are statistically different, SE: self-etch mode, ER: etch & rinse mode C: Control, ARP: Astringent Retraction Paste, RAC: Racegel, TC: thermocycling.
(Fig. 1 and 2, Tab. 1–3: S. Jacker-Guhr and A.-K. Lührs)

4. Discussion

Astringents are, by definition, substances which precipitate proteins, but without penetrating cells, so that only the superficial layer of the mucosa is involved [17]. These substances can be used in various ways and are suitable for superficial, local bleeding control in sufficiently high concentrations [17]. Aluminum chloride and ferrous sulfate are often used to arrest bleeding prior to taking impressions or restoring subgingival cavities with direct composites, thanks to their beneficial properties and minimal tissue irritation. Previous studies already showed that hemostatic agents have a certain influence on the bond strength of adhesively bonded restorations; also, marginal gap formation increased in cervical areas [2, 16, 20, 25]. A two-step self-etch adhesive system provided lower bond strengths after the use of a hemostatic agent containing ferrous sulfate, as compared to the uncontaminated control group [5]. In contrast, a hemostatic containing aluminum chloride did not influence dentin bond strengths [25]. These results are inconsistent with the results of our study, which showed significantly lower bond strengths to dentin contaminated with an aluminum-based agent versus uncontaminated dentin in the self-etch mode. The same applies to the results obtained after thermocycling (see Tab. 3 and Fig. 1). In a previous study, Land et al. showed that hemostatic agents containing 15.5 % ferrous sulfate completely remove the smear layer from prepared dentin and, after prolonged application (5 min), cause demineralization of the dentin surface with partial loss of peritubular dentin [13]. The investigators attribute this effect to the low pH-value (0.8–0.9) of the hemostatic agent applied to the dentin surface [13]. Kuphasuk et al. showed similar effects for a hemostatic agents containing 25 % aluminum chloride (pH = 0.8), which partially removed the smear layer without completely exposing the dentinal tubules after an application time of 30 sec [12]. The hemostatics used in our in-vitro study had mild pH-values (Racegel pH = 2.3–3.5 and Astringent pH = 3.2–4.0; Safety Data Sheet, Racegel, Septodont; Safety Data Sheet, Astringent Retraction Paste, 3M Oral Care, 3M Deutschland GmbH). As a consequence, these agents would only incompletely, if at all, remove the smear layer from the dentin surface. Kuphasuk et al. also showed that the use of a hemostatic containing aluminum chloride resulted in lower bond strength of a two-step self-etch adhesive system, as compared to the uncontaminated control group; however, this effect was not present for an etch & rinse system [12]. Our study confirms these findings. Kuphasuk et al. demonstrated that doubling the application time of the self-etch primer from 20 to 40 sec led to a significant increase in bond strength to contaminated dentin [12]. The investigators attribute this to an enhanced etching effect, which not only completely removes the smear layer, but also removes peritubular dentin and completely exposes dentinal tubules [12]. In scanning electron microscopic (SEM) imaging, this effect appeared to be similar to phosphoric acid etching [12]. We applied a universal adhesive with a relatively mild pH-value (Scotchbond Universal: pH = 2.7; Safety Data Sheet, Scotchbond Universal, 3M Oral Care, 3M Deutschland GmbH) for 20 sec in the self-etch mode; this may explain the low bond strengths obtained after contamination, in addition to the milder pH-values of the hemostatic agents used. Another explanation may be that a component of the hemostatic agents, namely aluminum, can replace the calcium contained in hydroxyapatite, which leads to the formation of insoluble calcium compounds [12, 15]. The weak acids of self-etch primers do not seem to be capable of dissolving these compounds, and as a result bond strengths are lower [12, 15]. Scotchbond Universal, the adhesive used in our study, has a pH-value of 2.7 and thus can be classified as an "ultra-mild" system (Safety Data Sheet, Scotchbond Universal, 3M Oral Care, 3M Deutschland GmbH). The above-mentioned interaction of the hemostatic with the dentin surface may considerably reduce monomer infiltration in the self-etch mode, as indicated by significantly lower bond strength of this group versus the control group (see Tab. 3 and Fig. 1). Hemostatic components remaining on the dentin surface may block dentinal tubules, preventing sufficient adhesive infiltration, and therefore negatively influencing the formation of a proper hybrid layer [1, 3, 20]. An

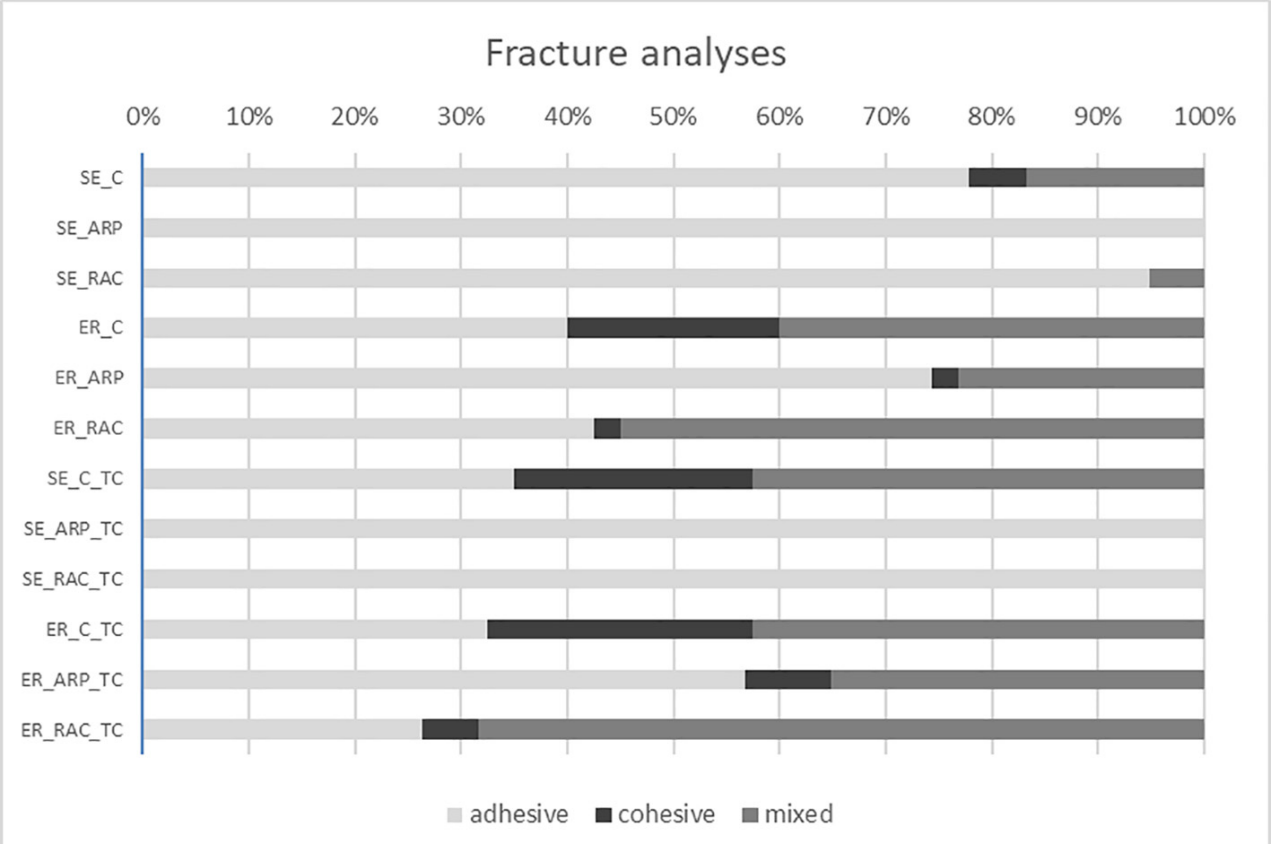

Figure 2 Bar graph depicting the percentages of adhesive, cohesive and mixed fractures, SE: self-etch mode, ER: etch & rinse mode C: Control, ARP: Astringent Retraction Paste, RAC: Racegel, TC: thermocycling,

SEM examination showed that an amorphous surface layer was formed after contamination of the dentin surface with a hemostatic fluid for 1 to 2 min. Two of the hemostatic agents tested contained aluminum chloride, like the two products we examined, and the application time was also identical with the times we used. Therefore, the hemostatic agents used in our study may have caused a change in the dentin surface similar to the effect described by Ayo-Yusuf et al. [2]. Analysis by energy-dispersive x-ray spectroscopy (EDS) and SEM showed that phosphoric acid etching for 20 sec removed almost all calcium and phosphate from the dentin surface, due to the demineralizing effect of the acid. After hemostatic contamination, however, phosphoric acid etching did not result in the same degree of demineralization, because the use of hemostatic agents had led to the formation of granular deposits, which cannot be completely removed by etching [2]. In our study, one group showed similar initial bond strengths as the control after hemostatic contamination and phosphoric acid etching (ER_RAC), whereas the other group showed values that were significantly lower (ER_ARP: –29 %, as compared to the control). An explanation may be that the two hemostatic agents interacted with dentin to different extents, causing that the etching effect differed as well. This was also shown in an EDS analyses by Ayo-Yusuf et al., in which the demineralizing effect of phosphoric acid differed significantly, depending on the hemostatic used [2]. However, thanks to its lower pH-value (< 2), phosphoric acid seems to more effectively remove residues or reaction products of hemostatic agents and dentin from the tubules and the surface during demineralization, as compared to adhesives used in the self-etch mode. Phosphoric acid allows adhesives to infiltrate the dentinal tubules and form a proper hybrid layer [3]. In addition to phosphoric acid, EDTA seems to be highly effective in removing residues from dentin surfaces after aluminum chloride contamination [1]. Using EDTA, an increase in bond strengths to the level of an uncontaminated control group without any pretreatment was achieved; for phosphoric acid, this effect was weaker [1].

The effects described by Ayo-Yusuf et al. und Kuphasuk et al. are also relevant when using universal adhesives in the self-etch mode: the acidic monomers of universal adhesives etch dentin surfaces covered with the above-mentioned amorphous layer to a lesser extent [2, 12]. Besides, as a result of the replacement of calcium by aluminum, less calcium is available for the formation of chemical bonds [12]. The hydrophobic functional monomer 10-MDP has a high potential to interact with the tooth structure by forming a stable salt with the calcium contained in hydroxyapatite [4]. So the lower bond strength of the universal adhesive in the self-etch mode in our

study may also be attributed to the fact that calcium reacts with and is bound by components of the astringents used, and therefore the dentin surface contains less reactive calcium ions [2, 12]. Studies conducted by O'Keefe KL et al. and Ajami et al. also indicate a negative influence of dentin contamination with ferrous sulfate or aluminum chloride on the bond strength of adhesives used in the self-etch mode, as compared to uncontaminated dentin [1, 20].

A comparison of the two application modes (self-etch vs. etch & rinse) without dentin contamination shows high bond strength in the self-etch mode, not significantly different from the one obtained in the etch & rinse mode. This may be attributed to the presence of 10-MDP, which reacts with the calcium contained in hydroxyapatite, establishing a chemical bond to dentin [23].

Although hemostatic agents may negatively influence bond strength, it is advisable for clinicians to use them to control gingival bleeding. Blood contamination affects the bond strength of self-etch adhesive systems to a varying extent, depending on the time at which it occurs and also on the "decontamination measures" taken, e.g. reapplication of a self-etch primer with or without prior rinsing [5]. Due to this diversity of outcomes, there is no standardized "decontamination protocol", which would reliably lead to an increase in bond strength to the level of the control group [5]. In addition to a decrease in bond strength to dentin, there is also an increase in adhesive fractures [3, 5]. In the present study, no blood contamination of the dentin surface was conducted. In the clinical situation, especially in deep cavities, a mixture of hemostatic agents and blood can be present on the dentin surface. Groddek et al. investigated the marginal adaptation of composite restorations in enamel and dentin using different adhesive systems (etch & rinse and self-etch) after blood contamination of the cavity and treatment with various hemostatic agents [8]. No significant effect on marginal adaptation after blood contamination and application of a hemostatic agent was present [8]. The etch & rinse procedure showed no adverse effect on margins located in dentin [8]. The results of our fracture analysis may also, in addition to the low bond strength in our microtensile test, be indicative of impaired interaction of the universal adhesive with dentin when used in the self-etch mode: Microscopic analysis showed high percentages of adhesive fractures (94.9–100 %, see Fig. 1).

Thermocycling significantly influenced the bond strength only for the etch & rinse RAC groups. ISO Standard 11450 specifies that 500 thermocycles between 5 °C and 55 °C will be sufficient for artificial aging (International Standards Organization, 1994). However, dwell times and numbers of cycles vary greatly between different in-vitro studies [7]. The literature does not provide any standardized thermocycling protocol, so we used the most common method, i.e. 5,000 thermocycles between 5 °C and 55 °C [6, 18]. It has to be proven whether a higher number of cycles has any influence on the bond strength, but a certain tendency is clearly recognizable [11].

5. Conclusion

Contamination of dentin surfaces with hemostatic agents containing aluminum chloride should be avoided as far as possible when using a universal adhesive. If dentin is contaminated with an aluminum-based hemostatic during treatment, it seems preferable to use a universal adhesive in the etch & rinse mode. Phosphoric acid etching after contamination will then have a cleaning effect, leading to sufficient bonding. In order to prevent contamination of the cavity with either saliva or blood, the use of rubber dam is still the best clinical approach because hemostatic agents might not be required.

Conflicts of Interest

The authors declare that there is no conflict of interest within the meaning of the guidelines of the International Committee of Medical Journal Editors.

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