



# An in vitro evaluation of microleakage of resin based composites bonded to chlorhexidine-pretreated dentin by different protocols of a universal adhesive system

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Received 8 August 2020; revised 20 September 2020; accepted 21 September 2020  
Available online 1 October 2020

## KEYWORDS

Universal bond adhesive;  
Microleakage;  
2% chlorhexidine;  
Bulk-fill;  
Conventional composite

**Abstract** *Purpose:* This study compared microleakage of different resin based composite (RBC) materials bonded to dentin, after chlorhexidine (CHX) application, by different adhesion protocols of a universal adhesive system.

*Methods:* Class V cavities were prepared on the buccal and lingual surfaces of 40 premolar teeth. The “etch-and-rinse” technique of a universal bond adhesive system (Single Bond Universal Adhesive) was used on buccal preparations, while the “self-etch” protocol was used on the lingual surfaces. Two RBCs, one bulk fill (Filtek Bulk Fill [FBF]) and one conventional (Filtek Z350 XT [Z350XT]), were used. Teeth were divided into two groups of 20 teeth each, 10 per each RBC (n = 10): (1) control; and (2) pretreatment with 2% CHX. For FBF groups, teeth were restored with a single increment; however, for Z350XT, a layering technique was used. Teeth were aged by thermo-cycling and prepared for microleakage testing. Dye penetration was evaluated and scored from 0 to 4. Data were analyzed at a significance level of  $P < 0.05$ .

*Results:* The highest microleakage mean scores were found in the control group of the etched margins for both RBCs ( $2.80 \pm 1.033$  FBF and  $2.10 \pm 1.370$  Z350XT). The CHX-pretreated group

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Peer review under responsibility of King Saud University.



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showed significantly lower microleakage than the control for FBF only ( $P = 0.008$ ). No significant difference was found between groups for the “self-etch” protocol ( $\chi^2 = 0.884$ ,  $P = 0.08$ ). No significant differences were found between FBF and Z350XT in all study groups ( $P > 0.2$ ).

**Conclusions:** When the “self-etch” protocol of the universal adhesive system was used, dentin microleakage was not affected by CHX-pretreatment when teeth were restored with bulk fill or conventional RBCs. In the “etch-and-rinse” protocol, CHX application improved the marginal seal before restoration with bulk fill material. However, in the absence of CHX, the “etch-and-rinse” protocol negatively affected marginal integrity.

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## 1. Introduction

During cavity preparations for Class V restorations, gingival margins may end up with little or no enamel, which becomes a challenge to achieve an adequate bond for tooth-colored restorations (Litonjua et al., 2003; Sensi et al., 2004). An important factor in the maintenance of clinically successful bonds between restorations and tooth structures is the prevention of bacterial passage between the cavity margins and the restorative material (Owens et al., 2018). Moreover, the bacteria remaining after cavity preparation, if not adequately cleansed, can survive, especially in the presence of microleakage, and lead to caries recurrence (Mutluay and Mutluay, 2019).

In addition, the location of these restorations makes them difficult for the patient to reach and keep clean. All these factors may aid in the production of a weak bond between the tooth and the restoration, therefore contributing to the formation of secondary caries and, hence, failure of the restoration (Ferracane, 2017; Jokstad, 2016).

For complete elimination of cariogenic bacteria before restoration of a tooth, efforts have been made by researchers to evaluate laboratory-proven potent antibacterial disinfectants. The most commonly used oral antimicrobial agent is chlorhexidine gluconate (CHX) (Bin-Shuwaish, 2016; Dionysopoulos, 2016), which is considered “the gold standard” of oral antiseptics (Matthijs and Adriaens, 2002). CHX is a biguanide biocide that inhibits the formation and progression of dental plaque (Puig Silla et al., 2008). Cavity pretreatment with CHX before dental restoration has been reported to increase the clinical success of restorative dental procedures (de Castilho et al., 2013; Rosenberg et al., 2013), because CHX was documented as having high antibacterial activity against Gram-positive bacteria, such as *Streptococcus mutans*, (Machado et al., 2011).

Lately, the introduction of bulk fill resin based composites (RBCs) was a significantly substantial improvement in the manufacture of RBC materials. The most important factor attracting the attention of clinicians and researchers to these materials is the number of increments required to restore a cavity preparation, since these materials can be built in 4- to 5-mm increments each (Bahbishi et al., 2020). The stress-decreasing resin technology in these materials was created to minimize shrinkage stress. Clinically, this would eliminate the need for increments, therefore reducing the clinical time needed for material placement (Garcia et al., 2014).

Universal adhesives are newly developed adhesive systems that simplify the adhesion procedure by combining all steps

into one. These systems can be used in multiple adhesion modes or protocols, including the “etch-and-rinse” mode, the “self-etch” mode, or the “selective-etch” mode. (Yamauchi et al., 2019).

Limited data are available on the effects of CHX cavity pretreatment on microleakage when universal adhesive systems were used to bond bulk fill RBCs to dentin margins. Therefore, the purpose of this study was to evaluate the effects of 2% CHX cavity disinfectant on the microleakage of different RBCs bonded to dentin by different adhesion protocols of the universal adhesive system.

When different RBCs were bonded to dentin by different adhesion protocols, the null hypotheses were that: (1) there would be no significant difference in dentin microleakage between groups of teeth; pretreated and not pretreated with 2% CHX; (2) there would be no significant difference in microleakage of 2% CHX-pretreated dentin between bulk fill and conventional composites; and (3) there would be no significant difference in microleakage of 2% CHX-pretreated dentin between “etch-and-rinse” and “self-etch” protocols of the universal bond adhesive system.

## 2. Materials and methods

Forty sound extracted premolar teeth were collected from oral and maxillofacial surgery clinics. Teeth with caries lesions, previous restorations, endodontic treatment, or fractures were excluded. Approval for this project was obtained from the ethics committee of the institutional review board (IRB) (Application No. E-18-3312), and the project was conducted at College of Dentistry, KSU, Riyadh, KSA. Teeth were cleaned and stored in distilled water at 37 °C until being tested. Class V cavities (2 mm deep, 4 mm wide, and 3 mm high) were prepared on the facial and lingual surfaces of each tooth, by means of 330 carbide burs (Columbia, Brasseler, Savannah, GA, USA) mounted in a high-speed handpiece with copious water-coolant spray. The cervical margins on each surface were in dentin, about 1 mm apical to the cement-enamel junction (CEJ).

Prepared teeth were randomly divided into two groups of 20 teeth each. In each group, teeth were further subdivided into the following groups of 10 teeth for each RBC material ( $n = 10$ ):

Group 1 (Control): Teeth were rinsed with distilled water and restored with the RBCs.

Group 2 (2% CHX): Teeth were pretreated with CHX (2% chlorhexidine, Consepsis®, Ultradent, South Jordan, UT, USA) before being bonded with the RBCs.

Two RBCs were used in this study; a bulk fill (Filtek™ Bulk Fill [FBF], 3M ESPE, St. Paul, MN, USA), and a conventional material (Filtek™ Z350 XT [Z35XT], 3M ESPE). A universal bonding adhesive system (3M™ Single Bond Universal, 3M ESPE) was used in two modes, “etch-and-rinse” and “self-etch”.

On each tooth, and before restorations with the assigned RBC materials, the buccal cavity preparation was treated with the “etch-and-rinse” mode of the universal bond system, while the lingual preparation was restored with the “self-etch” mode of the same system.

Acid etchant was applied to the facial margins with 35% phosphoric acid (Ultra-Etch®, Ultradent). Fig. 1 shows a flow chart for the groups and subgroups distribution.

In the CHX-pretreated group, CHX was applied after teeth were acid-etched and before bonding. The materials used in this study are presented in Table 1.

For the FBF groups, the cavity preparation was restored with a single layer of the material; however, for Z350XT, a layering technique of 1- or 2-mm thickness was used to build the restoration. Adhesive bonds and RBCs were light-cured, according to the manufacturer’s instructions, with a Light Emitting Diode Laser (LED) curing unit of 1200 mW/cm<sup>2</sup> radiant exposure (Bluephase® G2, Ivoclar Vivadent, Schaan, Liechtenstein). Restorations were finished with composite diamond finishing burs, then with Sof-Lex discs (Sof-Lex®, 3M ESPE).

Teeth were then stored in distilled water at 37 °C for 24 h, after which thermo-cycling aging was conducted for 1500 cycles (5 °C/55 °C) with a dwell time of 30 s and a transfer time of 10 s. Tooth surfaces were then covered with two layers of acid-resistant varnish to about 1 mm away from the cervical preparation margins on both surfaces.

Teeth were immersed in 2% methylene blue solution at room temperature for 24 h, then rinsed, gently air-dried, and embedded in a cold-curing orthodontic acrylic resin (Ortho-

plast, Vertex, Soesterberg, Netherlands) in preparation for being sectioned. Bucco-lingual longitudinal sections, one in the center and two close to the mesial and distal margins of the restoration, were created with a precision saw (Isomet 2000, Buehler, Lake Bluff, IL, USA) under water cooling. Dye penetration in each cavity and for both margins was examined by two evaluators, pre-calibrated at 85% reliability (Deliperi et al., 2006), using a digital stereomicroscope (HiRoX, Tokyo, Japan) at 50x magnification. In case of disagreement, a consensus measurement was forced (Wagner et al., 2008). The evaluators were blinded to scoring according to the following system (Owens and Johnson, 2005):

- 0 = No dye penetration.
- 1 = Penetration < 1/2 of the cavity wall depth.
- 2 = Penetration up to 1/2 of the cavity wall depth.
- 3 = Penetration > 1/2 of the cavity wall depth, but not including the pulpal floor.
- 4 = Penetration including the pulpal floor.

Data were collected and statistically analyzed by means of SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA), with the Kruskal-Wallis one way analysis of variance (ANOVA) test followed by paired-group comparisons by a Mann-Whitney *U* test at a significance level of  $P < 0.05$ .

### 3. Results

The microleakage scores for the different study groups are presented in Table 2. Fig. 2 shows microscopic images of different samples with different scores.

#### 3.1. Effects of CHX

Samples pretreated with CHX in both RBC materials showed less microleakage when the “etch-and-rinse” mode was used. Kruskal-Wallis one-way ANOVA indicated a significant difference between groups ( $\chi^2 = 9.496$ ,  $P = 0.02$ ) (Table 2). The Mann-Whitney test found significant differences between

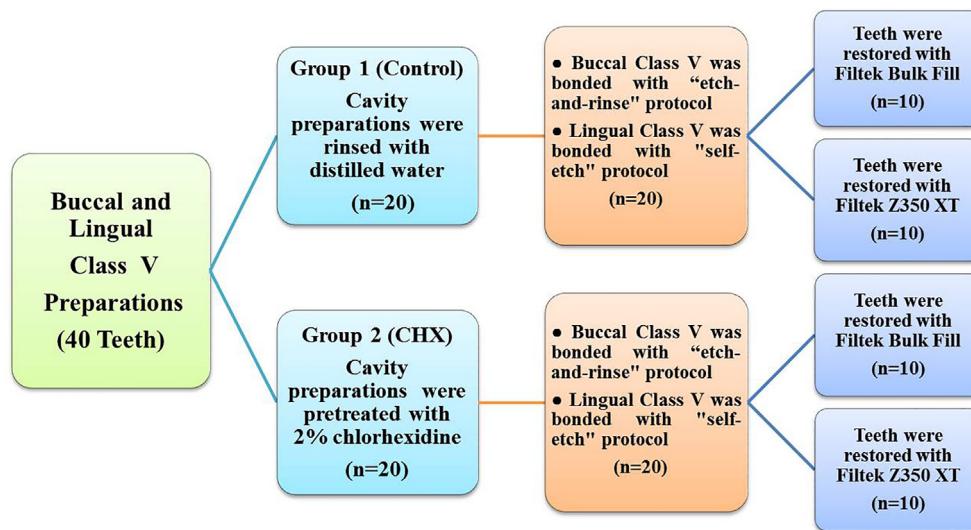


Fig. 1 Flow chart for the study groups.

**Table 1** List of the materials used in the study.

Material	Company	Composition
Filtek™ Z350 XT Universal Composite Resin Restorative Material Shade A2	– 3M ESPE, St. Paul, MN, USA	– Bis-GMA, UDMA, TEGDMA, PEGDMA and bis-EMA (6) resins. – Fillers: non-agglomerated/non-aggregated 20-nm silica filler, non-agglomerated/non-aggregated 4- to 11-nm zirconia filler, and aggregated zirconia/silica cluster filler (comprised of 20-nm silica and 4- to 11-nm zirconia particles). – 78.5% by weight (63.3% by volume).
Filtek™ Bulk Fill Posterior Composite Resin Restorative Material Shade A2	– 3M ESPE, St. Paul, MN, USA	– AUDMA, UDMA and 1, 12-dodecane-DMA. – Fillers: combination of a non-agglomerated/non-aggregated 20-nm silica filler, a non-agglomerated/non-aggregated 4- to 11-nm zirconia filler, an aggregated zirconia/silica cluster filler (comprised of 20-nm silica and 4- to 11-nm zirconia particles), and a ytterbium trifluoride filler consisting of agglomerate 100-nm particles. – 76.5% by weight (58.4% by volume).
3M™ Single Bond Universal Adhesive Bonding System	– 3M ESPE, St. Paul, MN, USA	– MDP phosphate monomer, HEMA, ethanol, Vitrebond copolymer, filler, water, initiators, dimethacrylate resins, and silane.
Ultra-Etch® Phosphoric Acid	– Ultradent, South Jordan, UT, USA	– 35% phosphoric acid in water, thickening agent, and colorants
Consepsis® Antibacterial Solution	– Ultradent, South Jordan, UT, USA	– 2.0% chlorhexidine gluconate solution

the FBF-control and FBF-CHX groups ( $P = 0.008$ ) and between the FBF-control and Z350XT-CHX groups ( $P = 0.013$ ). However, no significant differences were found between other pairs of groups ( $P > 0.16$ ).

In the “self-etch” mode groups, Kruskal-Wallis test indicated no significant differences between groups ( $\chi^2 = 0.884$ ,  $P = 0.083$ ), and therefore, no significant differences were found between control groups and CHX-pretreated groups for both RBC materials ( $P > 0.5$ ).

### 3.2. Adhesion protocol comparisons

When adhesion protocols were compared (Fig. 3), the “self-etch” mode showed mean scores with less microleakage than “etch-and-rinse” mode in all groups. Differences were significant for the control groups only ( $P = 0.001$ , FBF;  $P = 0.045$ , Z350XT) (Table 3). However, in CHX-pretreated groups, the differences were not significant for both RBC materials ( $P = 0.38$ ).

### 3.3. RBC comparisons

Z350XT material showed less marginal leakage than FBF in the control group of the “etch-and-rinse” protocol; however, this difference was not statistically significant ( $P = 0.26$ ). In contrast, in the CHX-pretreated groups of materials, FBF showed mean scores with less microleakage than Z350XT, which was also not significant ( $P = 0.73$ ).

For the “self-etch” protocol, both materials performed almost the same, with no significant differences between them regardless of disinfectant pretreatment ( $P > 0.70$ ).

## 4. Discussion

Microleakage at the dentin margin is considered a common problem in RBC restorations. One reason is that dentin con-

tains a high percentage of organic components and fluids, which make bonding more difficult than that to enamel, resulting in marginal microleakage (Mantri and Mantri, 2013).

Bacterial contamination at these margins can aggravate this problem, leading to a weaker dentin bond and eventual failure of the restoration (Ferracane, 2017). Therefore, 2% CHX has been used, in the present study, to disinfect cavity preparation walls, which has been documented to be a potent and biocompatible disinfectant (Athanasiadis et al., 2007).

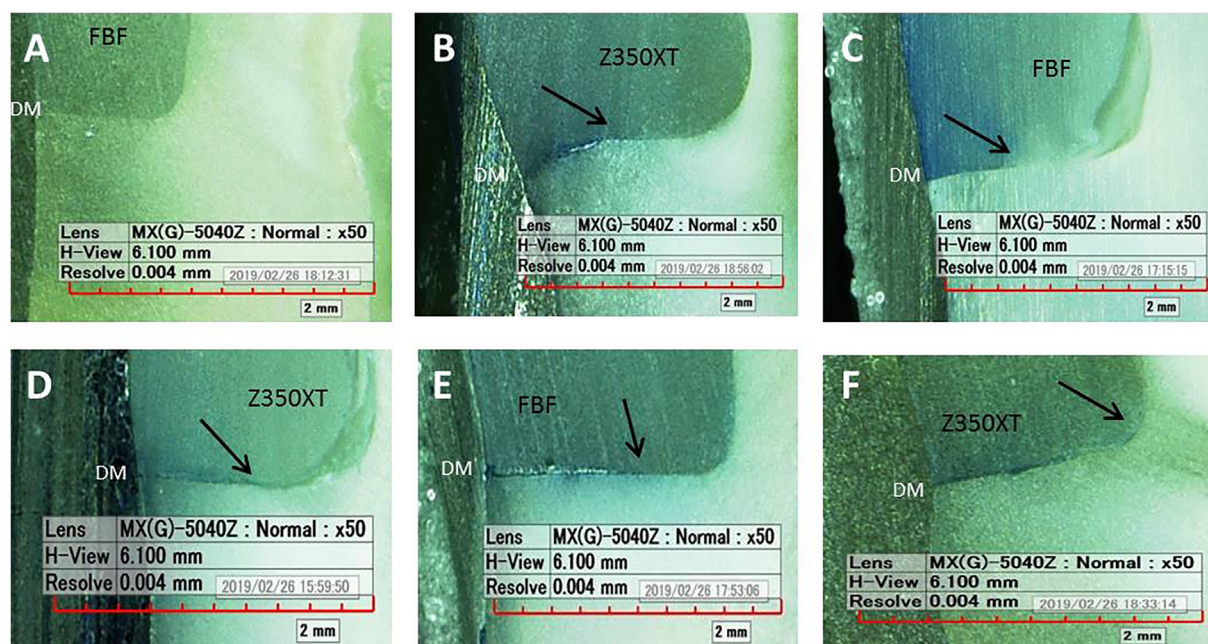
Universal adhesive systems possess self-etching capabilities with properties that are hydrophobic upon polymerization and hydrophilic during application. The results of the present study showed that the “self-etch” properties of the universal adhesive system were sufficient to condition the dentin surface and create an adequate seal. The “self-etch” mode has a pH value of 2.7, which is considered a mild acid, capable of enhancing dentin proteolytic activity without denaturation of the enzymes (Muñoz et al., 2013; Gunaydin et al., 2016). Furthermore, the single-bond universal adhesive system contains 10 methacryloxydecylphosphate (MDP), which is responsible for hydrogen-bonding between calcium ions of the dentin hydroxyapatite and the functional monomer, creating a strong micromechanical interlocking bond at the adhesive-dentin interface (Fukegawa et al., 2006; Loguercio et al., 2018).

In the current study, an extra step of dentin-etching yielded increased dentinal microleakage in the control group. Therefore, the null hypothesis, that there would be no significant differences in dentin microleakage between etched and non-etched margins, was rejected for the control group. This was in agreement with results from previous studies that reported better sealing ability at dentin margins when “self-etch” rather than “etch-and-rinse” adhesives were used (Pontes et al., 2002; Pushpa et al., 2009). This could be attributed to the increased etching depth that could not be completely infiltrated with resin, therefore resulting in a weak collagen network which subsequently increased microleakage.

**Table 2** Microleakage scores, mean scores, and Kruskal-Wallis one-way ANOVA analysis results for the study groups.

Adhesion protocol		C-FBF				C-Z350XT				CHX-FBF				CHX-Z350XT				Kruskal Wallis <i>H</i>	<i>df</i>	<i>P</i> value				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0				1	2	3	4
Etch-and-Rinse	Score	0	1	3	3	3	1	3	2	2	2	3	3	3	1	0	1	6	1	2	0	9.496	3	0.02*
	Mean ( $\pm$ SD)	2.80 ( $\pm$ 1.033) <sup>a</sup>				2.10 ( $\pm$ 1.370) <sup>a,b</sup>				1.20 ( $\pm$ 1.032) <sup>b</sup>				1.40 ( $\pm$ 0.966) <sup>b</sup>										
Self-etch	Score	2	7	1	0	0	1	9	0	0	0	5	3	1	1	0	2	7	0	1	0	0.884	3	0.83
	Mean ( $\pm$ SD)	0.90 ( $\pm$ 0.568)				0.90 ( $\pm$ 0.316)				0.80 ( $\pm$ 1.033)				1.00 ( $\pm$ 0.816)										

\* Indicates significant different ( $P < 0.05$ ). Different lower-case superscript letters indicate significant differences between groups within the same row. Abbreviations: C = Control, FBF = Filtek Bulk fill, Z350XT = Filtek Z350 XT, SD = Standard deviation.



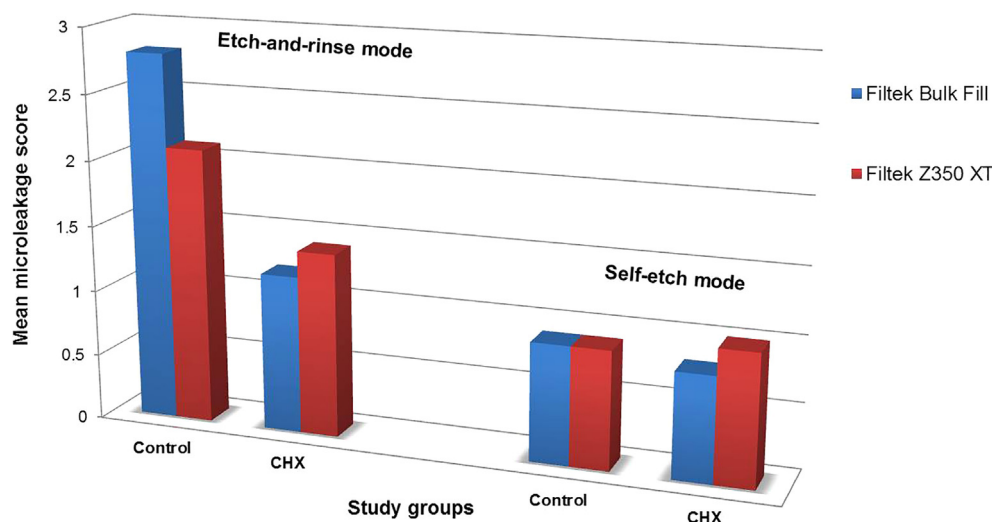
**Fig. 2** Samples restored with Filtek bulk fill [FBF] and Filtek Z350 XT [Z350XT], showing different dye penetration scores on the dentin margins [DM], with the microleakage extension indicated by arrow along the gingival wall of buccal surfaces [BS] bonded with the “etch-and-rinse” protocol of the universal bond system, and lingual surfaces [LS] bonded with the “self-etch” protocol: (A) LS of CHX-pretreated sample restored with FBF, scored 0; (B) LS of CHX-pretreated sample restored with Z350XT, scored 1; (C) LS surface of control sample restored with FBF, scored 1; (D) BS of CHX-pretreated sample restored with Z350XT, scored 2; (E) BS of control sample restored with FBF, scored 3; and (F) BS of control sample restored with Z350XT, scored 4.

FBF was reported to contain monomer components, in addition to the UDMA, that aid in decreasing the polymerization shrinkage compared to the conventional Z350XT with BisGMA (Gajewski, 2012). The results of this study showed no difference in microleakage between the conventional and bulk fill RBCs, regardless of the adhesion protocol. Therefore, the null hypothesis, that there would be no significant difference in microleakage between RBCs, cannot be rejected. These results were in agreement with those of Campos et al. (2014) and Benetti et al. (2015).

In the present study, 2% CHX, was applied after the acid-etching procedure in the “etch-and-rinse” protocol and before adhesive bonding application, as was recommended by the manufacturer and researchers (Fukegawa et al., 2006; Breschi, 2013). Pashley et al. found that the application of CHX to etched dentin prevented the degradation of collagen

fibrils, therefore stabilizing and preserving the hybrid layer (Pashley et al., 2004).

The application of CHX in the “etch-and-rinse” mode group resulted in significant improvement in the marginal seal, when restored with bulk fill RBC. Similar results were obtained with Z350XT, although the improvement was not significant. Therefore, the null hypothesis, that there would be no significant difference in dentin microleakage between groups of teeth pretreated and not pretreated with 2% CHX, was rejected for teeth restored with bulk fill RBC in the “etch-and-rinse” mode. These results were in agreement with those of Francisconi-dos-Rios et al. (2015), who found significant differences in the strength of dentin adhesion interfaces when 2% CHX was applied after the acid-etching procedure. However, Meiers and Shook (1996) found the effects of cavity disinfectants on RBCs to be material-specific. The improve-



**Fig. 3** Bar graph for mean microleakage scores of both adhesion protocols.

**Table 3** Comparison between mean microleakage scores of different adhesion protocols within each composite material in the study groups.

Group	Composite material	Adhesion protocols		Mann-Whitney <i>U</i>	<i>P</i> value
		Mean microleakage score ( $\pm$ SD)			
		Etch-and-rinse	Self-etch		
Control	FBF	2.80 ( $\pm$ 1.033)	0.90 ( $\pm$ 0.568)	6	0.001*
	Z350	2.10 ( $\pm$ 1.370)	0.90 ( $\pm$ 0.316)	23	0.046*
CHX	FBF	1.20 ( $\pm$ 1.032)	0.80 ( $\pm$ 1.033)	38	0.38
	Z350	1.40 ( $\pm$ 0.966)	1.00 ( $\pm$ 0.816)	38	0.38

\* Indicates significant difference ( $P < 0.05$ ).

ment in dentin marginal integrity may be explained by the well-documented ability of CHX to inhibit matrix metalloproteinases (MMPs) in etched dentin, since the MMP inhibition capability of the CHX can maintain hybrid layer integrity by preventing the degradation of collagen fibrils (Breschi et al., 2009; Stanislawczuk et al., 2009; Simões et al., 2014). In contrast, the application of CHX to non-etched dentin did not improve the seal for both RBCs compared with non-pretreated teeth. A possible explanation for these results is the documented behavior of CHX bonding ability to mineralized, or sound, dentin, which was found to be lower than that to demineralized, or etched, dentin (Fukeyawa et al., 2006; Kim et al., 2010).

In addition to the dentin status, the solvent type in the adhesive system plays an important role in the adhesion chemistry. Single bond universal adhesive is an ethanol/water-based solvent system. Water solvents increase the hydrophobicity of the monomers, and therefore, increase the compatibility of the acid-etched dentin for bonding (Deng et al., 2013). However, due to high ester contents, some hydrophilic monomers can excessively hydrolyze, and therefore, entrap water, which may lead to weak adhesion interface. This hydrolysis process was documented to affect the bonding durability of self-etch

adhesives. (Hashimoto et al., 2007). The ethanol component solvent has low hydrogen-bonding ability, which lead to dehydration of the collagen network, and therefore aids in hydrolysis prevention of the bonding interface and allows the proper infiltration of resin monomers for the formation of the hybrid layer (Souza et al., 2018).

## 5. Conclusions

Within the limitations of this study, the following can be concluded:

Dentin pretreatment with 2% CHX before bonding bulk fill composite, by using the “etch-and-rinse” protocol of the universal bonding system, significantly decreased the microleakage. However, when the “self-etch” protocol was used, microleakage was not affected by CHX-pretreatment when teeth were restored with bulk fill or conventional RBCs.

## Ethical statement

- (1) This material is the authors' own original work, which has not been previously published elsewhere.

- (2) The paper is not currently being considered for publication elsewhere.
- (3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- (4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- (5) The results are appropriately placed in the context of prior and existing research.
- (6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- (7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

### CRedit authorship contribution statement

**Mohammed S. Bin-Shuwaish:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing - original draft, Writing - review & editing. **Alhanouf A. AlHussaini:** Data curation, Investigation, Visualization, Writing - original draft. **Lina H. AlHudaithy:** Data curation, Investigation, Visualization, Writing - original draft. **Shamma A. AlDukhiel:** Data curation, Investigation, Visualization, Writing - original draft. **Abdullah S. Al-Jamhan:** Investigation, Methodology, Validation, Writing - review & editing.

### Acknowledgements

The authors thank the College of Dentistry Research Center and Deanship of Scientific Research at King Saud University, Saudi Arabia, for funding this research project (Ref. No. IR 0294).

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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