

# Effect of Chlorhexidine Application on Dentin Bond Strength Durability of Two Etch-and-Rinse Adhesive versus a Universal Bond System

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## Abstract

Application of matrix-metalloproteinases (MMP) inhibitors such as chlorhexidine (CHX) is capable of reducing bond strength loss over time by preventing collagen fibrils degradation.

This *in-vitro* study compared the effect of 0.2% CHX application on micro-tensile bond strength (micro-TBS) of Single Bond Universal (SBU) and Optibond Solo Plus (OSP) versus Peak Universal Bond (PUB) to dentin after one week and six months.

**Materials and Methods:** Thirty extracted premolars were divided into five groups and their buccal surfaces reduced to expose a flat surface. The groups were: 1. PUB; 2. SBU with CHX application; 3. SBU without CHX application; 4. OSP with CHX application; 5. OSP without CHX application. After etching, the adhesives were applied to the dentin. In groups 3 and 5, CHX was applied for 60 seconds after etching and prior to adhesive application. A resin composite (Z-250) was placed incrementally on the treated dentin. The specimens were sectioned and four slices were obtained per tooth. The slices were placed in distilled water at 37±1 °C for one week and assigned into two subgroups; the first subgroups (control) were immediately tested for bond strength using a universal testing machine, and the second ones (experimental) were tested after six months. Data were analyzed using parametric tests ( $P$  value<0.05).

**Results:** The micro-TBS of PUB declined slightly but there was no significant decline over time ( $p=0.087$ ); while, regardless of the treatment applied to the dentin, the micro-TBS of other groups were significantly decreased after six months ( $p<0.05$ ). There was no significant difference between PUB and SBU with CHX pretreatment and OSP with and without CHX pretreatment in both storage times ( $p>0.05$ ).

**Conclusion:** A reduction of the micro-TBS of PUB was not significant after aging but it had no additional bond strength than the other groups. Also, application of 0.2% CHX does not prevent the loss of micro-TBS of SBU and OSP.

**Keywords:** Matrix metalloproteinases; chlorhexidine; dentin bonding; bond strength; adhesive systems

## Introduction

The longevity and integrity of composite resin restorations depend on bond durability between adhesive systems and tooth structure. The formation of a perfect resin-infiltrated hybrid layer has been thought essential to provide durable and successful adhesion to human dentin [1-4]. The degradation of collagen fibrils at the bottom of the hybrid layer and breakdown of the hydrophilic polymers of the adhesive systems cause loss of bond strength and reduction of retention and marginal adaptation [5-8]. Bonding to dentin is still a major challenge and despite improvement in the chemistry of contemporary adhesive systems, there is a consensus that the hybrid layer degrades over time and results in a decrease in bond strength, thus influencing the durability and longevity of the adhesive joint [1,2,4,9,10]. As a result, the composite resin restorations fail. Therefore, the preservation of the collagen matrix integrity is critical to improving the dentin bond durability [4,11].

The dentin collagen network is exposed after acid primer etching in etch-and-rinse and self-etching adhesive systems. The incomplete resin infiltration to the bottom of the hybrid layer leads to incomplete collagen encapsulation [1,12,13]. This non-infiltrated collagen is susceptible to proteolytic degradation by collagenolytic host-derived enzymes, such as matrix metalloproteinases (MMPs) [14]. MMPs are a group of zinc-and calcium-dependent enzymes produced by odontoblasts and are trapped within mineralized dentin

matrix [4,12,15,16]. Within dentin, MMPs are physiologically inactive, but the pH changes caused by acid etching, acidic dental adhesive monomers or the pH fluctuations due to cariogenic challenges can activate these enzymes. As a result, collagenolytic activities increase, the organic matrix of demineralized dentin hydrolyzes, the hybrid layer degrades, the bond strength gradually decreases and finally the longevity of a composite resin restoration is shortened [1,4,5,9,11,12,15,16]. The collagenolytic activity of MMPs is inhibited with the use of specific inhibitors, such as chlorhexidine digluconate (CHX). CHX is a cationic, antibacterial and antiseptic agent widely used in oral health [5,14,16,17]. Besides its antimicrobial property, in previous studies when CHX was used in concentrations of 0.2% and 2.0%, it was found capable of preventing degradation of the collagen. Thus, maintaining integrity of the hybrid layer and increase the bonding durability between adhesives and dentin [1,5,6,11,18]. CHX is applied on acid etched dentin prior to the use of etch-and-rinse and self-etching adhesives to prevent bond strength loss. Improvement of the bond durability by using 0.2% CHX has been reported with various results [1,5,11,19]. Different strategies have attempted to improve the bond durability by applying MMP inhibitors as a pretreatment before resin infiltration or by admixing MMP inhibitors into adhesive [1,4,5,20]. Recently, a self-etching adhesive containing 7.5% filler and 0.2% CHX named Peak Universal Bond has been introduced to improve the integrity and long-term bond strengths [21]. The application of CHX with adhesives could suppress residual contamination after caries removal preventing the colonization of bacteria in gaps formed by resin shrinkage and interface degradation and discouraging secondary caries formation [9,11].

There are limited published studies examining the effectiveness of CHX pretreatment prior to application of adhesive and adhesives containing CHX on the durability of the bond between resin adhesive and dentin. The aim of this *in-vitro* study was to compare the micro-tensile bond strength (micro-TBS) of two self-etching adhesives (Single Bond Universal and Optibond Solo Plus) with and without 0.2% CHX pretreatment after acid etching and prior to application of adhesive versus a novel self-etch adhesive containing 0.2% CHX (Peak Universal Bond) at time intervals of one week and six months.

## Materials and Methods

### Tooth Selection and Preparation

This *in-vitro* study was conducted on 30 intact human maxillary premolars without fracture, structural anomalies, caries, or previous restorations. These teeth had been extracted for orthodontic purposes. The teeth were washed, scrubbed, scaled and cleaned with pumice; to remove any remnants of blood, mucous, shreds of periodontal ligaments, plaque, and calculus, then stored in saline solution at room temperature. The teeth were disinfected in an aqueous buffered solution of formaldehyde (Yekta Chem Co., Tehran, Iran) for two hours, then stored in distilled water at room temperature and utilized within three months of extraction. The study protocol was approved by the Research Ethics Committee of Rafsanjan University of Medical Sciences, Iran (register number: IR.RUMS.REC.1395.47).

The teeth were embedded in a cubic mold with self-cure acrylic resin (Acropars, Marlik, Tehran, Iran) so that the buccal surface faced upward and extended three mm above the resin surface. As the depth of dentin is a crucial factor affecting the dentin bond strength values and also for standardization, 1.5-mm depth holes were drilled in the middle of the buccal surface using a round diamond bur (D&Z, Lemgo, Germany) before grinding the dentinal surface flat (Figure 1). The enamel buccal surface was removed along the long axis using a cylindrical diamond bur (D&Z, Lemgo, Germany) and high-speed handpiece under water and air spray until the diameter of the exposed dentin surfaces was 4.5 mm. The bonding surfaces were verified for the absence of enamel and/or pulp chamber exposition using a laboratory magnification lens at 4X magnification. Subsequently, the flat exposed dentin surfaces were polished using wet coarse/medium aluminum oxide discs (Kerr Hawe, Bioggio, Switzerland) for 30 seconds to produce a homogenous smear layer; one disc for each five restorations.





**Figure 1:** The teeth were embedded in acrylic resin with the buccal surface facing upward. A resin composite rod was built on the treated surface (A&B)

## Bonding Procedure

The adhesive systems evaluated in this study were Peak Universal Bond (Ultradent Products Inc., UT, USA), Single Bond Universal (2M ESPE, St. Paul, MN, USA) and Optibond Solo Plus (Kerr Italia, S.P.A, Scafati, Italy) (Table 1). The 30 teeth were randomly divided into five groups of six teeth each; Group 1: Peak Universal Bond; Group 2: Single Bond Universal with 0.2% CHX application; Group 3: Single Bond Universal without 0.2% CHX application; Group 4: Optibond Solo Plus, Scafati, Italy) with 0.2% CHX application and Group 5: Optibond Solo Plus without 0.2% CHX application.

Adhesive	Composition	Application Procedure
Peak Universal Bond	Ethyl alcohol, HEMA, methacrylic acid, 0.2% CHX di(acetate).	<ol style="list-style-type: none"> <li>1. Wash thoroughly with water spray.</li> <li>2. Acid etching (15 s).</li> <li>3. Rinse thoroughly ensuring that all acid is removed.</li> <li>4. Dry lightly (do not desiccate).</li> <li>5. Apply adhesive and air stream (10 s).</li> <li>6. Light cure (20 s).</li> <li>7. Place composite and light cure for 20 s.</li> </ol>
Single Bond Universal	MDP monomer, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	<ol style="list-style-type: none"> <li>1. Wash thoroughly with water spray.</li> <li>2. Apply etchant (15 s).</li> <li>3. Rinse thoroughly (10 s) and air dry (2 s). Experimental groups only: 0.2% CHX application (60 s) and excess removal.</li> <li>4. Apply the adhesive with a micro-brush and rub it in for (20 s).</li> <li>5. Direct a gentle stream of air (about 5 s).</li> <li>6. Light cure (20 s).</li> <li>7. Place composite and light cure for 20 s.</li> </ol>
OptiBond Solo Plus	MDP Monomer, dimethacrylate resins, HEMA, vitrebond copolymer, filler, ethanol, water, initiators, silane	<ol style="list-style-type: none"> <li>1. Wash thoroughly with water spray.</li> <li>2. Acid etching (15 s).</li> <li>3. Rinse thoroughly ensuring that all acid is removed.</li> <li>4. Dry lightly (do not desiccate). Experimental groups only: 0.2% CHX application (60 s) and excess removal.</li> <li>5. Apply the adhesive and rub for 15 s.</li> <li>6. Air thin for 3 s.</li> <li>7. Light cure for 20 s.</li> <li>8. Place composite and light cure for 20 s.</li> </ol>

**List of Abbreviations:** 2-hydroxyethyl methacrylate (HEMA); Chlorhexidine (CHX); Methacryloyloxydecyl dihydrogen phosphate monomer (MDP monomer)

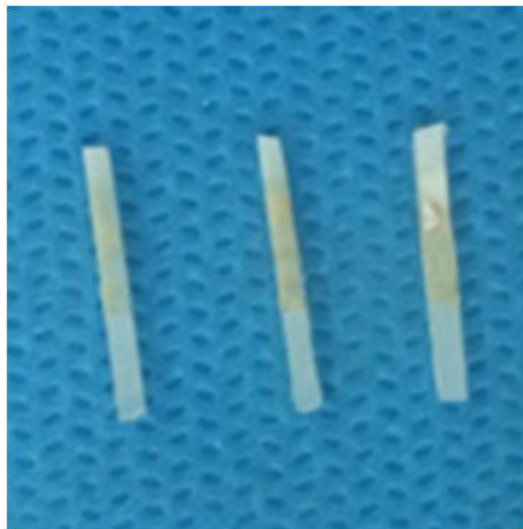
**Table 1:** Adhesive systems used in the study. The mode of their application was according to the manufacturers' instructions

The prepared specimens were etched with 37% phosphoric acid gel (3M ESPE) for 15 seconds, rinsed thoroughly and then blotted dry. The adhesive systems were applied with a micro-brush on the exposed dentin surface and light cured according to the manufacturer's instructions (Table 1). In groups 3 and 5, 0.2% CHX solution (Corsodyl, GlaxoSmithKline, Brentford, UK) was

applied with a micro-brush after dentin etching and prior to adhesive application for 60 seconds under a slight rubbing motion. The solution excess was removed through gentle air jet, leaving the dentin surface saturated with moisture.

Immediately after the adhesive procedures, a micro-hybrid resin composite (Feltek Z-250, 3M/ESPE, A2 Body Shade) was inserted inside a bipartite stainless steel matrix (4×3×3 mm) on the treated dentin in two increments of two mm. Each increment was light polymerized for 20 seconds, and then the stainless matrix was removed (Figure 1). A light-emitting diode (LED) curing unit (Demetron A.2, Kerr Italia, S.P.A.) with an output of 1000 mW/cm<sup>2</sup> was used to polymerize the adhesives and the composite. The light curing unit tip was placed 1-mm away from the surface of the restorative materials.

The specimens were serially sectioned using a cutting machine (Vafaei Co, Tehran, Iran) perpendicular to their long axis in the mesiodistal and buccolingual directions with a distance of one mm between the slices. Four slices were obtained approximately 1.0 mm<sup>2</sup> in cross section and 4.0 mm in length per tooth (Figure 2). Subsequently, the slices were placed in distilled water at 37 ± 1 °C for one week; the slices were then randomly assigned into two subgroups (12 beams per subgroup). The first subgroups (control groups) were immediately tested and the second ones (experimental groups) were tested after six months of storage in distilled water at room temperature and 1500 thermocycling (5 °C to 55 °C; dwell time one minute; transfer time five seconds) (Vafaei Co, Tehran, Iran); the distilled water was renewed weekly.



**Figure 2:** The sectioned ultrathin specimens with approximately 1.0 mm<sup>2</sup> in cross section and 4.0 mm in length

### Micro-tensile Bond Strength Test

For micro-tensile testing; each slice was fixed to a custom-made testing jig with cyanoacrylate glue (Universal Instant Adhesive; Henkel Adhesives Co. Ltd., Shantou, China) and tested in tension in a universal testing machine (Z010, Zwick/Roell, Ulm, Germany) at a cross-head speed of 0.5 mm/min until failure occurred (Figure 3). The cross-sectional area of each slice was measured to the nearest 0.01 mm with a caliper and the bond strength values (MPa) were calculated by dividing the peak tensile load (N) at failure by the cross-sectional area in mm<sup>2</sup>. To determine the failure modes, both surfaces of each slice were observed under an optical microscope (Olympus Tokyo, Japan) at 20x magnification and classified as 1) cohesive failure in the resin composite or dentin; 2) adhesive failure in the resin dentin interface; or 3) mixed, combination of adhesive and cohesive failure in dentin and/or resin composite.



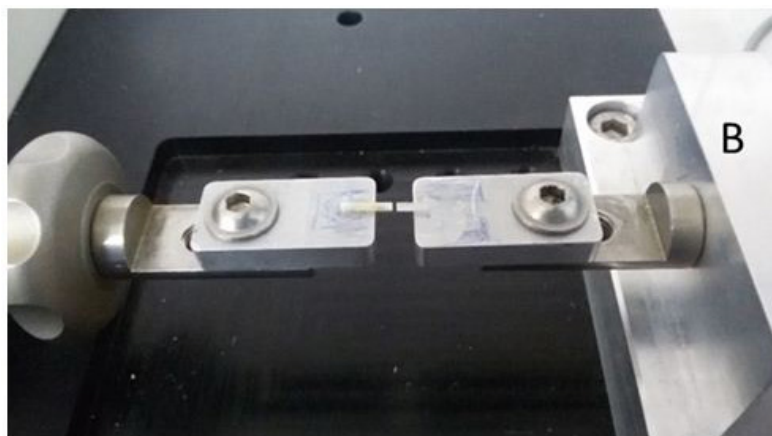


Figure 3: The specimen was attached to a Z10 universal testing device for tension test (A&B)

## Statistical Analysis

Data were statistically analyzed using SPSS software version 21 (SPSS Inc, Chicago, IL, U.S.A.). Descriptive statistics including the mean, standard deviation, maximum and minimum of the micro-TBS were computed for each group. Normal distribution of the data was confirmed using Shapiro-Wilk test ( $p > 0.05$ ); while, homogeneity of variances was not verified using Levene's test ( $p = 0.002$ ). Therefore, one-way analysis of variances (ANOVA) was used for comparison among all groups and post hoc Tamhane's T2 test for pairwise comparisons in each storage times. The failure mode frequencies were analyzed using the Fisher's exact test.  $P$  value  $< 0.05$  was considered statistically significant.

## Results

The minimum, maximum, mean and standard deviations of micro-TBS values (MPa) of the studied groups and percentage of bond strength reduction are presented after one week and six months of water storage (Table 2). The one-way ANOVA showed statistically significant differences between tested groups in one week and six months of storage ( $p = 0.001$ ). Tamhane's T2 test showed the micro-TBS of Peak Universal Bond declined slightly, but it was not significant over time ( $p = 0.087$ ); while, regardless of the treatment applied to the dentin, the micro-TBS of other groups were significantly decreased after six months of storage ( $p < 0.05$ ) (Table 2).

Groups	One week			Six months			Reduction	P-value*
	Min	Max	Mean±SD	Min	Max	Mean±SD		
PUB	19.04	39.25	29.09±7.64 <sup>a</sup>	15.90	25.78	20.15±3.18 <sup>a</sup>	31%	0.087
OSP	25.48	52.48	34.57±8.22 <sup>a</sup>	18.14	30.22	22.51±3.55 <sup>a</sup>	35%	0.014
OSP+CHX	22.26	41.21	29.84±5.43 <sup>a</sup>	15.85	31.93	20.59±5.52 <sup>a</sup>	31%	0.019
SBU	40.43	73.16	58.17±10.25 <sup>b</sup>	23.18	44.68	33.42±7.04 <sup>b</sup>	43%	0.001
SBU+CHX	27.54	53.66	35.75±8.58 <sup>a</sup>	18.17	29.08	23.28±3.90 <sup>a</sup>	35%	0.015

Different letters within the same column indicate statistical differences between pairwise groups according to Tamhane's T2 test ( $p < 0.05$ )

\*Statistical difference between one week and six months storage

Table 2: The Min, Max and Mean±SD of micro-TBS values (MPa) of studied groups after one week and six months of water storage and percentage of bond strength reduction (n=12)

The means of micro-TBS of Single Bond Universal without 0.2% CHX pretreatment were significantly higher than those with CHX pretreatment and the other groups in both storage times ( $p < 0.05$ ). Pretreatment with 0.2% CHX in Optibond Solo Plus revealed lower bond strength values than those non-pretreated, but these differences were not statistically significant in both non-stored and stored samples ( $p > 0.05$ ). The percentage of micro-TBS reduction varied between 31%-43% and the Single Bond Universal had the highest bond strength reduction.

Microscopic examination of adhesive joints revealed that adhesive failure was the most prevalent type of failure mode and no mixed failure was observed. The Fisher's exact test showed a significant difference in the frequency of the failure mode in each tested groups ( $p < 0.05$ ) (Table 3).

Groups	One week			Six months		
	Adhesive n (%)	Cohesive n (%)	Mixed n (%)	Adhesive n (%)	Cohesive n (%)	Mixed n (%)
PUB	12(100)	0(0)	0(0)	12(100)	0(0)	0(0)
OSP	12(100)	0(0)	0(0)	12(100)	0(0)	0(0)

Groups	One week			Six months		
	Adhesive n (%)	Cohesive n (%)	Mixed n (%)	Adhesive n (%)	Cohesive n (%)	Mixed n (%)
OSP+CHX	12(100)	0(0)	0(0)	11(91.7)	1(8.3)	0(0)
SBU	12(100)	0(0)	0(0)	12(100)	0(0)	0(0)
SBU+CHX	11(91.7)	1(8.3)	0(0)	12(100)	0(0)	0(0)

Table 3: Failure modes of the study groups (n=12)

## Discussion

The bond strength to dentin decreases over time was possibly due to water diffusion through the hybrid layer and/or collagen fibrils degradation that was not infiltrated by adhesives at the bottom of the hybrid layer [6,9-11,14,16,22]. This degradation has been attributed to MMP enzymes in the dentin matrix, which are activated both by acid-etchants and by acidic monomers used in adhesive systems that slowly degrade the collagen fibrils [1,4,12,15,19,23]. These enzymes are inhibited by protease inhibitors; however, it is suggested the use of MMP inhibitors (e.g., CHX) would increase the stability of resin dentin adhesive and reduce collagen fibrils degradation through time [1,4,5]. The present study evaluated the bonding efficacy of two two-step etch-and-rinse universal adhesive (Single Bond Universal and Optibond Solo Plus) both with and without 0.2% CHX pretreatment and a self-etch adhesive (Peak Universal Bond with 0.2% CHX incorporated).

The results of this study showed that differences between the experimental groups in one week and six months of storage were significant. Reduction of the bond strength of only Peak Universal Bond was not significant over time. Aging tends to reduce the bond strength after, which may be explained by water diffusion through the hybrid layer and degradation of demineralized collagen [7,18,22,23]. This study showed a drop in micro-TBS after six months storage in all groups. The Single Bond Universal without CHX application had the greatest bond strength reduction (43%). Nevertheless, the bond strength of Single Bond Universal without CHX application was significantly higher than other groups. Pashley, *et al.* reported that micro-TBS often fall 30% to 40% in 6 to 12 months. The cause of this poor durability is a combination of the activation of MMPs by weak acids such as lactic acid released by caries-producing bacteria, and acid-etchants used in adhesive bonding systems; these acids uncover and activate matrix-bound MMPs [23]. Rao, *et al.* concluded that Peak Universal Bond has no adverse effect on immediate resin dentin bond strength and it is comparable with that of Adper SE adhesive system (self-etch adhesive without CHX) [24]. He hypothesized that use of CHX incorporated in self-etch adhesives result in better preservation of resin dentin bonds than the application of 2% CHX after acid etching for an etch and rinse adhesive system. Also, Carvalho, *et al.* indicated that the dentin bond strengths of Peak Universal Bond is unaffected by water storage and no changes in interfacial micromorphology were observed [25]. Peak Universal Bond is a novel self-etch adhesive with 0.2% CHX incorporated in it and filled with 7.5% fillers, which may ensure long-term bond strengths. Its viscosity has been optimized for minimal film thickness (2µm) and superior strength. It is a multi-mode adhesive and can be used in etch-and-rinse and/or self-etch modes for indirect and direct enamel and/or dentin bonding [5,23,26,27].

Although Rao, *et al.* stated self-etch adhesives demineralize dentin and activate MMPs, CHX could simultaneously inactivate MMPs until the adhesive is cured [24]. But, Anusavice, *et al.* believes that incorporating CHX into the resin could hinder the polymerization process and result in a higher level of residual monomers [28]. In this study, the etch-and-rinse mode was selected to compare with other two etch-and-rinse adhesives (Single Bond Universal and OptiBond Solo Plus). Non-simplified adhesives are preferred to achieve adhesive interfaces that are less prone to degradation over time [5,6,9]. Based on our results, no differences in bond strength were distinguished between Peak Universal Bond with Optibond Solo Plus with or without CHX pretreatment and Single Bond Universal with CHX pretreatment. The MMPs are activated during the dentin etching procedure and may be inhibited by protease inhibitors, such as CHX. The CHX may be able to inactivate MMPs through cation chelating mechanisms and sequester metal ions such as calcium and zinc. CHX has the potential to bind to both organic (collagen) and inorganic (hydroxyapatite) components in dentin when applied after acid etching on the prepared tooth surface. It can also be incorporated into the acid etching agent, which is rinsed away from the surface, or within the adhesive system composition, or applied directly on the dentin surface after the etching [4,5,11,12,16,29-31].

The results revealed that after one week and six months storage, the micro-TBS of Optibond Solo Plus is not altered by 0.2% CHX application. However, the application of 0.2% CHX in Single Bond Universal group significantly reduced the bond strength. Based on this study, the application of 0.2% CHX does not preserve bond strength when compared to the controls. It has been stated that the application of CHX after dentin etching promoted a reduction in the degradation of the bond strength, but other studies have shown that priming the dentin substrate with MMP inhibitors can reduce the bond quality [18,23,32-34]. A meta-analysis presented lower bond strength values for 0.2% CHX pretreatment groups than controls (without use of CHX) for immediate results and higher bond strength values for 0.2% and 2% CHX when compared with the control group after aging [35]. Some studies have also revealed that the topical application of CHX after acid etching has no effect on the immediate bond strength [5,26,36-38]. Dutra-Correa, *et al.* reported that the application of CHX prior to the application of dentin adhesives did not influence their clinical performance up to 18 months of service [39].

The most common failures were adhesive failures; no mixed failures were observed and only two samples (Single Bond Universal with 0.2% CHX group at one week and Optibond Solo Plus with 0.2% CHX group after six months storage) had cohesive failure. Therefore, adhesive joint failure is still considered the principal mechanisms of the resin-dentin degradation [40,41].

Using 0.2% CHX as a therapeutic primer may inhibit dentin proteolytic activity [42]. However, Dionysopoulos, concluded that CHX had a negative effect on the bond strength of adhesive systems with dentin [43]. This would suggest that further *in-vitro* and *in-vivo* investigations are necessary to clarify the influences of CHX application on the longevity of dentin bond strength.

## Conclusions

Within the limitations of this *in-vitro* study it can be concluded:

1. That the reduction of micro-TBS of Peak Universal Bond (self-etch adhesive with 0.2% CHX incorporated in it) was not significant after six months water storage, and it had no bond strength greater than other experimental groups with and without 0.2% CHX application on one week and six months storage.
2. The application of 0.2% CHX does not prevent the loss of bond strength of Single Bond Universal and Optibond Solo Plus after aging.
3. Additional *in-vitro* and *in-vivo* investigations are necessary to clarify the effect of CHX application on the durability of dentin bonds.

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## References

1. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, et al. (2010) Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. *Dent Mater* 26: 571-8.
2. Hashimoto M, Fujita S, Nagano F, Ohno H, Endo K (2010) Ten-years degradation of resin-dentin bonds. *Eur J Oral Sci* 118: 404-10.
3. Muñoz MA, Luque-Martinez I, Malaquias P, Hass V, Reis A, et al. (2015) In vitro longevity of bonding properties of universal adhesives to dentin. *Oper Dent* 40: 282-92.
4. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, et al. (2013) Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. *Dent Mater* 29: 116-35.
5. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, et al. (2007) In vivo preservation of the hybrid layer by chlorhexidine. *J Dent Res* 86: 529-33.
6. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H (2003) In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. *Biomaterials* 24: 3795-803.
7. Manso AP, Bedran-Russo AK, Suh B, Pashley DH, Carvalho RM (2009) Mechanical stability of adhesives under water storage. *Dent Mater* 25: 744-9.
8. Toledano M, Osorio R, Osorio E, Aguilera FS, Yamauti M, et al. (2007) Effect of bacterial collagenase on resin-dentin bonds degradation. *J Mater Sci Mater Med* 18: 2355-61.
9. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, et al. (2003) Four-year water degradation of total-etch adhesives bonded to dentin. *J Dent Res* 82: 136-40.
10. Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, et al. (2002) Micromorphological changes in resin-dentin bonds after 1 year of water storage. *J Biomed Mater Res* 63: 306-11.
11. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, et al. (2004) Collagen degradation by host-derived enzymes during aging. *J Dent Res* 83: 216-21.
12. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, et al. (2006) Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. *Biomaterials* 27: 4470-6.
13. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, et al. (2006) Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. *Eur J Oral Sci* 114: 160-6.
14. Strobel S, Hellwig E (2015) The effects of matrix-metallo- proteinases and chlorhexidine on the adhesive bond. *Swiss Dent J* 125: 134-45.
15. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S (2006) The role of matrix metalloproteinases (MMPs) in human caries. *J Dent Res* 85: 22-32.
16. Zhang SC, Kern M (2009) The role of host-derived dentinal matrix metalloproteinases in reducing dentin bonding of resin adhesives. *Int J Oral Sci* 1: 163-76.
17. Ribeiro LG, Hashizume LN, Maltz M (2007) The effect of different formulations of chlorhexidine in reducing levels of mutans streptococci in the oral cavity: A systematic review of the literature. *J Dent* 35: 359-70.
18. Moon PC, Weaver J, Brooks CN (2010) Review of Matrix Metalloproteinases' Effect on the Hybrid Dentin Bond Layer Stability and Chlorhexidine Clinical Use to Prevent Bond Failure. *Open Dent J* 4: 147-52.
19. Zheng P, Zaruba M, Attin T, Wiegand A (2015) Effect of different matrix metalloproteinase inhibitors on microtensile bond strength of an etch-and-rinse and a self-etching adhesive to dentin. *Oper Dent* 40: 80-6.
20. Collares FM, Rodrigues SB, Leitune VC, Celeste RK, Borba de Araújo F, et al. (2013) Chlorhexidine application in adhesive procedures: a meta-regression analysis. *J Adhes Dent* 15: 11-8.
21. Ultradent Products, Inc. [Cited 2017 Apr 10].
22. Tay FR, King NM, Chan KM, Pashley DH (2002) How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *J Adhes Dent* 4: 255-69.
23. Pashley DH, Tay FR, Imazato S (2011) How to increase the durability of resin-dentin bonds. *Compend Contin Educ Dent* 32: 60-4.

24. Rao SA, Roopa D (2015) Comparative Evaluation of the Bonding Efficacy of Seventh Generation Bonding Agent and Peak Universal Bond: An In-Vitro Study. *J Interdiscipl Med Dent Sci* 3: 167.
25. Carvalho AO, Bacelar-Sá R, Wodevotzky O, Ambrosano GMB, Magne PC, et al. (2016) Bond strength and micromorphology of resin-dentin interface of etch-and-rinse dentin bonding agents after 1-year of water storage. *Appl Adhesion Sci* 4: 16.
26. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, et al. (2010) Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. *Dent Mater* 26: 320-5.
27. Gunaydin Z, Yazici AR, Cehreli ZC (2016) In Vivo and In Vitro Effects of Chlorhexidine Pretreatment on Immediate and Aged Dentin Bond Strengths. *Oper Dent* 41: 258-67.
28. Anusavice KJ, Zhang NZ, Shen C (2006) Controlled release of chlorhexidine from UDMA-TEGDMA resin. *J Dent Res* 85: 950-4.
29. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, et al. (2008) Dental adhesion review: aging and stability of the bonded interface. *Dent Mater* 24: 90-101.
30. Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L (2007) The role of matrix metalloproteinases in the oral environment. *Acta Odontol Scand* 65: 1-13.
31. Ricci HA, Sanabe ME, Costa CA, Hebling J (2010) Effect of chlorhexidine on bond strength of two-step etch-and-rinse adhesive systems to dentin of primary and permanent teeth. *Am J Dent* 23: 128-32.
32. Heintze SD, Rousson V (2011) Pooling of dentin microtensile bond strength data improves clinical correlation. *J Adhes Dent* 13: 107-10.
33. Hiraishi N, Yiu CK, King NM, Tay FR (2009) Effect of 2% chlorhexidine on dentin microtensile bond strengths and nanoleakage of luting cements. *J Dent* 37: 440-8.
34. Stanislawczuk R, Reis A, Loguercio AD (2011) A 2-year in vitro evaluation of a chlorhexidine-containing acid on the durability of resin-dentin interfaces. *J Dent* 39: 40-7.
35. Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, Cenci MS (2014) MMP Inhibitors on Dentin Stability: A Systematic Review and Meta-analysis. *J Dent Res* 93: 733-43.
36. Brackett MG, Tay FR, Brackett WW, Dib A, Dipp FA, et al. (2009) In vivo chlorhexidine stabilization of hybrid layers of an acetone-based dentin adhesive. *Oper Dent* 34: 379-83.
37. Komori PC, Pashley DH, Tjäderhane L, Breschi L, Mazzoni A, et al. (2009) Effect of 2% chlorhexidine digluconate on the bond strength to normal versus caries-affected dentin. *Oper Dent* 34: 157-65.
38. Loguercio AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD, et al. (2009) Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. *Eur J Oral Sci* 117: 587-96.
39. Dutra-Correa M, Saraceni CH, Ciaramicoli MT, Kiyan VH, Queiroz CS (2013) Effect of chlorhexidine on the 18-month clinical performance of two adhesives. *J Adhes Dent* 15: 287-92.
40. Sabatini C (2013) Effect of a chlorhexidine-containing adhesive on dentin bond strength stability. *Oper Dent* 38: 609-17.
41. Yang B, Adelung R, Ludwig K, Bössmann K, Pashley DH, et al. (2005) Effect of structural change of collagen fibrils on the durability of dentin bonding. *Biomaterials* 26: 5021-31.
42. Gendron R, Grenier D, Sorsa T, Mayrand D (1999) Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. *Clin Diagn Lab Immunol* 6: 437-9.
43. Dionysopoulos D (2016) Effect of digluconate chlorhexidine on bond strength between dental adhesive systems and dentin: A systematic review. *J Conserv Dent* 19: 11-6.

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