International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-4, 2017, pp1623-1628
http://www.bipublication.com

Research Article

The Effect of Chlorhexidine Application on Shear Bond Strength and Microleakage of Two Different ETCH and Rinse and Self-ETCH Adhesive Systems in Primary Dentition

Alireza Sarraf[1], Sara Maleki Kambakhsh[2], Hamideh Ameri[3], Fatemeh Mazaheri[1] and Razieh Jabbarian[4*]

[1]Associate Professor, Department of Pediatric Dentistry, Dental School, Mashhad University of Medical Sciences, Mashhad, Iran.
[2]Assistant Professor, Department of Pediatric Dentistry, Dental School, Qazvin University of Medical Sciences, Qazvin, Iran.
[3]Assistant Professor, Department of Pediatric Dentistry, Dental School, Mashhad University of Medical Sciences, Mashhad, Iran.
[4]Postgraduate Student of Pediatric Dentistry, Department of Pediatric Dentistry, Dental School, Qazvin University of Medical Sciences, Qazvin, Iran
[*]**Corresponding author**:. E-mail: r_jabbarian@yahoo.com, tel: +989128811244

ABSTRACT

Introduction: Despite of significant advancements in the field of optimal bonding of restorative materials to tooth, bond longevity is still challenging. Partially or totally demineralized collagen fibers of hybrid layer are prone to destruction by matrix metalloproteinase enzymes of dentin. It appears that application of cross-linking agents such as chlorhexidine (CHX) may contribute to maintain and improve the resin-dentin bond strength. The purpose of this study is to evaluate the effect of chlorhexidine 0.2% on bond strength and microleakage of two types of adhesives to dentin of primary teeth. **Materials and Methods:** 40 primary molars split into two halves to use 40 halves in each microleakage or bond strength study. Four groups defined: 1) Clearfil SE bond, 2) Clearfil SE bond+CHX, 3) Single bond, 4) Single bond+CHX. According to the manufacturer recommendation, Clearfil AP-X composite in groups 1 & 2 and Z250 composite in groups 3 & 4 used. After storage of samples in 37° C water for 3 months and 1000 thermal cycles, bond strength evaluated by Instron machine. Class V cavities prepared for microleakage study by the use of dye penetration and stereomicroscope. **Results:** Clearfil SE bond and Clearfil SE bond+CHX groups showed highest and lowest bond strength respectively. Bond strength of Single bond adhesive improved significantly by the use of CHX. Microleakage rate was not significantly different among groups. **Conclusion:** In the case of CHX application with Clearfil SE bond, bond strength of primary teeth reduced significantly. While using CHX with single bond lead to improvement of bond strength.

Keywords: bond strength, microleakage, self-etch adhesive, etch and rinse adhesive, chlorhexidine

[I] INTRODUCTION

In recent decades, esthetic tooth-colored restorations have been used widely all around the world. Despite the noticeable improvement in mechanical properties of dental adhesive materials and technology, durability and preservation of created bonding layer is still challenging[1, 2]. In primary dentition, in regard to low thickness of enamel and dentin and higher volume of pretubular dentin which cause larger tubular lumen following acid etching, bonding to dentin is more complicated [1, 3].

In etch and rinse adhesive systems, decreased diffusion of resin monomers to the bottom of hybrid layer result in susceptibility of denuded collagen fibrils to degradation by host derived matrix metalloproteinase enzymes [4].

For self-etch adhesives, nanoleakage was also observed because of incomplete removal of water that is associated with the hydrophilic resin monomers [3-5]. Water component besides incomplete resin protection makes collagen fibrils prone to time-related hydrolytic degradation; which leads to decrease of dentin-adhesive bond strength over time [4, 6].

Recent studies revealed resin elution and hydrolytic degradation of collagen matrices in aged resin- dentin bonds [6]. According to studies, Collagen-degrading matrix metalloproteinases (MMPs) and cysteine cathepsins of dentin contribute to proteolytic degradation of dentin-adhesive interfaces. Extensive efforts made to understand the mechanism and ways of inhibiting these activities [7].

On the other hand, some other studies have provided evidence which indicated chemical cross linkers such as Glutaraldehyde, Grape seed extract, Proantocianidines and Chlorhexidine digluconate solution (CHX) can improve collagen fibrils network stability in etched dentin. It also has been mentioned that Glutaraldehyde in comparison with grape seed extract and CHX solution has more potential for toxicity [7-10]. Despite the inhibiting effect of CHX on the MMPs, numerous studies addressed the changes of bond strength as a result of this treatment in permanent teeth leading to conflicting results.

According to different indications and availability of CHX in dentistry, the purpose of this study was to investigate the effect of CHX application as cross linking agent on shear bond strength and microleakage of two different etch and rinse and Self-etch adhesive systems in primary dentition.

[II]MATERIALS AND METHODS

Forty sound human primary molars were used in this in vitro study. The specimens were immersed in 0.1% thymol solution for a week and then stored in distilled water at 5° C and used within one month after extraction. Half of the specimens were used for shear hand strength test and the other half were used for microleakage analysis.

2.1. Shear Bond Strength

The specimens were randomly divided into 2 experimental groups according to the dental adhesive which was used (l: Adper Single Bond, II: Clearfil SE Bond). In both groups teeth were sectioned mesiodistally using a diamond disk (SS White, Washington, USA). Then buccal or lingual half of specimens were randomly assigned in 2 subgroups a or b.

A flat and superficial dentin surface was exposed on each specimen after wet grinding the buccal /lingual enamel with trimmer (Dentarum, Germany) and further polished with #600 grit silicon carbide paper under running water for 30 Sec. to create a standard Smear layer.

To increase the accuracy of shear bond strength test, dentinal slabs Were mounted in acrylic resin so that their surface placed quite parallel to the horizon. **[Figure 1]**

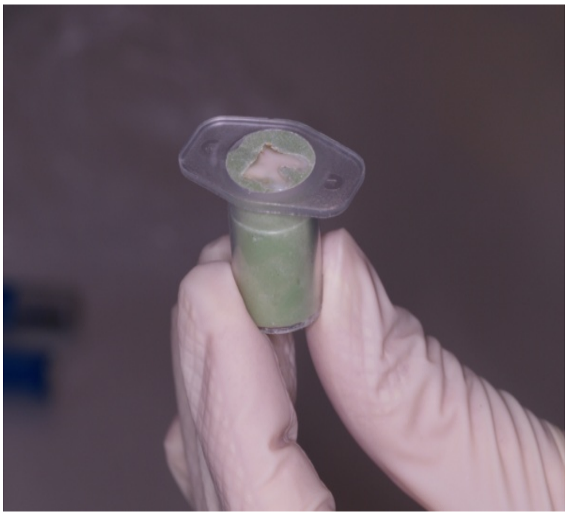

Figure 1- mounted sample for bond strength study

After cleaning with distilled water to remove any excess debris, the surface was dried with oil free compressed air and prepared for usage of two different adhesive Systems and rewetting solution (CHX 0.2%).

In group I, Adper Single Bond (SB; 3M ESPE; St Paul, MN, USA), an ethanol / water based etch and rinse adhesive system and in group II Clearfil SE Bond (C SEB; Kuraray Co., Osaka, Japan), a two-step self-etch primer adhesive system was applied according to manufacturer's instruction.

Chlorhexidine as a cross linking agent was applied in group lb, IIb for 30 Sec to rewet the surface after acid etching (I), conditioning (II). **[Table 1]**

Table 1- distribution pattern of samples in study groups

80 extracted primary molars (two groups of 40 each)	Adper Single Bond (A) N=40	Bond strength N=20	Without CHX N=10 (I)
			With CHX N=10 (II)
		Microleakage N=20	Without CHX N=10 (I)
			With CHX N=10 (II)
	Clearfil SE Bond (B) N=40	Bond strength N=20	Without CHX N=10 (I)
			With CHX N=10 (II)
		Microleakage N=20	Without CHX N=10 (I)
			With CHX N=10 (II)

The light curing procedure was performed by means of a Ivoclar Vivadent, Bluephase, Austria for the recommended time of 10 Sec. (600 mw/cm^2). A prefabricated transparent 2.2.2 mm mold was used to build up the resin composite on the surface. To accommodate the adhesive with composite resin Z250 (Filtek 22500, 3M ESPE Dental Product) in group I and Clearfil composite (Kuraray, Japan) was used in group ll.

The specimens were aged in distilled water for 3 months at 37° C and thermo-cycled for 1000 cycles in water bath between 5° C and 55° C, with a dwell time of 20 Sec in each bath.

The shear bond strength was measured using a universal testing machine (Instron, Zwick, Germany) at a crosshead speed of 1 mm per min. The failure modes were evaluated with a stereomicroscope (LEP 1450, Germany) at 40x magnification and classified as Cohesive (failure exclusively within the dentin or composite; C) Adhesive (failure at resin dentin interface; A) or Mixed (failure at the resin dentin interface that included cohesive failure of the neighboring substrate;M)

2.2.MicroLeakage

Class V cavities (2mm width, 2mm height and 1 mm depth) Were prepared at the buccal/lingual surface of each tooth using a cylindrical diamond burs (diamond fissure 330; S.S White, Washington, USA).

Each bur was used for five preparations. During preparations, the specimens were kept wet to avoid dehydration. No bevel was used on the margins. Grouping and procedures was similar to shear bond strength study.

After composite filling, the restorations were finished with diamond burs (finishing bur no. 820621; Teezkavan, Tehran, Iran) and polished.

After mentioned aging protocol, all tooth surfaces were covered with two layer of nail varnish except 1 mm around the restoration margins and immersed in 2% basic fuchsine dye for 24 hours at room temperature. (Apices of tooth were sealed with utility wax)

To assess the microleakage, the specimens were sectioned labiolingually through the center of restoration with a diamond disk (DiamantGmbh, D and Z, Berlin, Germany) and examined under the stereomicroscope with 40x magnifications. Degree of dye penetration was evaluated and scored as follow: 0: No dye penetration, I: Dye penetration along occlusal or gingival wall less than one third length, 2: Dye penetration along occlusal or gingival wall up to two third but not less than one third, 3: Dye penetration along the entire length of the occlusal or gingival wall as well as the axial wall.

Statistical Analysis

Obtaining data were analyzed using SPSS software version 19. To compare shear bond strength in different groups, T test and paired T test was performed for independent and dependent variables respectively. Mann Whitney and Wilcoxon tests were used to determine the significant differences of microleakage groups. Distribution of fracture pattern was assessed with Fisher Exact test. Statistical significance was considered as $P<0.05$.

[III]RESULTS

Comparison of Shear bond strength between experimental groups showed significant statistical difference (P value = 0.00< 0.05). Maximum mean of bond strength was related to Clearfil SE Bond, Single Bond + CHX, Single Bond and Clearfil SE Bond+CHX groups respectively.

More than 80% of specimens of each group (except Clearfil SE Bond+ CHX groups) had no microleakage. In other cases, microleakage score was reported equivalent 1 and in one case 2. Statistical analysis revealed no significant difference between groups (P value>0.05). Distribution of failure mode was similar in four groups (P value =0.084 >0.05)

[IV]DISCUSSION

Results of present Study showed that rewetting of etched dentin with CHX 0.2% for 30 Sec can improve shear bond Strength of Single Bond as an etch and rinse adhesive system in primary dentition which confirmed previously published findings of permanent teeth [11-13].

A 5 to 10 µm layer of demineralized dentin is formed following acid etching with 37% phosphoric acid in etch and rinse adhesive systems. Resin monomers usually cannot penetrate the full thickness of this layer, so a network of denuded collagen fibrils in the depth of hybrid layer will be at risk of degradation by intrinsic matrix metalloproteinase enzymes [4, 14].

Presence of water is necessary in most chemical and enzymatic reactions. Adding hydrophilic components to fifth generation of dental bondings like Single Bond and combination of primer to resin monomers to promote wetting ability of adhesives and simplifying of working steps, increased destruction of resin dentin bond following water resorption and occurrence of nanoleakage phenomenon [9, 10].

Another suggested role of water is inhibition of complete resin polymerization and plasticizing of resin component of bonding layer during time. Carriho and colleagues showed that in water free, mineral oil storage media, collagen fibril destruction do not take place [15].

With this rationale in some investigations [16], application of an additional hydrophobic resin layer like enamel bond was recommended to seal more and reduce water absorption by hybrid layer to result in bond destruction with more delay.

So CHX as a composition with long term stability, occupy porous regions on collagen network, create cross linkage between collagen fibrils and inhibit destructive action of MMP enzymes to increase strength and durability of resin-dentin [8, 17, 18].

Unlike Single Bond, bond strength of Clearfil SE Bond reduced noticeably by CHX application. This finding was in accordance with Campos and colleagues results in permanent teeth [6, 19].

Severe reduction of bond strength of self-etch adhesive systems following CHX application could interpret by susceptibility of these systems to moisture. CHX solution results in dilution of acidic property of self-etch primers and reduce etching capacity of these weak acids more.

On the other hand, CHX solution with its cationic property is able to bond with phosphoric groups of hydroxyapatite crystals of hybrid layer and interfere with acidic monomers function.

This problem is not critical in etch and rinse adhesive systems because of using a strong acid (phosphoric acid 37%) and also rewetting procedure takes place after etching not simultaneously.

According to the results of this in vitro study, Clearfil SE Bond showed the highest bond strength between other investigated groups which was in total agreement with previous studies [13, 20].

Previous studies suggested that etch and rinse adhesives during demineralization of dentin create a low PH medium which will denaturalize MMP enzymes and so cannot activate them [21]. But there is another statement which implies that short time of etching procedure, do the rinse, and high capacity of dentin, result in rising of PH value, so denaturation is relative.

Nishitani and colleagues[22] proposed that in self etch adhesive systems, the highest activation of MMPs is related to seventh generation.

Formation of a separate coupling resin layer and lower hydrophilicity of the cured resin as compared with the two step version may have resulted in low sensitivity to water degradation. In this regard, Clearfil SE Bond is comparable with three steps etch and rinse adhesives which considered as gold standard in dentin bonding. In addition, Clearfil SE Bond contains to some extent filler that promote its mechanical properties, make it a shock absorber and reduce water absorption.

In the present study, contemporary concentration of CHX (0.2%) was used for 30 Seconds, mentioned that lower concentrations and application time for rewetting may be sufficient for preserving resin dentin interface in permanent teeth [23].

Sadek et al [24] challenged long-term effectiveness of CHX anti-degradation strategy and found severe hybrid layer degradation; in contrast to ethanol-saturated dentin which preserved hybrid layer integrity over time. They believed that the degradation is related to the continuous release of CHX over time and consequent loss of the MMP-inhibitory effect.

There are some strategies other than CHX application and ethanol wet bonding to increase the longevity of dentin-adhesive bonds including increasing the degree of conversion and esterase resistance of hydrophilic adhesives, novel inhibitor functional groups grafted to methacrylate resin monomers and biomimetic remineralization of the water-filled collagen matrix using analogs of matrix proteins. Complete overcome of difficulties in bond longevity requires a combination of these strategies [7,8].

Microleakage scaling revealed more than 80% of specimens of each group (except Clearfil SE Bond + CHX) had no observable microleakage. Statistical analysis proved no significant difference between study groups. In this study, cavities prepared on dentin surface with no surrounded enamel. Composite restoration is not the first choice when facing this situation in clinic.

This result is in consistent with previous studies that there is no exact relevance between bond strength and microleakage/ nanoleakage[25]. Though it seems that Sealing property and Subsequent microleakage of dentin adhesive used in this study, do not affected by application of CHX 0.2% Solution.

[V] CONCLUSION

In conclusion: 1. Clearfil SE Bond as a two-step self-etch adhesive revealed the highest shear bond strength on the dentinal surface of primary teeth.

2. In primary teeth rewetting with CHX 0.2% in two steps etch and rinse adhesive system (Single Bond) result in promotion of shear bond strength, while in two step self-etch adhesive system (Clearfil SE bond) cause significant reduction.

3. CHX application for 30 sec. with Single bond or Clearfil SE bond had no effect on resin dentin microleakage of primary teeth.

REFERENCES

1. Ruschel HC, Ligocki GD, Flaminghi DL, Fossati AC,(2011), Microstructure of mineralized tissues in human primary teeth, The Journal of clinical pediatric dentistry. Vol-35, issue 3, pg 295-300.
2. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E, (2008), Dental adhesion review: aging and stability of the bonded interface, Dental materials : official publication of the Academy of Dental Materials.Vol-24, issue 1, pg 90-101.
3. Marquezan M, da Silveira BL, Burnett LH, Jr., Rodrigues CR, Kramer PF, (2008), Microtensile bond strength of contemporary adhesives to primary enamel and dentin, The Journal of clinical pediatric dentistry. Vol-32, issue 2, pg 127-32.
4. Andre CB, Gomes BP, Duque TM, Stipp RN, Chan DC, Ambrosano GM, et al, (2015), Dentine bond strength and antimicrobial activity evaluation of adhesive systems, Journal of dentistry. Vol-43, issue 4, pg 466-75.
5. Sarr M, Kane AW, Vreven J, Mine A, Van Landuyt KL, Peumans M, et al, (2010), Microtensile bond strength and interfacial characterization of 11 contemporary adhesives bonded to bur-cut dentin, Operative dentistry. Vol 35, issue 1, pg 94-104.
6. Abdalla AI, (2010), Effect of long-term water aging on microtensile bond strength of self-etch adhesives to dentin, American journal of dentistry. Vol-23, issue 1, pg 29-33.
7. Hedenbjork-Lager A, Hamberg K, Paakkonen V, Tjaderhane L, Ericson D, (2016), Collagen degradation and preservation of MMP-8 activity in human

dentine matrix after demineralization, Archives of oral biology.Vol-68, pg 66-72.
8. 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, The Open Dentistry Journal. Vol-4, pg 147-52.
9. De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G, et al. (2009), Inhibition of enzymatic degradation of adhesive-dentin interfaces,Journal of dental research. Vol-88, issue 12, pg 1101-6.
10. Kramer N, Tilch D, Lucker S, Frankenberger R, (2014), Status of ten self-etch adhesives for bonding to dentin of primary teeth, International journal of paediatric dentistry. Vol-24, issue 3, pg 192-9.
11. Campos EA, Correr GM, Leonardi DP, Pizzatto E, Morais EC, (2009), Influence of chlorhexidine concentration on microtensile bond strength of contemporary adhesive systems, Brazilian oral research. Vol-23, issue 3, pg 340-5.
12. Stanislawczuk R, Amaral RC, Zander-Grande C, Gagler D, Reis A, Loguercio AD, (2009), Chlorhexidine-containing Acid Conditioner Preserves the Longevity of Resin-dentin Bonds, Operative dentistry. Vol-34, issue 4, pg 481-90.
13. Jung Y-J, Hyun H-K, Kim Y-J, Jang K-T, (2009), Effect of collagenase and esterase on resin-dentin interface: A comparative study between a total-etch adhesive and a self-etch adhesive, American journal of dentistry. Vol-22, issue 5, pg 295.
14. Zhang S-c, Kern M ,(2009), The role of host-derived dentinal matrix metalloproteinases in reducing dentin bonding of resin adhesives, International journal of oral science. Vol-1, issue 4, pg 163-76.
15. Carrilho M, Carvalho RM, Tay FR, Yiu C, Pashley DH, (2005), Durability of resin-dentin bonds related to water and oil storage, American journal of dentistry. Vol-18, issue 6, pg 315-9.
16. Toledano M, Osorio R, Osorio E, Aguilera FS, Yamauti M, Pashley DH, et al, (2007), Effect of bacterial collagenase on resin-dentin bonds degradation, Journal of materials science Materials in medicine. Vol-18, issue 12, pg 2355-61
17. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjaderhane L, et al, (2007), In vivo preservation of the hybrid layer by chlorhexidine, Journal of dental research. Vol-86, issue 6, pg 529-33.
18. Li H, Li T, Li X, Zhang Z, Li P, Li Z, (2015), Morphological effects of MMPs inhibitors on the dentin bonding, International journal of clinical and experimental medicine. Vol-8, issue 7, pg 10793-803.
19. Abdalla AI, Feilzer AJ, (2008), Four-year water degradation of a total-etch and two self-etching adhesives bonded to dentin, Journal of dentistry. Vol-36, issue 8, pg 611-7.
20. Hiraishi N, Yiu C, King N, Tay F, (2010), Effect of chlorhexidine incorporation into a self-etching primer on dentine bond strength of a luting cement, Journal of dentistry. Vol-38, issue 6, pg 496-502.
21. Tay FR, Pashley DH, Loushine RJ, Weller RN, Monticelli F, Osorio R, (2006), Self-etching adhesives increase collagenolytic activity in radicular dentin, Journal of endodontics. Vol-32, issue 9, pg 862-8.
22. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, et al, (2006), Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives, European journal of oral sciences. Vol-114, issue 2, pg 160-6.
23. Loguercio AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD, Reis A, (2009), Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability, European journal of oral sciences. Vol-117, issue 5, pg 587-96.
24. Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR, (2010), Ethanol wet-bonding challenges current anti-degradation strategy, Journal of dental research. Vol-89, issue 12, pg 1499-504.
25. Yang B, Adelung R, Ludwig K, Bossmann K, Pashley DH, Kern M, (2005), Effect of structural change of collagen fibrils on the durability of dentin bonding, Biomaterials. Vol-26, issue 24, pg 5021-31.