Effect of 2% Chlorhexidine application on the bonding effectiveness of different dentin adhesives in normal and caries affected dentin in primary teeth: An in vitro study

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**Running title**: effect chlorhexidine bonding strength primary teeth

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**Abstract**

*Objectives:* The bond strength of adhesives to dentin is one of the most important parameters for evaluating the efficacy of dental adhesives.The aim of this study was to evaluate the effect of 2% chlorhexidine on 24 hour shear bond strength of different adhesives in normal and caries affected dentin in primary teeth.

*Methods:* Sixty extracted human primary molars were selected and sectioned in two halves and after preparing a flat surface of superficial dentin, were randomly divided into 12 groups; according to dentin condition (normal and caries affected), type of adhesive (Clearfil SE Bond [SE], Clearfil S3Bond [S3], Single Bond2 [S2]) and treatment (with chlorhexidine [2%CHX] and without 2%CHX ). In S2 group, after etching, CHX were applied and in SE and S3 groups were applied before use of bonding agents. Then composite was attached to the prepared surfaces and cured. The samples were maintained in distilled water at 37 ˚C for 24 hours and then thermocycled. Shear bond strength was calculated. Mode of failures was examined by stereomicroscope. Data were analyzed using t-test and ANOVA with p<0.05 as the level of significance.

*Results:* There were no significant differences in shear bond strength between the different types of adhesives (P=0.9), use of CHX (P=0.22) and type of dentin (P=0.9). The dominant fracture pattern was mixed.

*Significance:* 2% CHX had no effect on 24 hour shear bond strength of adhesive systems to normal and caries affected dentin in primary teeth.

**Key words:** Adhesive, Caries affected dentin, Chlorhexidine, Normal dentin, Primary tooth, Shear bond strength

**Advances in Knowledge:**

1. The effects of chlorhexidine mouthwash administration prior to dentin bonding in primary teeth was evaluated.
2. There was no significant difference in shear bond strength of different adhesives.
3. Administration of chlorhexidine did not affect bond strength of different adhesives.

**Application to patient care:**

1. Applying chlorhexidine prior to administration of bonding agents does not affect bond strength of different adhesives.
2. There is no need to use chlorhexidine prior to restoration of primary teeth with resin composites.

**Introduction:**

Dental caries is the most common pathological change in the dentin. Studies have shown that caries dentin has two distinct layers. The outer layer (caries infected dentin) consists of bacterially infected dentin with high levels of demineralization which cannot be remineralized and is accompanied by irreversible changes and the inner layer (caries affected dentin [CAD]) is affected by caries but is not infected and is relatively demineralized with the capacity to remineralize and thus should be maintained during clinical treatments. For preparing cavities for adhesive restorations, large areas of the cavity floor consists of CAD after removing the outer layer; therefore, the bonding substrate is mostly CAD, not normal dentin (ND) [1].

In general, the efficacy of an adhesive restoration is directly influenced by the quality and stability of the hybrid layer and the main defect of current adhesive systems is their limited durability [3]. Moreover, it seems that the matrix metalloproteinases (MMPs) present in the host's dentin are involved in damaging the quality of the hybrid layer in long term[4]. MMPs are a group of zinc-calcium dependent enzymes in the dentin substrate that regulate the pathological and physiological changes in collagen [3]. In acidic pH situations such as caries or etching, the activity of the MMPs caused the decomposition of denuded fibrillar collagen networks [5].

Previous studies have shown that collagenolytic and gelatinolytic activity could be suppressed using protease inhibitors such as chlorhexidine, sodium hypochlorite, and ethylene diamine tetra acetic acid (EDTA) and they can improve the long term durability and bond strength of hybrid layer. Human dentin contains gelatinase (MMP-8), collagenase (MMP-2,9) and enamelysin (MMP-20) [6]. It has been shown that chlorhexidine has an inhibitory effect on MMP-2,8,9 [3].

Bond strength of adhesives to dentin is one of the most important parameters for evaluating the efficacy of dental adhesives [2].

Most previous studies were done on ND whereas clinicians mostly work on CAD [5]. Moreover, CAD creates weaker bond strength as well as lower quality hybrid layer. Therefore, improving the bonding potential to CAD could enhance the durability of restorations in long term [1].

The aims of this study was to evaluate the effect of 2%chlorhexidine treatment on shear bond strength of self-etch and etch & rinse dentin adhesives in normal and caries affected dentin in primary teeth.

Materials And Methods:

All the procedures were approved by ethics committee of Kerman university of medical sciences, Kerman, Iran (Ethics code: KMUEC/92/333 ). Patients whom extracted teeth were used in the current study gave an informed consent for the use of their teeth in the current research.

This experimental in vitro study was done on sixty non-fractured primary molars extracted due to several reasons. After extraction, all the teeth were immediately washed and disinfected in thymol 0.1% and kept in distilled water at room temperature. Each tooth was sectioned mesiodistally in two halves (buccal and lingual) using a low speed diamond disk with water spray. We selected teeth with sound dentin in one side and superficial dentinal caries on the other side.The cutted surfaces of each sample were sealed with nail varnish.

In order to reach the healthy superficial surface dentin, the teeth were prepared using fissure diamond burs (Tizkavan, Iran) at a depth of 1-1.5 mm. In CAD samples, we prepared samples with superficial dentin caries in total depth of 1-1.5 mm from intact surface. Then, in order to create a flat surface with a standard smear layer, the dentin surfaces of the samples were ground with 400 and 600 grit sand papers under running water. The teeth were mounted in self-cured acrylic resin (AcroPars, Iran) up to the cementoenamel junction (CEJ) so that the prepared and cutted buccal or lingual surfaces were in a vertical position using a surviuor vertical rod. The samples with ND as well as the CAD were randomely divided into six groups according to three adhesives and application or non-application of 2% CHX. Adhesive materials were applied according to the manufacturer’s instructions (table1). In Single Bond2 group, after etching with phosphoric acid, CHX were applied for rewetting of dentin for 30 seconds and in Clearfil SE Bond and Clearfil S3 Bond were applied before use of these adhesive systems. Adhesive were cured with a quartz-tungsten-halogenunit with 600mW/cm2 intensity (Demetron LC, Kerr, USA).

In the next stage, a transparent plastic mold (2 mm internal diameter and 2 mm in height) was externally fixed with compound for placing the composite on the bonding surface and Tetric N-Ceram composite (shade:A1) was placed in two increments to the dentin surface and cured for 40 secends. After curing, the cylinder was gently removed and the composite was cured again for 60 seconds. The samples were stored in distilled water at 37 ˚C in the incubator for 24 hours and then thermocycled (500 cycles between 5-55˚C). Then, the samples were tested for shear bond strength using Universal Testing Machine (M350-10CT Testometric, Lancashire, United Kingdom) at the composite-dentin interface with the cross-head speed of 0.5mm/min[7].

The shear bond strength was calculated in mega Pascal (MPa). For examining the mode of failure, the samples were observed by two examiners under a stereomicroscope (Olympus, DP 12, Germany, 40x) and mode of failures were classified as follows: Adhesive failure (failure occuring within the adhesive between the composite and tooth), Cohesive failure in the dentin, Cohesive failure in the resin composite, and Mixed failure (adhesive and cohesive failures occuring simultaneously) (Table 1).

Data were analyzed using SPSS software, version 18. Three-way multivariate ANOVA was used to assess the effect of different variables (dentin type, adhesive type, and use of chlorhexidine) on bond strength. P<0.05 was considered as statistically significant.

Results:

Mean shear bond strength was summerized in table 2. We found no significant difference in shear bond strength between the different types of adhesives (P=0.9), use of chlorhexidine 2% (P=0.22) and type of dentin (P=0.9). Table 3 shows the failure modes. The dominant fracture pattern was mixed.

Discussion

Compared with permanent teeth, primary teeth have micromorphological, physiological and chemical differences such as less mineralization, smaller dentin tubules, continuous dentin permeability, and higher reactivity to acidic conditioners, all constituting to the lower bond strength to dentin in primary teeth [8].

In etch & rinse adhesives, remaining denuded vulnerable collagen layer at the base of the hybrid layer promotes to degrading them by active host-drived endogenous MMPs.

The reaction between chlorhexidine molecules and the cross-linking groups in the active areas of the demineralized collagen could re-expand exposed collagen fibrils. The impregnated guanidinium groups in chlorhexidine assist the regulation of created changes in the demineralized collagen network [6]. Researchers have confirmed the use of chlorhexidine after etching and before applying any adhesive because it not only reduces bacteria but also inhibits MMPs and thus slows down the degeneration of the adhesive joint interface. In vivo and in vitro studies have previously assessed the application of chlorhexidine with varying concentrations (0.002-5%) and shown lesser decrease in long-term bond strength due to hybrid layer durability [9]. In CAD (compared with ND) more collagenolytic activity has been reported which indicates faster bonding degradation [5,6,9,10].

We found that 2% chlorhexidine did not have a significant effect on 24 hour shear bond strength of Clearfil SE Bond, Single Bond 2, and Clearfil S3 Bond to healthy and caries affected dentin in primary molars,

but Vieira et al. study concluded that cavity treatment with 2% chlorhexidine led to reduced shear bond strength of Single Bond to primary dentin [11]. We found similar results in our study with respect to the different adhesive systems, but some studies have shown that the mean bond strength is lower in CAD compared with ND [11- 13]. Wei and colleagues found Single bond 2 have a high bond strength to both healthy and caries affected dentin in permanent teeth but SE Bond had a higher bond strength in ND compared to CAD [2]. The specific characteristics of CAD are as follows: 1) caries intertubular dentin has a higher amount of porosity compared to ND which is directly correlated with demineralization of the decay. 2) Due to demineralization, CAD is softer than healthy dentin. 3) The existence of whithlockite mineral in the tubules which could interfere with the infiltration of resin monomers [2].

Nakajima and colleagues found that one of the reasons for reduced bond strength to CAD compared with ND was the reduced cohesive strength in CAD in permanent teeth. Even if caries affected and ND has similar intertubular hardness, bond strength is still considerably weaker in CAD as a result of morphological and chemical changes [1].

In CAD, the wet bonding technique is more suitable than dry bonding techniques for the infiltration of monomers in deeper demineralized etched layers since it leads to higher bond strength [6]. In this study, we use wet bonding technique that probably caused equal bond strength to ND and CAD. CAD contains free radicals that interfere with the polymerization of the adhesives in deeper areas. It has been reported that the infiltration adhesives in acid etched CAD layers is less than that of ND [6]. The role of microcanals or large tubules of primary dentin in the bonding process is unclear, but it could be related to weaker bond strength in primary dentin [14]. Considering the characteristics of CAD, stronger acid, and more time needed for dissolving the mineral deposits in CAD. Studies have shown that by increasing the etching time of phosphoric acid 35% from 15 seconds to 45 seconds, higher bond strength to CAD would be reached, although the bond strength would still be lower than ND. Increased etching time caused higher depth of demineralization but the gap between the demineralized region and resin monomer infiltration cannot be filled and this weaken bond strength [1].

Nor and co-workers suggested that lesser time for etching the primary dentin could lead to thinner hybrid layer with more complete resin infiltration [15]. If we suppose that the lower bond strength in CAD is related to the resin infiltration to the tubule in the form of resin tag, longer etching time and stronger etchant is used. If a longer etching time is used to dissolve the tubular deposits, the longer etching time would also produce a deeper and more complex demineralized layer, which could destroy the bond interface after time [16].

In the self-etch adhesive system, less difference is observed between the depth of the demineralized region and resin monomer penetration because demineralization and monomer penetration occur simultaneously. This system cannot dissolve acid-resistant mineral deposits in CAD tubules because of a higher pH. Although in our study there were not significant differences between different adhesives, theetch & rinse system because of their, were advocated although they cannot completely dissolve them [1].

In our study the dominant failure pattern in most groups was the mixed and adhesive types. If bond strength to enamel or dentin's higher than 20 MPa, mode of failure happened cohesive predominantly in composite or tooth [17].

**Conclusion:**

The results of this study showed that 2% CHX had no effect on the 24 hour shear bond strength of adhesive systems to normal and caries affected dentin in primary teeth.

**Conflict of interest:**

The authors declare no conflict of interest.

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**Table 1.** **Materials used in this study.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Application** | **Composition** | **Manufacturer** | **Type** | **Material** |
| Etching: 15 seconds, Rinse:10 s, Blot dry, CHX application, apply 2 coats of adhesive:15 s with gentle agitation, Gently air thin:5s, cure for 10 s | BisGMA, HEMA, dimethacrylates, ethanol, water, a novel photoinitiator system and a methacrylate functional copolymer of polyacrylic and  polyitaconic acids | 3M, ESPE, USA | Two-step etch & rinse Adhesive | **Single Bond2** |
| CHX application, Apply primer, leave undistributed for 20 s, apply bond , air thin :5 s, cure for 10 s | **Primer:**10-MDP, HEMA, hydrophilic dimethacrylate,  photo-initiator and water  **Adhesive:** 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate  and microfiller | Kuraray, Japan | Two-step etch& rinse Adhesive | **Clearfil SE Bond** |
| CHX application, Apply adhesive for10 s, Gently air thin:5s, cure for 10 s | MDP, HEMA, bis-GMA, water, ethanol, DL-camphorquinon , silinated colloidal silica | Kuraray, Japan | One-step self-etch  Adhesive | **Clearfil S3 Bond** |
|  | Chlorhexidine gluconate ,ethyl alcohol | Consepsis, USA | Antibacterial Solution | **Chlorhexidine2%** |
|  | Dimethacrylates, fillers:barium glass, yettrbium trifloride, mixed oxide and copolymers ,catalysts,stabilizers,pigment | Ivoclar Vivadent , Schaan,  Liechtenstein | Light curing composite | **Tetric N-ceram** |
|  | 35% phosphoric acid | Ultradent,  USA | Etching agent | **Ultra Etch** |

**Table 2.** **Shear bond strength data in mega Pascal (mean±SD) for each tested group.**

|  |
| --- |
| SingleBond2 Clearfil SE Bond Clearfil S3Bond  CAD N D CAD ND CAD ND |
| With 2% CHX 14.57[ 3.32] a 13.42[4.73]a 14.41[4.33]a 11.72[6.09]a 11.04[4.78]  a 15.70[2.24]a |
| Without 2%CHX 14.59[6.11]a 13.81[5.40]a 16.99[2.89] a 12.17[4.58]a 17.27[4.52]a 12.49[5.57]a |
| a : for horizontal row and vertical column were not significantly different were labeled by the same word |

**Table 3: Mean percentage of failure mode after shear bond strength test**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Mixed | Adhesive | Cohesive in composite | Cohesive in dentin | Mode of failure  Groups | | |
| 3(30%) | 7(70%) | 0(0%) | 0(0%) | With 2% CHX | Clearfil S3Bond | **Normal dentin** |
| 6(60%) | 3(30%) | 0(0%) | 1(10%) | Without 2%CHX |
| 9(90%) | 1(10%) | 0(0%) | 0(o%) | With 2% CHX | Single Bond2 |
| 4(40%) | 5(50%) | 0(0%) | 1(10%) | Without 2%CHX |
| 7(70%) | 3(30%) | 0(0%) | 0(0%) | With 2% CHX | Clearfil SE Bond |
| 5(50%) | 4(40%) | 0(0%) | 1(10%) | Without 2%CHX |
| 3(30%) | 6(60%) | 1(10%) | 0(0%) | With 2% CHX | Clearfil S3Bond | **Caries affected dentin** |
| 3(33%) | 4(44%) | 1(11%) | 1(11%) | Without 2%CHX |
| 6(60%) | 3(30%) | 0(0%) | 1(10%) | With 2% CHX | Single Bond2 |
| 4(40%) | 4(40%) | 1(10%) | 1(10%) | Without 2%CHX |
| 7(70%) | 3(30%) | 0(0%) | 0(0%) | With 2% CHX | Clearfil SE Bond |
| 6(60%) | 1(10%) | 0(0%) | 3(30%) | Without 2%CHX |