Effect of Chlorhexidine Application on Bond Durability of a Filled-Adhesive System

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Abstract

Introduction: The aim of this study was to evaluate the effect of chlorhexidine (CHX) application on immediate and two-month bond strength of a filled adhesive system. **Methods:** Forty eight non-carious human third molars without enamel of occlusal surface were randomly divided into two main groups according to CHX application: control and CHX. Each main group was further subdivided into 2 subgroups depending on storage time: immediate or two-month collagenase storage. In CHX groups, 2% CHX was applied for 30 s. Filled adhesive resin (PQ1) was applied on the etched dentin surfaces and cured in all experimental groups. Composite resin (Amelogen) was placed on the dentin surfaces and shear bond strength (SBS) was evaluated immediately and after two-month storage in collagenase solution. Failure modes were determined by stereomicroscope. **Results:** There was no significant difference in immediate SBS between groups ($P>0.05$). After two-month collagenase storage, although SBS was significantly reduced in control group ($P<0.05$), no significant reduction was observed in CHX group ($P>0.05$). SBS in CHX group was significantly higher than the control group ($P<0.05$). **Conclusion:** The use of CHX had no adverse effect on immediate bond strength of filled adhesive and could preserve the bond strength.

Key Words: Bond durability, chlorhexidine, collagenase, filled adhesive.

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Introduction

Nowadays, lack of durability of bond to tooth structure is one of the most important problems in application of dental adhesive systems especially in the etch-and-rinse adhesives. A variety of *in vivo* and *in vitro* studies have provided evidences that, load cycling, thermocycling, fatigue and hydrolysis phenomenon can degrade the bond (1,2). Also, incorporation of ionic or polar monomers in single step etch-and-rinse adhesive systems may reduce the bond durability by enhancing water sorption and hydrolysis phenomenon (2). In other aspect, Pashley et al. (3) showed that matrix metalloprotenase enzymes (MMPs) can degrade the collagen fibril of dentin substrate which does not protect with resin infiltration during the bonding procedure, so the bond strength or durability of bond will decrease.

Matrix metalloproteinases (MMPs) are zinc- and calcium-dependent proteolytic enzymes, which in oral environment exist in saliva, dentinal fluid, and bacterial products (2,3). MMPs can degrade the extracellular matrix of connective tissue such as collagen and gelatin as one of their physiologic roles (2,4). Five types out of 23 different MMPs exists in dentin matrix including MMPs 2, 3, 8, 9, 20 (4). They release as proenzymes and change into active form by different mechanisms such as activation of other enzymes and alteration in temperature or PH; the latter is a condition, which may be provided during etching process (4). An accepted method for decreasing the activity or inhibiting the MMPs is the application of MMPs inhibitors. Various studies have been demonstrated the efficacy of these materials for preserving the bond strength (5,6).

Recently, some studies have confirmed that chlorhexidine (CHX) which has been used as disinfectant in dentistry (7), can act as MMPs inhibitors and improves the durability of bond (8,9). However,

there is contrary in literature about the effect of CHX on immediate bond strength of composite to dentin. Some investigators concluded that CHX decreases the bond strength (7,10) but, others reported that CHX has no adverse effect on it (11,12).

There is a little information about effect of CHX on filled adhesives that have more viscosity than unfilled-adhesive systems. It was assumed that CHX application may more interfere with resin infiltration of these adhesives as a result of their viscosity.

The aim of current study was to evaluate the effect of CHX application on immediate bond strength and durability of a filled etch-and-rinse adhesive. The first null hypothesis was that CHX application has no adverse effect on immediate bond strength. Another null hypothesis was that CHX pretreatment has no effect on durability of bond after collagenase storage.

Materials and Methods

Forty eight non-carious human third molar teeth were collected, cleaned, and stored in 4% thymol solution at 4°C. Then the samples were mounted perpendicular to the horizontal plane in the self cure acryl resin (Acropars 200, Marlic Medical Industries Co, Tehran, Iran). Enamel of occlusal surfaces were removed by using a diamond disk, and dentin surfaces were flattened and polished with silicon-carbide (400, 600, 800, 1200, and 2000 grit). Specimens randomly divided into two groups of 24 samples by using the randomization table according to the numbers written on each block.

Dentin surfaces were etched by 37% phosphoric acid gel (Ultra-etch, Ultradent, USA) for 20 seconds, rinsed with distilled water for 10 seconds, and dried with air spray for 10 seconds. Adhesive resin (PQ1, Ultradent, USA) was applied on the surfaces according to the manufacture's instruction and cured (Blue Phase C8, Ivoclar Vivadent, Schaan, Lichtenstien) for 20 seconds. The first layer of composite resin (Amelogen, Ultradent, USA) was placed with 1 mm thickness inside a washer with 2 mm diameter, which mounted on the dentinal surface, and cured for 40 seconds. Two other composite increments of 1.5 mm were placed and cured for 40 seconds. Bulk of composite was post-cured for additional 40 seconds.

Prepared specimens were randomly divided into two subgroups. In the first subgroup, specimens were stored in 100% humidity for 24 hours and shear bond strength (SBS) was evaluated by universal testing machine (Zwick/Z250, Zwick Roell Group, Ulm, Germany) with crosshead speed of 1 $^{mm}/_{min}$. In the second subgroup, teeth were stored in 100 µg/ml collagenase solution (Sigma-Allorich, USA) for two months, and SBS was assessed with the same method of the former subgroup.

Second group (CHX group) had the same process of the first group but, before applying adhesive, aqueous solution of 2% CHX (Consepsis, Ultradent, USA) was placed on the dentinal surface with micro brush (Pegasus, Astek Innovations, England) and after 30 seconds, remnants were removed by a clean micro brush.

Failure modes were observed by using stereomicroscope (LEO, 1450 UP, Germany), and classified as adhesive, cohesive of dentin, cohesive of composite and mixed. Data were analyzed by two-way ANOVA and Tukey's post-hoc test at the pre-set significance level of 0.05 by SPSS 16 software. Chi-square test was used for evaluation of failure modes.

Results

The mean shear bond strength of experimental groups is presented in Table 1. Kolmogorov-Smirnov test showed normal distribution of data ($P<0.05$). There was no significant difference in immediate SBS among groups ($P=0.164>0.05$). After two-month storage in collagenase solution, SBS of CHX group was significantly higher than the control group ($P=0.00<0.05$). In the control group, SBS after two-month storage in collagenase solution was significantly lower than immediate SBS ($P=0.00<0.05$). However, there was no significant difference in SBS of immediately and after two-month storage in CHX group. ($P=0.261<0.05$).

Results of failure modes were displayed in Table 2. The most frequently observed failure mode was adhesive failure in all experimental groups. Chi-square test showed failure modes and bond strength were independent ($P=0.756>0.05$).

Table 1. The mean shear bond strength (MPa) of experimental groups

Groups		N	Mean	Std. Deviation	Std. Error
Control	Immediate	12	14.670	1.506	0.434
	Collagenase	12	10.334	1.448	0.418
CHX	Immediate	12	15.609	1.683	0.485
	Collagenase	12	14.746	1.969	0.568

There was no significant difference in immediate SBS among groups ($P=0.164>0.05$). After two-month storage in collagenase solution, SBS of CHX group was significantly higher than the control group ($P=0.00<0.05$)

Table 2. Distribution of failure mode in experimental groups

Groups		Adhesive	Mixed	Cohesive (dentine)	Cohesive (composite)
Immediate	Control	7	3	2	0
	CHX	6	3	3	0
Collagenase storage	Control	9	3	0	0
	CHX	8	2	2	0

Chi-square test showed failure modes and bond strength were independent (P=0.756>0.05)

Discussion

Application of 2% CHX prior to applying the adhesive resin had no adverse effect on immediate bond strength and; therefore, the first hypothesis was accepted. This result is supported by other studies (2,13). The increase in the surface energy of tooth structure and the wetting-ability of adhesives by application of CHX may be an explanation for this (14). By the contrast, some studies showed that CHX can interfere with bonding procedure and reduce the bond strength or increase the microleakage especially in self etch adhesive systems (15,16). Owing to cationic properties, CHX can bind to phosphate groups of apatite in smear layer or dentin surface that may have adverse effect on the resin infiltrations (16). Furthermore, one should keep in mind that the most ingredient of CHX solution is water and application of it on dentin surface after acid-etch may make an over wetted surface so; wetness control should be done carefully after application of CHX (14).

The result of current study showed that application of CHX can preserve the bond strength and therefore the second hypothesis was rejected. The same result was obtained by previous studies (8,9). Inhibition of MMPs 2, 8, 9 by CHX via chelating mechanism is the most accepted explanation for it. Moreover, CHX can inhibit the cycteine cathepsins enzyme, another collagenolytic enzyme recently has been found in dentin (17).

In the present study, CHX with 2% concentration was used. Campus et al. (18) showed that lower concentration (0.2%) of CHX cannot preserve the bond strength and may decrease immediate bond strength. On the other hand, another study concluded that CHX concentration have no effect on bond strength value (2). Carrilho et al. showed that substantivity of CHX to human dentin depends neither to concentration nor time of CHX applied and it can bind to tooth structure via cationic part of its molecule (19). One of the disadvantages of CHX application is the augmentation of the bonding procedure steps. Although, some studies showed that adding the CHX to acid-etch solution or primer of self etch adhesives may inhibit the bond degradation without any additional step (20,21).

Previous studies demonstrated that PQ1 adhesive, an ethanol-based filled adhesive, has a slightly better durability (22), bond strength (23), and less microleakage (24) in comparison with unfilled adhesives. The outcome of present study supports the theory of preservation of bond of this filled adhesive by CHX without any adverse effect on immediate bond strength.

The collagenase solution in this study was prepared according to previous study that used the same enzyme (25). This enzyme either releases from dentin (endogenous MMPs) (26) or exogenous MMPs (9,25), has ability to cleave the clinically exposed collagen and degrade the bond strength. This theory was supported by result of current study that specimens exposed to both endogenous and bacterial collagenase. By the contrast, Toledano et al. (27) concluded that exogenous collagenase has not more adverse effect on bond durability than water storage. Large size of collagenase and infiltration of resin among the demineralized collagen fibril make some limitation for the enzyme to reach to the cleavage site (28). Although, collagenase seems to have the ability to penetrate into resin-dentin interface via porous and non-resin infiltrated sites (29). It should be considered that MMPs need water for activation (2), and one of most important factors that decreases the durability of bond is hydrolytic degradation phenomenon (3). Without water, the bond degradation may not progress or slightly increased. Various studies showed that oil storage can preserve the bond strength (3,30). Furthermore, water can leach out uncured or hydrophilic monomer especially in simplified adhesive systems and exposed more collagen fibril for degradation by MMPs (2). In oral environment, elimination of water is not possible. So, other alternatives such as application of hydrophobic resin layer (31) or use three step etch and rinse (32), use ethanol-wet bonding (33), MMPs inhibitors (5-6), collagen cross linkers (28) , increasing the degree of conversion and use of esterase resistant adhesive may be helpful (17).

Although after two months storage in collagenase, the adhesive failure mode was slightly increased in both groups, there was no significant difference in failure

modes among the experimental groups. Previous studies showed there is no relation between bond strength and failure modes that support by results of current study (34).

Due to limitation of this study only a filled ethanol-base adhesive was evaluated. So, the results of this study may not attribute to all filled adhesives. Also, the authors suggested a further study to evaluate the effect of mixed solution of MMPs inhibitor with collagen cross linker on durability of bond to demonstrate whether or not it has cumulative preserving effect compared with MMPs inhibitor or cross linker alone.

Conclusion

The application of CHX as MMPs inhibitor has no adverse effect on immediate shear bond strength of filled adhesive and can preserve the bond strength.

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