Original Article

Effect of Chlorhexidine on Micro-Shear Bond Strength of Two Adhesive Systems After Aging

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Abstract

Background and Aim: Recurrent caries and low bond strength are the main causes of composite restorations failure. This study sought to assess the effect of chlorhexidine (CHX) on micro-shear bond strength (MSBS) to dentin of a fourth generation adhesive system and a universal bonding agent after aging.

Materials and Methods: This in vitro study used 32 extracted third molars and 3-5mm thick dentin slices were cut out of each tooth. Specimens were randomly divided into 8 groups. After etching, Scotchbond fourth generation bonding agent or Single Bond universal adhesive was applied on dentin surfaces and Z250 composite cylinders were bonded to the surfaces. In groups B, D, F and H, CHX was applied for 1 minute after etching and prior to the application of bonding agent. The MSBS was measured after 24 hours and four months of water storage at 37°C using a micro-tensile tester. The collected data were analyzed using three-way ANOVA.

Results: The Scotchbond yielded significantly higher MSBS than the universal adhesive at 24 hours and four months ($P<0.001$). Application of CHX significantly increased the MSBS of both adhesives to dentin at 24 hours and four months ($P<0.001$). The bond strength decreased over the time irrespective of the type of bonding system ($P<0.001$).

Conclusion: The MSBS of Scotchbond fourth generation bonding agent was generally higher than that of Single Bond universal adhesive. The MSBS of both adhesives decreased over time, however, the application of CHX decelerated the deterioration rate.

Key Words: Adhesives, Chlorhexidine, Shear Strength

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Introduction

Low bond strength, microleakage, and recurrence of caries are the main causes of failure of composite restorations. Dentin is rich in organic materials and minerals and has higher water content than enamel. Due to this complex structure, bond to dentin is more challenging than the bond to enamel. Researchers have long been in search of strategies to increase resin-dentin bond strength. However, early loss of bond strength and subsequently decreased the durability of adhesive restorations remains a challenge in adhesive dentistry [1,2]. Despite recent advances in the formulations of dental adhesives, evidence shows that bond strength significantly decreases over time due to the aging of restorations [3].

The fourth generation etch-and-rinse bonding agents contain three separate components of

etchant, primer, and adhesive. Despite being time-consuming, this bonding system has a high success rate. However, the risk of over-etching and/or over-drying of dentin surface are among its drawbacks which make it very technique sensitive [4]. In an attempt to overcome these shortcomings, one-step universal adhesives were introduced to the market, which can be used as self-etch, total-etch or selective-etch for direct and indirect restorations [5]. Nonetheless, due to their relatively recent introduction to the market, their durability in the oral environment needs to be further evaluated [6].

Chlorhexidine (CHX) has long been used as an effective antibacterial agent in periodontal therapy and endodontic treatment [7]. It has been shown that application of CHX decreases the activity of matrix metalloproteinases (MMPs) due to its anti-proteolytic activity and consequently increases the durability of bonding agents [8-12]. However, some studies showed that application of CHX after acid etching and prior to the application of bonding agent had no positive effect on bond strength of restorative materials to tooth structure [13,14]. Considering the gap of information on the strength and durability of the bond provided by universal adhesives and the existing controversy regarding the effects of CHX on bond strength, this study sought to assess the effect of CHX on the micro-shear bond strength (MSBS) of a fourth generation bonding agent as the gold standard and a universal adhesive to dentin after aging by water storage for 24 hours and four months.

Materials and Methods

This in vitro experimental study was used 32 third molars which were extracted within six months of the experiment from patients between 20-40 years old. The teeth were selected using consecutive sampling and examined to be free from cracks, fractures, and caries. The sample size was calculated to be a minimum of 16 samples in each group based on the study by Deng et al, [15] using Minitab software. In this calculation, α=0.05, β=0.2, and minimum significant difference and standard deviation were 6 and 3.1 respectively.

Teeth were randomly divided into eight groups, crowns were cut at the cementoenamel junction, and then their crowns were sectioned mesiodistally into buccal and lingual halves. The enamel on the buccal and lingual surfaces was ground with a long-shank cylindrical bur and high-speed handpiece in order to expose dentin, followed by polishing the surfaces with a series of polishing discs from P800 to P1200 (3M ESPE, St. Paul, MN, USA). From each tooth, at least 4 dentinal slices were prepared and the final thickness of dentin slices was between 3-5mm. Dentine slices were randomly divided into 8 groups each containing 16 specimens.

Sample Preparation of the eight experimental groups:

The specifications of the materials used in the present study and their composition are summarized in Table 1. In all experimental groups, the external surface of dentin slices was etched with 37% phosphoric acid (Alpha Etch, Nova DFL, Rio de Janeiro, Brazil) for 15 seconds followed by rinsing for 30 seconds. Etched dentin was dried gently with air spray until its surface remained slightly moist. In group A and E Scotchbond (3M ESPE, St. Paul, MN, USA) was then applied according to the manufacturer's instructions and cured for 10 seconds using a light curing unit (Coltolux 2.5, Coltene, USA) with a light intensity of 600mW/cm^2. While in the group B and F, 2% CHX (Concepsis solution, Ultradent Products Inc., South Jordan, UT, USA) was applied on the surface for one minute after etching and prior to the application of bonding agent.

The same procedure as group A, B, E, and F was followed in the group C, D, G, and H respectively except Single Bond universal adhesive (3M ESPE, St. Paul, MN, USA) was used as the bonding agent. Then, an A2 shade of Z250 composite resin (3M ESPE, St. Paul, MN, USA) was packed into Tygon tubes with a round cross-section of 0.7mm internal diameter and 2mm height. The tubes were placed on the dentin surface and light cured for 40 seconds. After curing, Tygon tubes were separated from the composite cylinders using a scalpel. In the present study, group A served as the control.

Specimens in groups A to D were stored in saline for 24 hours before MSBS testing. Whereas in group E to H, MSBS was measured after four months in which, specimens were kept in saline at 37°C. The test groups' descriptions of the present study are summarized in Table 2.

Table 1. The specifications of the materials used in the present study

Materials	Composition	Manufacture
Scotch bond	Primer: HEMA, polyalkenoic acid polymer, Water Bonding :Bis-GMA, HEMA Tertiary amines, photo initiator	3M ESPE, St. Paul, MN, USA
Single Bond universal	MDP phosphate monomer, Dimethacrylate resins, HEMA, Vitrebond™Copolymer,Filler, Ethanol, Water ,Initiator ,Silane	3M ESPE, St. Paul, MN, USA
Z 250	Martix: Bis GMA, UDMA TEGDMA, Bis EMA Filler: Zirconia/ silica (0/01 – 3/5 μm) 84/5%WT	3M ESPE, St. Paul, MN, USA
Concepsis solution	Cholorhexidine Digluconate 2%. Water	Ultradent products, South Jordan, UT, USA

Table 2. The test groups' specifications in the present study

Groups	Type of Treatment	Storage time
A	Scotchbond	24 hours
B	Scotchbond + CHX	24 hours
C	Single Bond universal adhesive	24 hours
D	Single Bond universal adhesive + CHX	24 hours
E	Scotchbond	four months
F	Scotchbond + CHX	four months
G	Single Bond universal adhesive	four months
H	Single Bond universal adhesive + CHX	four months

Bond strength test:

For MSBS testing, the samples were mounted on a test block jig of a Bisco micro-tensile tester (Bisco Inc., Schaumburg, IL, USA). An orthodontic wire was looped around the composite cylinder at the composite-dentin interface and load was applied at a crosshead speed of 1mm/minute until the bond failure. The fracture load (recorded in N) was divided by the cross-sectional area of the bonding surface and the bond strength was reported in MPa.

Statistical analysis:

The data were analyzed using SPSS version 20 (SPSS Inc., IL, USA). Three-way ANOVA was used to assess the collected data. $P<0.05$ was considered statistically significant.

Results

Descriptive statistic results of the MSBS measurement are shown in Table 3. Statistical analysis revealed that MSBS of Scotchbond (fourth generation bonding agent) significantly decreased after four months of aging ($P<0.001$). The results also showed that the groups used Single Bond universal as the adhesive agent had significantly lower mean MSBS compare to counterpart groups which used Scotchbond ($P<0.001$).

The use of CHX to dentin prior to applying either bonding system significantly increased the MSBS regardless of their storage time ($P<0.001$).

In the group A, the mean MSBS of Scotch Bond was 16.78±2.36 Mpa while in the group E, which similar specimens were stored for 4 months, the mean MSBS of the Scotch Bond was 11.95±1.75Mpa. Accordingly, the bond strength of Scotch Bond (4th generation bonding) significantly decreased after 4-month aging ($P<0.001$). The mean MSBS of the Single Bond universal adhesive (group C) was 12.73 ±1.84 Mpa after 24 hours, however, after 4 months of storing at 37°C, the mean MSBS of Single Bond decreased to 10.57±1.72Mpa ($P< 0.001$).

Group B and F were the counterparts of group A and E, respectively, except chlorhexidine was applied before bonding agent. The mean shear bond

Table 3. The descriptive statistics of microshear bond strength of four tested groups

Chlorhexidine	Bonding agent	Group n=16	Time	Mean	Standard deviation	Minimum	Maximum
With Chlorhexidine	Scotch bond	B	24 hours	17.55	1.98	13.42	20.52
		F	Four months	13.67	2.94	10.00	20.26
	Single Bond universal	D	24 hours	14.46	2.84	9.47	19.47
		H	Four months	12.36	2.19	8.42	16.57
Without Chlorhexi-dine	scotch bond	A	24 hours	16.78	2.36	12.89	19.21
		E	Four months	11.95	1.75	10.00	14.73
	Single Bond universal	C	24 hours	12.73	1.84	10.00	15.78
		G	Four months	10.57	1.72	8.15	13.42

strength of group B was 17.55±1.98 Mpa and 13.67±2.94 Mpa in group F. The mean shear bond strength of group D and H, which are the counterparts of group C and G, were 14.46 ± 2.84 MPa and 12.36±2.19Mpa respectively. Statistical analysis revealed CHX significantly increase the MSBS (P<0.001). Results of the present study have also show that the application of CHX slowed the bond strength reduction process during 4 months storage time (P<0.001). Additionally, the type of bonding has significantly influenced on MSBS; the 4th generation adhesive system was significantly higher than the universal bond (P<0.001).

Discussion

The present study evaluated the effect of CHX on MSBS of a fourth generation bonding agent and a universal adhesive after 24 hours and four months of storage. The results have shown that the use of CHX after etching had no adverse effect on bond strength and yet improved it. After four months of saline storage, micro-shear bond measurement revealed that CHX increased the bond durability and reduced the rate of bond diminution in both groups of Scotchbond fourth generation bonding agent and Single Bond universal adhesive [16,17].

Our results have shown that regardless of dentin treatment and storage time, Scotchbond three-step total-etch adhesive system had higher bond strength compared to self-etch universal adhesive. Universal adhesives contain MDP, which forms a chemical bond to dentin. Yoshida et al. [18] have demonstrated that chemical interactions between MDP and hydroxyapatite created a nano-layer at the adhesive interface, which was a strong phase and increased the bond strength of the adhesive system. Universal adhesives have a moderate pH of 3 [16]; this level of acidity does not remove the smear layer and thus, the smear layer remains and serves as a barrier against resin penetration into dentin and consequently prevents the formation of hybrid layer [5,16]. Moreover, polyalkenoic acid is present in the composition of one-step universal adhesives. This copolymer competes with the MDP in bonding to the hydroxyapatite crystals of dentin and interferes with the bond of MDP to dentin [5,16]. Furthermore, the polyalkenoic acid copolymer has high molecular weight and prevents the approximation of monomers during polymerization which results in the reduction of the degree of polymerization of the bonding system and subsequently, the bond strength [17]. The amount of MDP is lower in one-step systems compare to two-step self-etch and total-etch systems since the priming and bonding agents are blended in the one bottle [18]. This might explain the lower bond strength of one-step universal

adhesive compared to the three-step total-etch system in the current study [17].

As a disinfecting agent, CHX can be used after cavity preparation and prior to etching [19]. The use of CHX, as an antibacterial agent, has been suggested for cleaning the cavity surface prior to the restoration. Researchers believe that 2% CHX decreases the microbial count, particularly mutans streptococci, in the dentinal tubules in short-term [20]. In the current study, CHX increased the MSBS in both adhesive groups. However, some previous studies reported a negative effect of CHX on bond strength in the first 24 hours [13,14]. de Castro et al. [19] showed that CHX had no adverse effect on bond strength, which may be attributed to the fact that CHX increased the surface energy of the tooth structure and consequently enhanced the wettability of dentin by adhesive. However, most CHX formulations are water soluble, therefore, after etching, moisture control would be difficult following the application of CHX. The difference in the moisture content of the specimens could be a factor in the lack of uniformity in the results of various studies [19]

Furthermore, CHX has an inhibitory effect on collagenolytic MMPs. It has been confirmed that application of CHX after etching in total-etch bonding systems, would increase the durability of the bond to dentin due to its inhibitory effects on these enzymes [8, 21]. In the current study, application of CHX declined the bond strength reduction in both adhesive systems after four months. Brackett et al. [22] assessed the effect of using 2% CHX after etching on the durability of the microtensile bond strength of Single Bond total-etch system and reported that in CHX group, deterioration of the hybrid layers was slightly slower than the control group, however, its effect on bond strength was not significant.

De Munck et al. [23] observed that the application of CHX mainly altered the total-etch bonding systems and had no significant effect on the self-etch systems. It appeared that in self-etch systems with mild acidity, the release of endogenous collagenolytic MMPs does not occur while in total-etch systems, the release of endogenous enzymes is significant. In the other words, factors such as water sorption at the bonding interface were the main contributing factor for the deterioration of the hybrid layer although, in total-etch systems, the release of proteolytic enzymes enhanced this destructive process [23].

Microshear bond strength test was performed in the current study. In this method, a higher number of samples can be prepared from each tooth compared to other methods. Moreover, the load is better distributed in this method due to the small dimensions of specimens [16]. In an earlier study on micro-bond strength measurement, it was reported that the mean MSBS values were approximately one-third of the microtensile bond strength values [24].

Despite extensive studies on the mechanism of hybrid layer diminishing, a definite conclusion has yet to be drawn and further investigation is needed. Adequate sample size, evaluation of a highly debated topic with the use of a gold standard bonding agent, and newly introduced universal adhesive were among the strengths of this study. However, this was an in vitro study and therefore, a generalization of the results to the clinical setting must be done with caution since many factors are involved which could not be completely simulated in vitro. Future in vitro studies may consider the use of artificial saliva for the storage of specimens and the use of thermocycling and cyclic loading for aging the samples in order to better replicate the clinical setting. Furthermore, the effect of CHX on bond strength of other bonding systems to dentin and in longer periods of time should be evaluated in future studies.

Conclusion

Within the limitations of this in vitro study, the results showed that the MSBS of Scotchbond fourth generation bonding agent was generally higher than Single Bond universal adhesive. The MSBS of both adhesives decreased over time, however, the application of CHX results in decreasing the rate of this course.

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