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Effect of Chlorhexidine Application Methods on Microtensile Bond Strength to Dentin in Class I Cavities

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Clinical Relevance

In order to secure high bond strength in clinical practice, 2% chlorhexidine application after etching with 37% phosphoric acid is the recommended procedure.

SUMMARY

Objective: To evaluate the influence of chlorhexidine with different application methods on the microtensile bond strength (μTBS) to dentin in Class I cavities.

Methods: Flat dentinal surfaces with Class I cavities (4 mm long, 4 mm wide, 2 mm deep) in 50 molars were bonded with Adper Single Bond 2 etch-and-rinse adhesive system after: 1) Chlorhexidine (CHX) + rinsing + etching; 2) CHX + no rinsing + etching; 3) Etching + CHX + rinsing; 4) Etching + CHX + no rinsing; 5) Etching only as a control group. Resin composite buildups were constructed with Z-350 (3M ESPE) using a bulk method and they were polymerized for 40 seconds. For each condition, half of the specimens were immediately submitted to microtensile bond strength and half of the same group was submitted to 10,000 cycles of thermocycling between 5°C and 55°C before testing. The data were analyzed using the two-way ANOVA and Student *t*-test at a 95% significance level.

Results: Chlorhexidine pretreatment did not affect the bond strength of specimens tested at the immediate testing period, regardless of the application method used, compared to the control group. However, after 10,000 thermocycles, a significant bond strength reduction was found in the control group.

In general, the chlorhexidine application method did not demonstrate a significant difference among the groups treated with chlorhexidine. However, there was a significant difference after thermocycling in the groups treated with chlorhexidine before etching and no significant difference in the groups treated with chlorhexidine after etching.

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Conclusion: Considering the amount of reduction in bond strength after thermocycling, the current study showed improved dentin bond strength with chlorhexidine when used after etching.

INTRODUCTION

The longevity of resin restorations is currently an area of interest in adhesive dentistry. Throughout the last two decades, chemical and technical advances have contributed to increased resin-dentin bond strength. However, premature loss of bond strength is one of the problems that still affects adhesive restorations[1] and markedly reduces their durability.[2] It had been widely stated that resin-dentin bonds obtained with contemporary adhesive systems can deteriorate over time,[3] and durability of the bond between dentin and resinous adhesives may not be as durable as was previously assumed.[4-5] The loss of bond strength has mainly been attributed to degradation of the hybrid layer at the dentin-adhesive interface and deterioration of the dentin collagen fibrils. Numerous publications have demonstrated the lack of bond stability.[6-9] The notion that deterioration of dentin collagen fibrils contributes to the mechanism responsible for bond degradation has been established.[10]

It has been speculated that a decreasing concentration gradient of resin monomer diffusion within acid-etched dentin and a subsequent resin elution from hydrolytically unstable polymeric hydrogels within the hybrid layers, leaves the collagen fibrils unprotected and vulnerable to degradation by endogenous matrix metalloproteinases (MMPs), a class of zinc-and calcium-dependent endopeptidases.[6] These MMPs are capable of degrading the organic matrix of demineralized dentin[11] and can reach the exposed collagen fibrils at the base of the hybrid layer that were originated from the deficient resin infiltration within the demineralized dentin matrix, resulting in hydrolytic degradation and a reduction in bond strengths.[12]

Chlorhexidine (CHX) is widely used as an antimicrobial agent and possesses a broad spectrum of activity against oral bacteria.[13] It has been stated that the currently accepted disinfection technique, which applies CHX to acid-etched dentin, may prevent the degradation of collagen fibrils.[3] Thus, apart from being a commonly known disinfectant, CHX also functions as a potent MMP inhibitor.[14] CHX also prevents or minimizes the auto-degradation of exposed collagen fibrils within incompletely-formed hybrid layers, thereby contributing to the long-term stability of the hybrid layer and bond strength.[4] Additionally, CHX may also be a useful complementary method to other techniques of proven efficacy for rehydrating dried mineralized dentin and, therefore, preserving the humidity necessary for keeping the collagen network expanded.[15] However, any positive benefits would be negated if CHX interferes with a hydrophilic resin's ability to wet and micromechanically bond to dentin.

The current *in vitro* study evaluated the influence of CHX with different application methods on microtensile bonds strength (µTBS) to dentin in Class I cavities. The tested hypotheses were: 1) CHX does not cause a detrimental effect on microtensile bond strength to dentin; 2) the CHX application method does not influence microtensile bond strength after thermocycling, regardless of the point in time when it was applied (before or after acid etching the dentin).

METHODS AND MATERIALS

Cavity Preparation

Fifty freshly-extracted caries-free molars, stored in saline, were employed in this study. The occlusal enamel was ground flat using a model trimmer (Model Trimmer 1/2 HP, SeJong, Korea) under running water, then abraded with wet 600-grit silicon carbide abrasive paper to expose a flat dentin surface that permitted placing the occlusal cavity margins in dentin. Class I cavities (4 mm long, 4 mm wide, 2 mm deep) were prepared in dentin using a diamond bur in a high-speed handpiece with copious air-water spray. At this time, the specimens that showed visible pulp exposure were excluded from the study.

Restorative Procedure

The materials used in this study and their compositions are listed in Table 1. All the cavities were submitted to the bonding protocols using a two-step etch-and-rinse (Adper Single Bond 2, 3M ESPE, St Paul, MN, USA) adhesive system and a light-curing composite (Z-350, 3M ESPE). All the materials were handled according to the manufacturer's instructions.

The specimens were randomly divided into five groups of 10 teeth each. Table 2 shows the experimental groups with their respective modalities.

In group 1, Consepsis (Ultradent Products, Inc, South Jordan, UT, USA), a 2% CHX cavity disinfectant was applied for 20 seconds, rinsed with water spray for 20 seconds and etched with 37% phosphoric acid for 10 seconds. The surface was cleaned with water spray for 10 seconds and gently dried for 10 seconds. Three coats of the adhesive were then applied.

In group 2, Consepsis was applied before acid etching, as in group 1, except that it was air dried without rinsing.

In group 3, Consepsis was applied after acid etching with blot drying; it was placed in contact for 20 seconds, then rinsed with water spray for 20 seconds. The acid etching and bonding procedures were performed as previously described.

Table 1: *Material Brand Names, Composition and Manufacturers*

Material Brand Name	Composition	Manufacturer
Scotchbond Etchant	37% phosphoric acid	3M ESPE Dental Products, St Paul, MN, USA
Adper Single Bond 2	Ethyl alcohol, Bis-GMA, HEMA, Glycerol, 3-dimethacrylate, Acrylic acid copolymer, Itaconic acid, Diurethane dimethacrylate, Water, Colloidal filler	3M ESPE Dental Products, St Paul, MN, USA
Filtek Z-350	Bis-GMA, UDMA, TEGDMA, Ethyl methacrylate, inorganic fillers	3M ESPE Dental Products, St Paul, MN, USA
Consepsis 2%	2% Chlorhexidine	Ultradent Products, South Jordan, UT, USA

Bis-GMA= Bisphenol A-glycidyl methylmethacryrate
HEMA = Hydroxyethyl methacrylate
UDMA=Urethane dimethacrylate
TEGDMA=Triethylene glycol dimethacrylate

Table 2: *Experimental Groups and Their Respective Application Mode*

Group	Treatment
1	CHX with rinsing, acid etching and bonding
2	CHX without rinsing, acid etching and bonding
3	Acid etching, CHX with rinsing, bonding
4	Acid etching, CHX without rinsing, bonding
5	No CHX, acid etching and bonding

X=Chlorhexidine

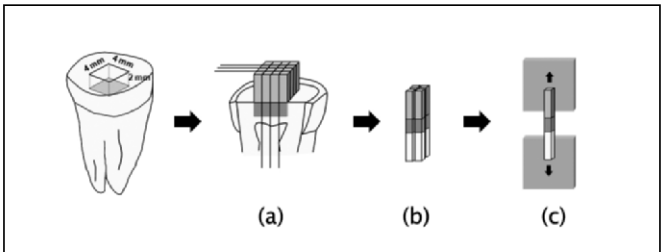

Figure 1. *Specimen preparation for the microtensile bond test: a) bonding substrates were restored with adhesive and composite, and excess composite was built-up on the outer surface of the restoration;(b) resin-dentin sticks with a rectangular cross-sectional area approximately 1 mm x 1 mm; c) microtensile bond strength test at a crosshead speed of 0.5 mm/minute.*

In group 4, Consepsis was applied after acid etching, as in group 3, except that it was air-dried without rinsing.

In group 5, the Control group, just the conventional bonding procedure was used without CHX.

Following the pretreatment sequences of the individual groups, the cavities were filled with Z-350 resin composite using a bulk method. An additional 2-mm thick resin composite was built-up and each increment was polymerized for 40 seconds. The resin composite was then built-up for the microtensile bond strength.

Half of the specimens for each group were stored in water for 24 hours, and the remaining half were submitted to 10,000 cycles of thermocycling, with the temperature changing from 5°C to 55°C, with a dwell time of 15 seconds and an interval time of 10 seconds each.

Measurement of Microtensile Bond Strength

After 24 hours of storage in water at 37°C, the restored specimens were serially sectioned into 1-mm thick resin slabs (Figure 1a); they were then rotated 90° and sectioned again to obtain resin-dentin sticks from the cavity floor with a rectangular cross-sectional area of approximately 1 mm^2 using Accutom 50 (Struers, Copenhagen, Denmark). Three to four sticks were obtained from each restoration, therefore, 15 to 18 specimens were used in each group (Figure 1b). The bonded surface area was calculated before each test by measuring the narrowest portion to the nearest 0.01 mm using a digital caliper (Mitutoyo, Tokyo, Japan). The ends of the sticks were glued to a testing machine (EZ Test, Shimadzu Co, Kyoto, Japan) using cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA, USA), and they were subjected to microtensile bond testing at a crosshead speed of 0.5 mm/minute (Figure 1c).

Statistical Analysis

To analyze the effects of the chlorhexidine treatment on bond strength before and after thermocycling, two-way ANOVA and the Scheffe's post-hoc test were used. Another Student *t*-test was used to determine, when using chlorhexidine, which application method was better. Statistical significance was preset at α=0.05.

RESULTS

The Effect of Chlorhexidine on Microtensile Bond Strength

The results of the microtensile bond strength values are summarized in Table 3. The factors of chlorhexidine treatment (p=0.000) and thermocycling (p=0.000) showed a significant effect, and their interaction (p=0.000) was also significant. Chlorhexidine pretreatment did not affect *in vitro* bond strength of the tested specimens during the immediate testing period, com-

Table 3: *Overall Means and Standard Deviations of Microtensile Bond Strength Values (MPa)*

Group	Mean µTBS No Thermocycling	Mean µTBS Thermocycling (10,000 cycles)
1	30.84 (3.86)[a]	26.48 (3.09)[a]
2	31.05 (5.56)[a]	26.19 (2.66)[a]
3	29.97 (6.81)[a]	25.86 (3.16)[a]
4	29.35 (3.82)[a]	26.86 (2.67)[a]
5	29.43 (3.37)[a]	19.12 (2.49)[b]

CHX=Chlorhexidine
In each group, the different letters indicate statistically significant differences (p<0.05).

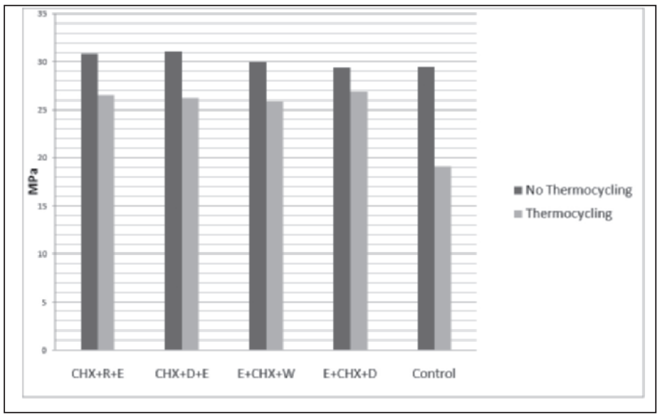

Figure 2. *Microtensile bond strength tested immediately and after thermocyling.*

pared to the control group (p=0.905). However, 10,000 cycles of thermocycling resulted in a significant bond strength reduction in the control group (p<0.001), but less of a reduction in groups treated with CHX (Figure 2).

Effects of the Chlorhexidine Application Methods on Microtensile Bond Strength

When bond strengths were compared on the basis of each test time, the chlorhexidine application method did not have any significant difference among the groups treated with chlorhexidine (immediate; p=0.910/after thermocycling; p=0.840). However, when the amount of reduction in bond strength after thermocycling was analyzed with the Student's t-test, a significant difference was found in groups treated with chlorhexidine before etching (group 1; p=0.007/group 2; p=0.015), and no significant difference was found in groups treated with chlorhexidine after etching (group 3; p=0.074/group 4; p=0.055).

DISCUSSION

Since the 2% CHX application produced no negative effects on bond strength of the etch-and-rinse adhesive system to dentin, the primary tested hypothesis is supported. After 10,000 cycles of thermocycling, the groups treated with CHX showed some degree of reduction in bond strength, compared to the immediate groups. However, this reduction was less than that observed in the control group, demonstrating that CHX was able to diminish, but not completely eliminate, the loss of bond strength over time. This reduction of bond strength in specimens treated with CHX was also observed *in vitro*[16-17] and *in vivo*[18] studies using the etch-and-rinse adhesive Single Bond. Analysis of the current data apparently indicates beneficial effects of CHX on the preservation of dentin bond strength.

The dental literature shows the association between the application method of cavity disinfectant and bond strength values to be a contentious issue. The current *in vitro* study showed no significant differences among µTBS values of the CHX-treated groups, irrespective of the CHX application sequence when the bond strengths were compared on the basis of each test time before thermocycling. Previous studies have demonstrated that CHX applied before or after acid-etching had no adverse effects on composite-adhesive bonds to dentin,[19-22] which is in agreement with the results of the current research. However, after 10,000 cycles of thermocycling, which represent a service year,[23] a significant reduction in bond strength was seen in groups treated with CHX before etching, and there was no significant reduction of bond strength in groups treated with CHX after etching, resulting in a partial rejection of the secondary tested hypothesis. In fact, certain properties of chlorhexidine–a strong positive ionic charge, ready binding to the phosphate groups, a strong affinity to tooth surfaces that are increased by acid etching, increases in the surface-free energy of enamel and perhaps that of dentin–could lead to the assumption that its application after dentin acid-etching would increase the dentin-wetting ability of primers, thus improving adhesion.[22]

Another important aspect related to the use of chlorhexidine is the influence of matrix metalloproteinases (MMPs) on bonding durability. Currently, 24 MMP genes have been identified in humans and 26 well-characterized members have been reported. By *in situ* hybridization or immunohistochemistry, collagenase MMP-1, gelatinases MMP-2 and MMP-9, stromelysin-1 (MMP-3) and enamelysin (MMP-20) have all been identified in either odontoblasts or in the predentin/dentin compartment.[14] CHX has been shown to inhibit MMP-2, -8 and -9 activities directly at extremely low concentrations (0.02% for MMP-8, 0.002% for MMP-9 and 0.0001% for MMP-s),[14] thus strongly inhibiting the inherent collagenolytic activity of mineralized dentin. It could be speculated that the CHX-saturated demineralized matrix becomes sequestered from interstitial fluids by resin tags that occlude dentinal tubules by adhesive resin coating collagen fibrils and by an overlying adhesive layer, which may produce prolonged retention of CHX and inhibition of MMPs.

It is well known that stress from polymerization shrinkage is influenced by restorative techniques, modulus of resin elasticity, polymerization rate and cavity configuration or "C-factor," which is defined as the quotient between bonded and unbonded resin composite surface area.[24] Three-dimensional cavity preparation (Class I cavities) has the highest (most unfavorable) C-factor, where polymerization stresses may be greater.[25] The incremental technique is recommended for Class I cavities to reduce the C-factor, and therefore minimize the harmful effects of stress developing at the adhesive interface. In the current study, the bulk-filling technique was performed, and these factors could explain the relatively low bond strength compared to previous studies of the etch-and-rinse system.

Although many studies, including the current one, show the merits of CHX, there are some side-effects in the long-term use of CHX, which limit its widespread acceptance. These side-effects include brown staining of the teeth, an increase in calculus deposit and difficulty in completely masking its taste when used as a rinse. However, due to the short duration of its use in a restorative procedure, CHX does not result in these potential negative aspects. The current results do not prove the cause of therapeutic action on the preservation of bond strength of etch-and-rinse adhesive to dentin; therefore, further *in vitro* and *in vivo* studies are needed to analyze the morphologic changes after using CHX, the association of these solutions to other adhesive systems, such as self-etching and the influence of CHX application methods on bond strength over time.

CONCLUSIONS

Based on the results of this *in vitro* study, the following conclusions were drawn:

1. Chlorhexidine pretreatment did not affect the *in vitro* bond strength of specimens tested before thermocycling compared to the control group.
2. Thermocycling for 10,000 cycles resulted in a significant bond strength reduction in the control group ($p<0.05$), but it showed less reduction in groups treated with chlorhexidine.
3. Considering the amount of reduction in bond strength after thermocycling, a significant difference was found in groups treated with chlorhexidine before etching and no significant difference in groups treated with chlorhexidine after etching ($p<0.05$).

In summary, a 2% chlorhexidine application after etching with 37% phosphoric acid is the recommended procedure in clinical practice.

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