

Effect of adhesive mode and chlorhexidine on microtensile strength of universal bonding agent to sound and caries-affected dentins

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ABSTRACT

Objective: The main objective of this study is to assess the effect of the adhesive mode and chlorhexidine (CHX) on the microtensile strength of a universal bonding agent to sound and caries-affected dentins. **Materials and Methods:** Six intact third molars and six decayed third molars were sectioned to obtain two middle dentin specimens per tooth. Prime and Bond Elect Universal (Dentsply) was applied to specimens in two different modes: Etch-and-rinse (ER, phosphoric acid for 15s) and self-etch (SE, no phosphoric acid). 2% CHX (Cavity Cleanser, Bisco) was applied to both dentins for 30 s. Specimens were randomly assigned into eight groups ($n = 3$) according to the treatments applied to sound and caries-affected dentins: SE; ER; SE + CHX; and ER + CHX. All specimens were restored with TPH Spectra High Viscosity (Dentsply) composite resin and sectioned to obtain specimens of $0.8 \pm 0.1 \text{ mm}^2$. The microtensile bond strength test was conducted at 1 mm/min. The data were statistically analyzed by ANOVA and multiple comparisons' test ($\alpha = 0.05$). **Results:** There was no significant difference between sound dentin and caries-affected dentin ($P = 0.132$). The highest bond strength was obtained with the application of the ER (31.81MPa). SE + CHX promoted the lowest bond strength with no statistical difference to ER + CHX ($P > 0.05$). **Conclusions:** ER mode for the universal bonding agent yielded the highest bond strength to sound and caries-affected dentins. CHX reduced the bond strength to both dentins regardless the application of phosphoric acid. **Clinical Significance:** The universal bonding agent increased the bond strength to sound and caries-affected dentins when applied by the ER mode.

Key words: Acid etching, chlorhexidine, dentin, dentin adhesives, tensile strength

INTRODUCTION

One of the highest challenges in adhesive dentistry is to find a versatile bonding agent that promotes adequate bond strength to both enamel and dentin,^[1] low technique sensitivity,^[1,2] and high

longevity.^[3-5] Although universal bonding agents have been introduced to clinical dentistry recently,

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satisfactory results have been observed for both enamel and dentin due to their composition and multimode techniques (etch-and-rinse [ER]; self-etch [SE]; and selective etch).^[1]

It is already known that ER mode is the chosen technique to bond enamel, due to complete removal of the smear layer and demineralization of enamel rods by phosphoric acid and penetration of resin monomers in the demineralized area, resulting in chemical-mechanical retention and consequently high bond strength to enamel.^[1,4,5] However, the most appropriate adhesive mode to dentin is still controversial due to the complex morphology and physicochemical characteristics of sound and caries-affected dentin.^[6] The dentin bond strength is affected by the degradation of collagen that occurs in the dentin-resin interface.^[7] This is partly due to the activity of matrix metalloproteinases (MMPs),^[8-11] a class of endogenous enzymes of proteolytic action of collagen, which can become active by contact even with weak acids.^[10,12-14]

To prevent the loss of bond strength to dentin by hydrolysis and to maintain the integrity of the restoration in long-term, some strategies have been studied for inhibiting the MMPs.^[15] They include the incorporation of agents such as polymerizable quaternary ammonium methacrylates and benzalkonium chloride in self-etching primers,^[16,17] the use of calcium and zinc with ethylene diamine tetraphosphoric acid as a conditioner,^[18-20] the production of synthetic MMP inhibitors as the chemically modified tetracyclines,^[21] and the use of chlorhexidine (CHX) as an antimicrobial agent.^[22]

CHX has demonstrated the most outstanding results for reducing the residual risk of infection and increasing the bond strength to dentin by inhibition of activated MMP enzymes.^[22] This outcome depends on CHX concentration^[15,22] and type of substrate (sound or caries-affected dentin) used.^[23] Although CHX has increased the bond strength to dentin, its effect on different dentin substrates when applied previously to universal bonding agents on SE and ER modes has not been completely studied.

Therefore, the aim of this study was to assess the effect of the adhesive mode (SE or ER) and CHX on the microtensile strength of one-step universal bonding agent to sound and caries-affected dentins. The null hypotheses tested were: (1) the factor “type of dentin” has no significant effect on bond strength; (2) the factor

“type of treatment used” has no significant effect on bond strength; and (3) the interaction between the factors “dentin” × “treatment” has no significant effect on the bond strength.

MATERIALS AND METHODS

Specimens preparation

This study was reviewed and approved by the Ethics Committee for Human Studies (CORIHS#: 2016-3766-F), Stony Brook University, NY, USA. Six intact third molars and six decayed third molars were cleaned and stored in 0.5% chloramine solution (Fisher Scientific, Pittsburg, PA, USA) at 4°C for 7 days.^[24] After the disinfection process, all teeth were sectioned 2.5 mm above and 4.0 mm below the cementum-enamel junction with a precision low-speed diamond saw (ISOMET 1000, Buehler, Illinois, USA). A buccolingual section perpendicular to the others was done to obtain two dentin specimens per tooth [Figure 1]. An explorer and a stereoscopic microscope at ×10 magnification (Zeiss West Germany-type 475200/9901) were used to detect sound and caries-affected dentins. Affected dentin was described as a dentin exposed to bacterial acids but is not yet infected by cariogenic bacteria.^[25] Caries-affected dentin was diagnosed as discolored or stained^[25] by visual inspection with the stereoscopic microscope and leathery and softer than sound dentin by tactile inspection with an explorer. Coronary and radicular pulp was removed with hand instruments at the root apex. The root canals were etched with 34% phosphoric acid (Dentsply, Milford, DE, USA) for 15 s and sealed with a

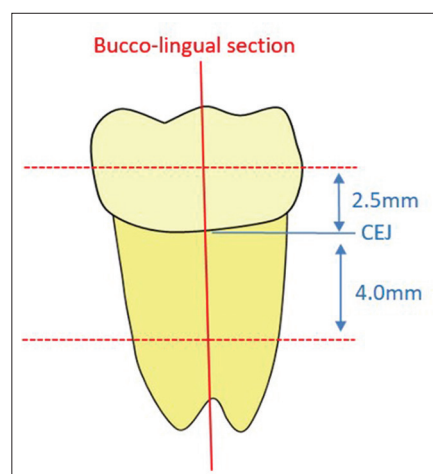


Figure 1: Specimens preparation. Third molars were sectioned 2.5 mm above and 4 mm below the cementum-enamel junction. A buccolingual section perpendicular to the others was done to obtain two dentin specimens per tooth

universal bonding agent (Prime and Bond Elect, Dentsply) and a flowable composite (TPH Flow, Dentsply) following the manufacturers' instructions.

Restorative procedures

To standardize the smear layer, sandpaper silicon carbide with grain 600 was used on the dentin for 1 min.^[26]

Specimens were randomly assigned into eight experimental groups according to the adhesive mode (SE or ER) and application of 2% CHX (Cavity Cleanser, Bisco Inc., Schaumburg, IL, USA) to sound and caries-affected dentins: SE; ER; SE + CHX; and ER + CHX.

Prime and Bond Elect was applied to dentin specimens following the manufacturers' instructions and two different adhesive modes: (1) ER: Application of 34% phosphoric acid for 15 s and (2) SE: No application of 34% phosphoric acid. 2% CHX was applied for 30 s^[27] with a microbrush on dentin specimens after the acid etching in the ER mode and before the universal bonding agent in the SE mode.

After the surface treatment of the substrates, 5 mm restorations were built following the incremental technique with a universal resin composite (TPH Spectra High viscosity, A2 shade, Dentsply). Each 2 mm increment was light cured for 20 s (SmartLite Focus light curing unit, 1000 mw/cm², Dentsply) following the manufacturer' recommendations.

All restored specimens were stored in distilled water at 37°C for 24 h and then assigned to microtensile bond strength test.

Microtensile bond strength test

Specimens were sectioned with a precision low-speed diamond saw to obtain sticks with a cross-sectional area of $0.8 \pm 0.1 \text{ mm}^2$. An absolute digital caliper (Mitutoyo Corp., Kanagawa, Japan) with an accuracy of 0.001 mm was used to measure both sides of the adhesive interface. The bonding area was calculated in mm² automatically by the Instron software. Each stick was tested individually by attaching them to a microtensile jig using a cyanoacrylate glue (Zapit Accelerator Dental Ventures of America Inc., Corona, CA, USA). All sticks were then subjected to a microtensile load (100N) using a universal testing machine (Instron Co., Canton, MA, USA) at 1 mm/min crosshead speed. The load (N) and the bonding surface area were registered and the microtensile bond strength calculated in MPa

by the BlueHill program software. Failures were evaluated using the Zeiss stereoscopic microscope at $\times 10$ magnification and classified as adhesive, cohesive, mixed, or debonded.

RESULTS

The interaction between the factors "dentin" \times "treatment" had no significant effect on the bond strength ($P = 0.475$). In addition, there was no statistically significant difference between sound and caries-affected dentin, regardless of the adhesive mode and CHX application ($P = 0.132$). The type of treatment affected the bond strength regardless of the type of dentins ($P < 0.001$) [Table 1].

The highest bond strength was obtained with the ER mode (31.81 MPa). SE + CHX had the lowest bond strength with no statistical difference for ER + CHX ($P > 0.05$).

Figures 2 and 3 present the percentage of failures according to the type of treatment applied to sound and caries-affected dentins, respectively.

The adhesive failure was predominant for all treatments on sound and caries-affected dentins, except for SE + CHX.

Table 1: Homogeneous groups, median, and quartiles (quartile 1; quartile 3) in MPa according to the type of treatment applied to both sound and caries-affected dentins

Treatments	Median (quartiles)	Homogenous groups*
SE	16.01 (0.01;29.99)	B
ER	31.81 (22.36;49.23)	A
SE + CHX	0.00 (0.00;25.82)	C
ER + CHX	17.28 (0.00;24.71)	BC

Dunn test (5%). Dentin: $F=0.512$, $P=0.475$, Treatment: $F=22.054$, $P<0.001$, *Multiple comparisons' *post hoc*: letters indicate statistical similarity, Dentin \times treatment: $F=1.883$, $P=0.132$. SE: Self-etch, ER: Etch-and-rinse, CHX: Chlorhexidine

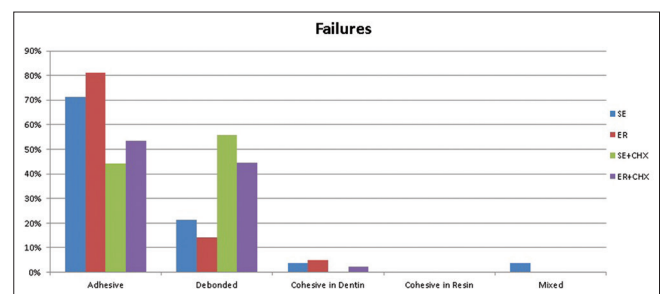


Figure 2: Frequency of failures according to the treatment applied to sound dentin

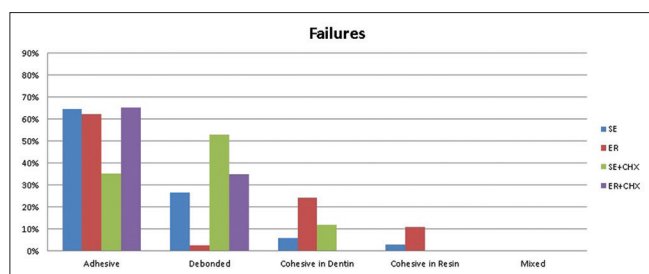


Figure 3: Frequency of failures according to the treatment applied to caries-affected dentin

DISCUSSION

The effect of adhesives modes and CHX on bond strength to sound and caries-affected dentin was evaluated in this study. Sound dentin is a complex substrate composed by hydroxyapatite (50%), collagen (30%), and water (20%), and it is exposed to structural changes by physiological^[28] and pathological processes.^[29] Although studies^[30] have reported that caries-affected dentin exhibits increased collagenolytic activity when compared to sound dentin, which suggests faster bonding deterioration,^[23] no statistical difference was observed between both dentin regardless the adhesive mode and CHX application. Therefore, the first and third null hypotheses of this study were accepted. Similar finding was observed by Tosun *et al.*^[31] through the scanning electron microscopy. Although caries-affected dentin is more porous and softer than sound dentin, no morphological difference was observed in the thickness of the hybrid layer between sound and caries-affected dentin, which explains the reason the bond strength was not affected by the dentin substrate.^[31]

The second null hypothesis was rejected because the bond strength was modified by the type of treatment: Adhesive mode and CHX application. Although studies have shown that the bond strength of universal adhesive systems to dentin was not affected when the phosphoric acid was used before the application of adhesives,^[29,32] in the present study, the ER mode increased significantly the bond strength of the universal bonding agent, regardless dentin substrate. Rosa *et al.*^[29] suggested that the differences in the compositions of the universal adhesives systems may be the reason for the different performances of phosphoric acid on bond strength. Our results are also in accordance to Muñoz *et al.*^[33] and Tekçe *et al.*^[34] who found lower values of bond strength to dentin in the SE mode for different universal adhesive systems used when compared to ER mode. This can be explained by the characteristics of the acidic

primer contained in the Prime and Bond Elect, which can be classified as ultra-weak according to its pH and depth of penetration to dentin (pH >2.5, depth 0.2–0.5 µm penetration). As the smear layer and the dissolved calcium phosphates^[1] are only partially removed by the ultra-weak acidic monomers, they become a physical barrier for the formation of the hybrid layer.^[35,36]

In contrast, 30%–40% phosphoric acid exposes collagen fibers through the dentin demineralization with simultaneous removal of the smear layer and the dissolved calcium phosphate.^[2,30] In addition, other studies have shown that the use of phosphoric acid before the SE adhesive system^[34,35] and the universal adhesive system (ER mode)^[32] has provided a deeper penetration of resin monomers, generating longer tags, and thicker hybrid layers.

It is known that MMPs are present in the exposed and noninfiltrated collagen beneath in the hybrid layer and can be activated by the presence of weak acids in both ER or SE systems.^[36–38] High bond strength to dentin is obtained when the bonding agent is able to infiltrate the exposed collagen by acid or inhibit the MMPs located in the demineralized area.^[39] CHX works as a therapeutic primer and a nonspecific inhibitor modifying the three-dimensional structure of the MMPs and competing with metal ions (Ca^{2+} , Zn^{2+}) necessary for their functions.^[12,40] Although there is evidence that CHX is capable of inhibiting the action of the MMPs, it is not clear its effect on the bond strength of universal adhesive systems because the results have been contradictory.^[34]

In this study, the application of CHX resulted in a significant reduction of the bond strength to dentin for both adhesive modes (ER, SE) 24 h after the restorative procedure. Similar results were reported by Campos *et al.*^[41] and Gunaydin *et al.*^[42] The low bond strength can be explained by the formation of precipitates on the dentin surface after the application of 2% CHX as observed by Di Hipólito *et al.*^[43] through the scanning electron microscopy. The two positive charges of the symmetric CHX molecule^[44] can be electrostatically attracted to the phosphate anions present in the hydroxyapatite,^[45] forming crystals in the form of “birefringent needles.”^[43] 2% CHX affects the bond strength to dentin by decreasing the amount of calcium ions available on the dentin for chemical bonding to the adhesive system and also by forming crystals that act as a mechanical barrier to adequate hybrid layer formation.^[43]

Although the application of CHX has affected the bond strength for both ER and SE modes, adhesive failures were predominant for the ER mode (53% for sound dentin and 65% for caries-affected dentin) and debonded for the SE mode (56% for sound dentin and 53% for caries-affected dentin). The phosphoric acid can have promoted a greater demineralization and removal of the smear layer on dentin surface and facilitated the penetration of the universal adhesive system, even with the presence of precipitates by the application of CHX. As for the SE mode, the shallow penetration of the universal adhesive system associated with the effects of CHX on the dentin surface may have resulted in the formation of an inadequate hybrid layer, which would explain the higher number of premature failures in the adhesive interface.

Considering that this study assessed the effect of the adhesive mode and the CHX on the bond strength to dentin 24 h after a restorative procedure, other studies are needed to determine the effect of these treatments on bond strength to dentin in long-term and evaluate the morphology of the hybrid layer through microscopy analysis.

CONCLUSIONS

It was concluded that the ER mode for the universal bonding agent yielded the highest bond strength to sound and caries-affected dentins. CHX reduced the bond strength to both dentins regardless the adhesive mode.

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Conflicts of interest

There are no conflicts of interest.

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