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Effect of Chlorhexidine Application on Microtensile Bond Strength to Dentin

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Clinical Relevance

Based on the results of this study, the application of chlorhexidine solution at a concentration of 0.12 and 2% before, after or associated with phosphoric acid etching, presented similar behavior with no adverse effect on 24-hour bond strength.

SUMMARY

This study evaluated the effect on microtensile bond strength (μTBS) of chlorhexidine application to dentin at different times during an indirect restoration luting procedure. Sixty bovine incisors had their superficial dentin subjected to 0.12% and 2% chlorhexidine solutions for 15 seconds before, during and after 37% phosphoric acid etching, resulting in six groups (n=10): 1) 0.12% chlorhexidine + etching; 2) 2% chlorhexidine + etching; 3) etching + 0.12% chlorhexidine; 4) etching + 2% chlorhexidine; 5) etching with 2% chlorhexidine; 6) etching without chlorhexidine (control). An adhesive system (Adper Single Bond 2) was applied and an indirect resin composite restoration (Filtek Z250) was luted using dual cured resin cement (Rely X ARC). After 24 hours of water storage, the specimens were tested by microtensile bond test (μTBS) at 0.5 mm/minute in a universal testing machine. The data were analyzed by one-way ANOVA (α=0.05), demonstrating no significant differences among the groups. The μTBS values in MPa were: 6: 22.83±3.53; 5: 22.4±3.52; 2: 21.62±2.5; 1: 21.28±3.17; 3: 19.62±2.05; 4: 19.55±2.34. The use of chlorhexidine at concentrations of 0.12% and 2% before, after or associated with acid etching did not significantly affect the μTBS values to dentin.

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INTRODUCTION

The inadequate removal of all cariously affected dentin has been shown to be the reason for secondary or residual caries, resulting in irritation and inflammation of the pulp.[1] To reduce the potential for residual caries and sensitivity, an antibacterial solution, which could be placed after cavity preparation and has the ability to disinfect the dentin, would be valuable.[2] Chlorhexidine remains the gold standard as an antiplaque and anti-gingivitis agent,[3] and its efficacy in caries prevention had been established in clinical trials.[4] Its molecule is symmetric, consisting of a hexamethylene bridge with terminal 4-chlorophenyl groups.[3] It has been shown that chlorhexidine has an affinity to bacteria, probably because of an interaction between the positively charged chlorhexidine molecule and negatively charged groups on the bacterial cell wall.[5] This interaction increases permeability of the bacterial cell wall and thus permits the agent to penetrate into the cytoplasm and cause death of the microorganism.[3] Different modes of administration are recommended; high and low concentrations of chlorhexidine have been reported to reduce the number of microorganisms in plaque and saliva for considerable periods of time.[6]

During the last two decades, chemical and technical advances have contributed to increasing resin-dentin bond strength; however, premature loss of bond strength is one of the problems that still affects adhesive restorations[7] and markedly reduces their durability.[8-10] The loss of bond strength has been attributed primarily to degradation of the hybrid layer at the dentin-adhesive interface[11-14] and deterioration of the dentin collagen fibrils.[15-16] Although the current strategies of incorporating ionic and hydrophilic resinous components into total-etch and self-etch adhesives arise from the need to bond to an intrinsically wet substrate, these strategies have created potentially unstable resin matrices that slowly degrade via water sorption.[13] Moreover, temperature change, chewing loads and chemical attacks by acids and enzymes in the oral cavity have represented a significant challenge to tooth-composite bond survival for some time.[17] Studies have shown that collagen degradation occurs over time via host-derived matrix metalloproteinases (MMPs), a class of zinc- and calcium-dependent endopeptidases[16,18] and has revealed that chlorhexidine also functions as a potent MMP inhibitor.[16,19-20]

Chlorhexidine 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.

The above-mentioned points allow for the hypothesis that the application of different substances containing chlorhexidine before hybridization would not influence the bond strength of an indirect resin restoration to dentin. Therefore, this study evaluated the influence of chlorhexidine application on the dentin substrate at different times during the indirect composite restoration bonding protocol on bond strength to bovine dentin.

METHODS AND MATERIALS

Sixty bovine incisors of similar age were extracted and stored in 0.2% thymol solution (Biopharma, Uberlândia, Brazil); the roots were cut off with a diamond disc (Kg Sorensen, Barueri, Brazil) and the pulp was removed under water irrigation. Flat dentinal surfaces were obtained by grinding the buccal tooth surfaces with 600-grit carbide paper (Norton, Campinas, Brazil) to create a standardized smear layer. The specimens were randomly divided into six groups: 1) 1 ml application of 0.12% chlorhexidine solution (Biopharma, Uberlândia, Brazil) for 10 seconds, rinsed with water spray for 15 seconds and etched with 37% phosphoric acid (FGM, Joinville, Brazil) for 15 seconds. The surface was then cleaned with water spray for 10 seconds; 2) 1 ml application of 2% chlorhexidine solution, rinsed with water spray for 10 seconds and etched with 37% phosphoric acid for 15 seconds. The surface was then cleaned with water spray for 10 seconds; 3) etching with 37% phosphoric acid for 15 seconds, rinsed with water spray for 10 seconds and an application of 1 ml of 0.12% chlorhexidine solution applied, then gently dried for 10 seconds; 4) etching with 37% phosphoric acid for 15 seconds, rinsed with water spray for 10 seconds and 1 ml of 2% chlorhexidine solution was applied, then gently dried for 10 seconds; 5) etching with 37% phosphoric acid associated with 2% chlorhexidine (FGM, Joinville, Brazil) for 15 seconds and rinsed with water spray for 15 seconds; 6) no chlorhexidine application, just conventional etching of the control group.

Indirect resin restorations (3.0 mm x 5.0 mm) were incrementally built-up with three layers of a microhybrid resin composite (Filtek Z250, 3M ESPE, St Paul, MN, USA) in a silicon matrix (Aerojet, São Paulo, Brazil). Each increment was halogen light cured at 600mW/cm^2 (XL 3000, 3M ESPE) for 40 seconds. Complementary polymerization was done in an autoclave for 15 minutes at 110°C.[21] The intaglio surfaces of indirect resin restorations were airborne particle abraded with 50 µm aluminum oxide particles (Bioart, São Carlos, Brazil) for 10 seconds at 4 bars pressure, a source-to-sample distance of 10.0 mm and silanized with a pre-hydrolyzed silane solution (Rely X Ceramic Primer, 3M ESPE) for one minute.[22] Two layers of a one bottle adhesive system (Adper Single Bond 2, 3M ESPE) were applied and the restorations were bonded with dual-cured resin cement (Rely X ARC, 3M ESPE) in accordance with the manufacturers' instructions.

This set was submitted to a load of 500g for five minutes to standardize the luting agent thickness. Visible-light activation of superior and all lateral surfaces on the bonded blocks was performed for 40 seconds using a halogen light-curing unit (XL3000, 3M ESPE). The specimens were stored in 100% relative humidity at 37°C for 24 hours.

The bonded samples were serial cut with a precision cutting machine (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) perpendicular to the bonded interface, obtaining four slices approximately 1 mm thick from each sample. Each slab was trimmed with a cylindrical diamond bur (#1090, Kg Sorensen, Barueri, SP, Brazil), resulting in an hourglass-shaped specimen with a cross-sectional bonded area of approximately 1 mm^2. Each specimen was bonded to the microtensile testing device with cyanoacrylate glue on all surfaces (Loctite Super Bonder, Henkel Loctite Corporation, Düsseldorf, Germany) and the microtensile bond strength (μTBS) was recorded at 0.5 mm/minute in a mechanical testing machine (EMIC 2000 DL, São José dos Pinhais, PR, Brazil) until complete failure.

After fracture, the specimen was removed from the testing apparatus and the cross-sectional area at the fracture site was measured with a digital caliper (S235, Sylvac, Switzerland). The means of μTBS were expressed in MPa. Data was submitted to the Shapiro-Wilk test and one-way ANOVA (α=0.05). Statistical analysis was conducted, and each tooth was considered to be a sample in the microtensile test. From this, an average of the results of the four hourglass-shaped specimens from each tooth was extracted, considering the existence of a correlation between slices from the same tooth.

RESULTS

The Shapiro-Wilk test of normality revealed that data presented a normal and homogeneous distribution, enabling a parametric analysis. One-way ANOVA showed no significant difference among groups (p=0.097). Table 1 shows the μTBS means and standard deviations for all the experimental groups.

DISCUSSION

The hypothesis tested was accepted. Chlorhexidine did not produce a negative effect on bond strength to dentin, irrespective of the solution concentration or moment of its application. Etching the dentin surface with an acidic solution, such as phosphoric acid, during bonding procedures may reduce the number of bacteria in the cavity; however, it is limited and should not be regarded as reliable.[23] In many cases, an antiseptic may be useful for eliminating the effects caused by bacteria and contribute to better prognoses for the minimal restorative treatment of dental caries. Chlorhexidine is a broad spectrum antiseptic with pronounced antimicrobial effects[24] and has been shown to be effective in reducing cariogenic bacteria.[25] If the bacterial challenge is reduced, then the protective factors have a greater chance of halting or reversing dental caries.[26] Thus, some clinicians have recommended the use of chlorhexidine as a complement to restorative procedures.[27]

The most common orally administered chlorhexidine digluconate is water soluble and readily dissociates at physiologic pH, releasing the positively charged chlorhexidine component.[6] The bactericidal effect of chlorhexidine solution is due to the cationic molecule binding to extra microbial complexes and negatively charged microbial cell walls, thereby altering the osmotic equilibrium of the cell.[28] Chlorhexidine solution is active against a wide range of microorganisms, because it is bacteriostatic at low concentrations and bactericidal at higher concentrations.[3] Chlorhexidine may also be involved in inhibiting bacterial adherence to surfaces and to each other by competing with calcium for retention sites, and thus may prevent the formation of calcium bridges between bacteria and oral surfaces or between bacteria.[5] Therefore, the use of solutions such as chlorhexidine, which have an antibacterial or bactericidal effect, provide an adjunct treatment that contributes to the suppression of residual infection, increasing survival of the restored tooth.

Chlorhexidine has been widely used as an antimicrobial agent, as well as for disinfection before the placement of restorations.[29] The current *in vitro* study showed that chlorhexidine application in concentra-

Table 1: *Ultimate Bond Strength Values (MPa) and Statistical Categories Performed with One-way ANOVA (p=0.097)*

Experimental Groups (pre-treatment of dentin surface before adhesive system application)	TBS Mean (Standard Deviation)
6—Control Group	22.83 (3.53)[a]
5—Etching with 37% phosphoric acid associated with 2% chlorhexidine	22.4 (3.52)[a]
2—2% Chlorhexidine solution application before etching with 37% phosphoric acid	21.62 (2.50)[a]
1—0.12% Chlorhexidine solution application before etching with 37% phosphoric acid	21.28 (3.17)[a]
3—Etching with 37% phosphoric acid followed by 0.12% chlorhexidine application	19.62 (2.05)[a]
4—Etching with 37% phosphoric acid followed by 2% chlorhexidine solution application	19.55 (2.34)[a]
A total of 40 specimens were evaluated in each group. *Same letters showed no difference among all groups observed by one-way ANOVA (p<0.05).*	

tions of 0.12% and 2% before acid etching did not significantly affect μTBS values of a water-based dentin bonding system (Table 1). Previous studies have demonstrated that chlorhexidine application prior to acid-etching had no adverse effects on immediate composite-adhesive bonds to dentin.[1,27,30-31] The antimicrobial efficacy of chlorhexidine used to disinfect a preparation may, however, be questioned if the surface is subsequently conditioned.[32] Recent studies have examined the use of chlorhexidine after acid-etching, demonstrating initial bond strengths comparable to those of the controls,[1,29,31,33] as was also observed in the current study. In addition, Carrilho and others[29] indicated that evidence of the antimicrobial efficacy of chlorhexidine, when used after acid-etching, needs to be demonstrated.

Another important aspect related to the use of chlorhexidine is the influence of metalloproteinases (MMPs) on bonding durability. It is well-established that resin-dentin bonds deteriorate over time.[17,34] For total-etch adhesives, a decreasing gradient of resin monomer diffusion within acid-etched dentin[35] results in incomplete infiltrated zones along the bottom of hybrid layers that contain denuded collagen fibrils.[36] This may result in incomplete hybridization, leaving the collagen unprotected and vulnerable to hydrolytic degeneration.[37] Recent studies revealed the contributions of host-derived MMPs to the breakdown of collagen matrices in the pathogenesis of dentin caries[18] and have suggested that incomplete infiltrated zones within hybridized dentin may be degraded by MMPs in the dentin matrix, in the absence of bacterial enzymes.[16] MMPs are a family of zinc-dependent proteolytic enzymes that are capable of degrading the dentin organic matrix after demineralization.[18] Enzymes with gelatinolytic (MMP-2 and MMP-20) activities are present within the intact dentinal matrix[38-39] and in carious dentin.[18]

Since release and activation of host-derived MMPs may be associated with the degradation of acid-demineralized denuded collagen fibrils, these enzymatic activities may also occur within incomplete resin-infiltrated subsurface regions of hybrid layers created by contemporary adhesives, despite the better surface seal achieved with these adhesives.[40] It was recently shown that the use of chlorhexidine, even in very low concentrations, strongly inhibited the inherent collagenolytic activity of mineralized dentin.[20] Carrilho and others[29] indicated the beneficial effects of chlorhexidine on the preservation of dentin bond strength as an MMP inhibitor when applied before bonding and without further rinsing. When applied in this manner, the naked collagen fibrils were exposed to chlorhexidine, which was then sealed into the fibrils by adhesive resins, leading to better preservation of the collagen fibrils. The study results by Carrilho and others[29] showed a significantly lower percentage of failure mode in the hybrid layer, especially in the bottom section after six months of chlorhexidine treatment, indicating that the higher bond strengths observed in this group reflected the preservation of a hybrid layer collagenous matrix, especially in the bottom zone, where partially exposed collagen fibrils are most prone to initial enzymatic degradation. These study results may also reflect the better preservation of sub-hybrid layer dentin, in which both progressive demineralization and degradation of the dentin collagenous matrix may occur with time.[41]

New phosphoric acid formulations have been developed in association with chlorhexidine, and this study did not demonstrate any negative effect on bond strength. In this context, it can be suggested that the application of chlorhexidine associated with phosphoric acid, as was conducted in the current study, would also lead to better exposure of the naked collagen fibrils to chlorhexidine, which could then be sealed into the fibrils by adhesive resins, providing the fibrils with better preservation. However, the real benefits of this procedure may be investigated in future studies.

From a clinical perspective, it would be advantageous to prevent the degradation of incomplete resin-infiltrated collagen fibrils by host-derived MMPs in dentin hybrid layers. Thus, apart from its widely known antimicrobial property[16] and it not significantly affecting bond strength to dentin, the use of chlorhexidine solution on acid-etched dentin may have the additional potential of preventing collagen fibril degradation in dentin hybrid layers. Furthermore, an alternative approach is the use of chlorhexidine water-based solutions for rehydrating dried mineralized dentin, preserving the necessary humidity needed to maintain the collagen network in an expanded condition, as well as reducing technique sensitivity, while utilizing its antimicrobial effects. Thus, the protocol may be of clinical value, because the application of a chlorhexidine solution would prevent collagen degradation, prevent and control micro-organisms and rehydrate dried mineralized dentin.

Some of the side effects of using chlorhexidine, which limit its widespread acceptance, include brown staining of the teeth, an increase in calculus deposit and difficulty in completely masking its taste when used as a rinse.[42] However, using chlorhexidine to complement a restorative procedure is, in the short time, without realization of these potential negative aspects. Further studies need to be conducted to analyze the effects of high concentration chlorhexidine solutions, the association of these solutions to self-etching adhesive systems and the influence of chlorhexidine application on bonding stability over time.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following conclusions were drawn:

1. Chlorhexidine solution application before or after phosphoric acid did not influence the μTBS to dentin;
2. Etching with 37% phosphoric acid gel, associated with 2% chlorhexidine, had a similar effect to that of conventional phosphoric acid gel and
3. A concentration of 0.12 and 2% chlorhexidine presented similar behavior, with no adverse effect on bond strength.

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