Effect of chlorhexidine on the bond strength of a self-etch adhesive system to sound and demineralized dentin

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Abstract: This study evaluated the effect of a 2% chlorhexidine-based disinfectant (CHX) on the short-term resin-dentin bond strength of a self-etch adhesive system to human dentin with different mineral contents. Dentinal mineralization was tested at 4 levels (sound, and after 2, 4, or 8 days of demineralization-remineralization cycles) and disinfectant at 2 levels [deionized water (DW, negative control) and CHX]. Dentin demineralization induced by pH-cycling was characterized by cross-sectional hardness (CSH). Each dentin surface was divided into halves, one treated with DW and the other with CHX (5 minutes). Each surface was bonded with a self-etch adhesive system and restored. The specimens were sectioned and subjected to microtensile bond testing. CSH and microtensile bond strength (μTBS) data were analyzed by regression analysis and ANOVA-Tukey tests ($\alpha = 5\%$), respectively. The groups treated with CHX resulted in mean μTBS similar to those found for the groups in which the dentin was exposed to DW ($p = 0.821$). However, mean μTBS were strongly influenced by dentin mineralization ($p < 0.05$): the bond strength found for sound dentin was lower than that found for dentin cycled for 8 days, which was even lower than the bond strengths for dentin cycled for 2 or 4 days. The results suggest that the degree of dentin demineralization affects the bond strength of self-etching adhesives, but the use of CHX does not modify this effect.

Descriptors: Dentin-Bonding Agents; Chlorhexidine; Tooth Demineralization; Dentin.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Submitted: Sep 05, 2012
Accepted for publication: Jan 31, 2013
Last revision: Feb 13, 2013

Introduction

Minimally invasive cavity designs have been proposed, and the excavation of dentin caries may eliminate only the more superficial zone of irreversibly damaged dentin,[1] facilitating the preservation of tooth structure.[2] It has been suggested that the temporary sealing of cavities may isolate residual acidogenic bacteria from dietary sources of fermentable carbohydrates, making them dormant.[3] However, the restoration of infected tissues requires careful evaluation, since studies have demonstrated that microorganisms are capable of surviving even extreme environmental conditions.[4] Therefore, antibacterial treatment of the dentin may suppress the growth of bacteria under existing restorations, thus minimizing the risk of recurrent caries. The use of 2% chlorhexidine digluconate has been recommended for cleaning tooth preparations, due to its ability to significantly reduce the levels of oral microorganisms in a short period of time,[5] including *S. mutans* present in dentinal tubules.

Chlorhexidine has also been shown to have an inhibitory effect on dentin matrix metalloproteinases (MMPs), thus reducing the degradation of adhesive interfaces.[6] However, the scientific literature reports conflicting results, since *in vitro* studies have demonstrated decreased bond strengths with self-etch adhesive systems applied after CHX-based cavity disinfectant,[7-9] while analysis of other data[9-11] demonstrated no adverse effect on dentin bonding compared with a control group.

In the most commonly encountered clinical situations, especially when a cavity disinfectant is applied, bonding procedures are carried out in abnormal dentin, such as caries-affected dentin. Several studies have investigated the durability and quality of the adhesive bonds not only to sound dentin substrates but also to carious dentin substrates,[9,10] since different degrees of mineralization may challenge effective bonding.[12] Since acceptable bond strength may depend on the type of dentin substrate, cavity disinfectant application, and the composition of adhesive systems, comprehensive evaluations of dentin-type-dependent bond strength of self-etch adhesives and their relations with CHX-based cavity cleaners are still lacking. No previous study has evaluated the short-term effect of the commonly used 2% concentration of chlorhexidine-based disinfectant on bonding to artificially demineralized dentin as substrate.

This study aimed to evaluate the short-term effect of a chlorhexidine-based disinfectant, applied previously to a self-etch adhesive system, on the bond strengths of composite resin to sound and demineralized dentin.

Methodology

This *in vitro* study involved a 2 × 4 factorial design, where the factors under evaluation were

- pre-restoration treatment at 2 levels:
  - deionized water (DW),
  - 2% chlorhexidine digluconate (CHX), and
- dentinal status at 4 levels:
  - sound dentin,
  - dentin demineralized by pH-cycling for 2 days,
  - dentin demineralized by pH-cycling for 4 days, and
  - dentin demineralized by pH-cycling for 8 days.

Tooth preparation

The Ethical Research Committee of the School of Medicine of the Federal University of Ceará approved the use of 47 extracted caries-free human third molars (protocol # 185/08), which were stored in 0.01% (w/v) thymol solution at 4°C and used within 6 months after extraction.[13] A flat dentin surface was exposed on each tooth after wet grinding of the occlusal enamel on #100-grit silicon-carbide (SiC) paper mounted in a polishing machine (Arotec SA, Cotia, Brazil). Dentin surfaces were exposed and inspected under ×8 magnification to ensure that no enamel remnants were left (Leica M60, Heerbrugg, Switzerland). The enamel-free exposed dentin surfaces were further polished on wet #600-grit SiC paper for 60 s to produce a standardized smear layer.

Using a computer-generated list, we randomly assigned the teeth to one of 4 groups (n = 8), according to the number of days of pH-cycling (0, 2, 4 or 8). The samples in the control non-cycled group were kept under refrigeration and humidity until being subjected to bonding procedures.

pH-cycling

The teeth to be demineralized were coated with acid-resistant nail varnish except for the occlusal area and subjected to demineralization-remineralization cycles at 37°C. The pH-cycling protocol proposed by Argenta *et al.*[14] was modified for a higher pH of demineralizing solution, Tris buffer, and different numbers of cycles to preserve the dentin surface, producing demineralization that could be evaluated by cross-sectional hardness testing (CSH) measurements. Each cycle consisted of a 4-h immersion in 20 mL of demineralizing solution, followed by a 20-h immersion in 10 mL of remineralizing solution. The demineralizing solution contained 2 mM of calcium ($CaCl_2 2H_2O$), 2 mM of phosphate (KH_2PO_4), 75 mM acetic acid, 0.030 ppm F, and 0.1 mM Tris buffer adjusted to a pH of 4.6. The remineralizing solution contained 1.5 mM of calcium ($CaCl_2$), 0.9 mM of phosphate (KH_2PO_4), 0.050 ppm F, and 0.15 M KCl adjusted to a pH of 7.4.[14]

Cross-sectional hardness testing (CSH)

To determine the different mineral contents among groups, we recorded lesion depths and integrated demineralization of the groups subjected to pH-cycling. Fifteen additional teeth (5 per group cycled) were analyzed with regard to integrated area of microhardness (kg/mm²) loss versus lesion depth (ΔS), according to Sousa *et al.*[13] In addition, we obtained the hardness profile of each group by plotting hardness values as a function of distance from the dentin surface.

Bonding procedures

Eight teeth in each group were sectioned, under water, parallel to the long axis, by means of a diamond disk mounted on a cutting machine to create 2 identical halves. One half was used as control, exposed to DW, while the dentin surface of the other half was treated with CHX (Sigma Chemical Co., St. Louis, USA). Both DW and CHX were scrubbed for 5 minutes by means of a disposable applicator; for CHX, this timing was proved to be effective in reducing *S. mutans in vitro* and *in situ*.[5]

Dentin surfaces were bonded with a two-component all-in-one self-etch adhesive, All-Bond SE, pH = 2.2 (Bisco Inc., Schaumburg, USA; batch # 0800008924), that was applied according to the manufacturer's recommendations, after the surfaces were dried by means of a compressed air syringe at a distance of 5 cm from the tooth. Composite resin (Filtek Z250; 3M ESPE, St. Paul, USA; batch # 0N141392BR) was then used incrementally to build up the specimen to a thickness of 5 mm. Each 1-mm increment was individually light-activated for 20 s by means of a Light Emitting Diode Optilight LD Max (Gnatus, Ribeirão Preto, Brazil) with a power density of 600 mW/cm². The specimens were stored in deionized water at 37°C for 24 h.

Microtensile bond strength testing

The bonded teeth were serially sectioned, with a water-cooled diamond saw in a cutting machine, in mesial-distal and buccal-lingual directions, to obtain sticks with a cross-sectional area of approximately 0.8 mm², measured with a digital caliper.[11] Each stick was glued with cyanoacrylate-based adhesive (Zapit Base and Accelerator, Dental Ventures of America Inc., Corona, USA), attached to opposing arms of the testing device and finally stressed until failure with a tensile force in a microtensile testing machine[8] (Micro Tensile Tester, T-61010 K, Bisco, Schaumburg, USA) at a crosshead speed of 1 mm/minute. The bond strength (MPa) of each specimen was determined as the failure load (N) divided by the cross-sectional area of the bonded interface.

Debonding pathway determination

Both surfaces of each fractured specimen were observed under a stereomicroscope (Leica Zoom 2000; Leica Microsystems GmbH, Wetzlar, Germany) at ×40 magnification to record the types of failure, which were classified as mixed, adhesive, cohesive failure in dentin, and cohesive failure in composite resin.

Statistical analysis

The normality of error distribution and the degree of non-constant variance were checked for each response variable. To assess the degrees of demineralization produced by the pH-cycling regimens, we analyzed the dependent variable ΔS and hardness profile. Since there was no violation of assumptions, hardness at different depths was analyzed by one-way analysis of variance (ANOVA) and Tukey tests. The μTBS means were analyzed by two-way ANOVA and a *post hoc* Tukey test. A regression analysis was used to correlate the number of cycles with each ΔS. Statistical significance was pre-set at $\alpha = 5\%$. Statistical analysis was performed with SPSS 17.0 statistical software (SPSS, Chicago, USA).

Results

The mean ΔS after pH cycling are reported in Figure 1. When these data were studied by regression analysis, significance was found, with a linear fit for the number of pH-cycling days ($p < 0.05$, Figure 1).

Figure 2 displays the hardness profile related to the studied groups. When the hardness differences in each studied depth from the surface were com-

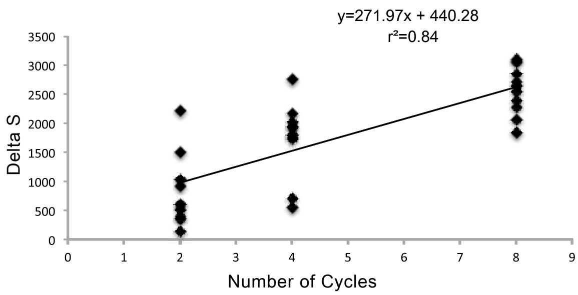

Figure 1 - Linear regression of ΔS as a function of number of pH-cycling days.

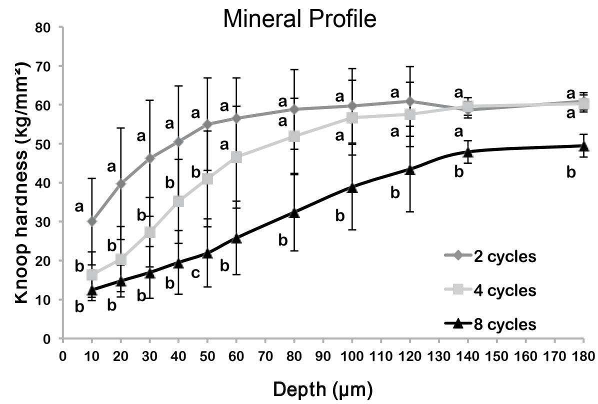

Figure 2 - Graphic chart of the descriptive statistics of hardness number related to each pH-cycling group as a function of depth. Groups identified with different letters were statistically significantly different ($p < 0.05$).

pared among the 3 pH-cycling groups, the groups subjected to 2 and 4 days of pH-cycling presented similar mineral hardness profiles at depths deeper than 50 µm. The group subjected to 8 days of pH-cycling did not reach the hardness of sound dentin (around 60), even at a depth of 180 µm.

The mean µTBS and respective standard deviations are summarized in Figure 3. A two-way ANOVA found a significant effect for mineral content ($p < 0.001$) but no significant effect for CHX ($p = 0.82$) or for the interaction between these factors ($p = 0.95$). The Tukey *post hoc* test ($p < 0.05$) revealed the following significant differences: the sound dentin group presented the lowest bond strength, which was different from that of all other groups. The highest µTBS was found for the 2- and 4-day groups, which were statistically similar to each other, but different from the 8-day as well as the sound groups.

Most of the observed failures were mixed (Table 1). The fracture surfaces of the specimens in all groups were predominantly mixed cohesive fractures in both resin and dentin, and cohesive fractures in both resin and dentin were also observed in some debonded specimens. However, adhesive fractures at the resin-dentin interface were mainly found in the group which had undergone 8 days of pH-cycling, regardless of the use of CHX.

Discussion

The current study demonstrated, for the first time, that CHX, used as a cavity disinfectant, did not affect the short-term bonding of self-etch adhesive systems to artificially demineralized dentin.

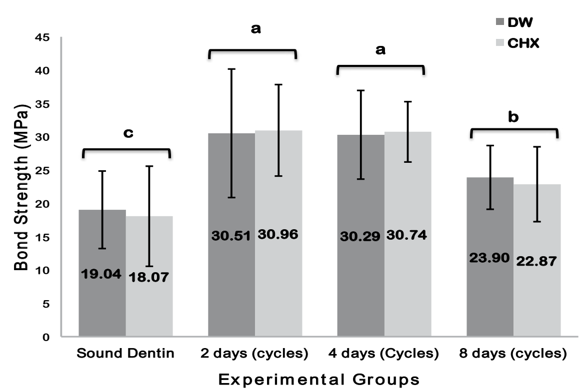

Figure 3 - Means and standard deviations of bond strengths (MPa) for all groups in which dentin was either control (DW) or CHX-pretreated (2% CHX) specimens that were tested immediately. Groups identified with different letters were statistically significantly different ($p < 0.05$).

Our results corroborate those of previous studies[9,10] reporting that disinfection did not adversely affect bonding to dentin compared with a control group. Nevertheless, these results do not agree with earlier reports showing decreased bond strengths when self-etch adhesive systems were applied after CHX-based cavity disinfectant.[7,8,10] This divergence may be due to the different adhesive tested in the earlier studies (two-step self-etch Clearfill SE Bond), which has a composition different from that of the two-component all-in-one self-etch All-Bond SE, thus interacting differently with CHX and the smear layer.

Table 1 - The fracture-type proportions of the debonded specimens in each group.

Group	Type of failure (%)							
	Deionized water groups				Chlorhexidine groups			
	M	A	CD	CC	M	A	CD	CC
Sound dentin	48.24%	24.14%	12.05%	12.05%	64.44%	15.09%	2.40%	18.07%
2 cycles (DE/RE)	66.97%	22.19%	6.67%	4.17%	63.92%	28.15%	4.76%	3.17%
4 cycles (DE/RE)	41.67%	27.79%	12.96%	17.59%	47.95%	27.73%	11.71%	12.61%
8 cycles (DE/RE)	69.33%	29.32%	0%	1.35%	69.85%	30.15%	0%	0%

*M, mixed failure; A, adhesive failure; CD, cohesive failure in dentin; CC, cohesive failure in resin composite.

Our data also diverge from those of another study, which demonstrated increased bond strength of a self-etch adhesive system after carious dentin was treated with a 5% CHX solution. It can be suggested that this discord was due to the different CHX concentrations and testing times (2 years) evaluated in both studies, reinforcing the fact that the substantivity of CHX is concentration-dependent.[15]

We were unable to determine if CHX would improve the bond strengths of self-etch adhesives. Additional long-term studies may be necessary to determine the long-term effects of CHX on the bond strengths of self-etch adhesive systems, because their acidity provides a low-pH medium that may trigger the action of latent endogenous MMP enzymes.[10] It is possible that the effects of CHX can be shown only after longer periods of evaluation.

Although, in clinical situations, carious dentin is the common tissue requiring treatment, sound dentin is more available for use in testing the performance of dental adhesives.[12] Nevertheless, sound dentin is not the most important residual dentin substrate that remains after caries removal. Frequently, the remaining substrate presents peculiar chemical and mechanical characteristics, causing differences in its receptiveness to adhesion.[12] Therefore, because different conditions of caries development co-exist within a given lesion, this research tested bonding in artificially demineralized dentin with different mineral contents. One of the detectable changes in caries-affected dentin was a lower degree of mineralization caused by repeated cycles of demineralization.

Analysis of our data demonstrated that the pH-cycling model used in the present study was able to produce different levels of demineralization in human dentin, since the ΔS, an indicator of the area of mineral loss in caries lesions, significantly increased with an increase in the number of days of pH-cycling. The lesion depth demonstrated by analysis of the hardness profile of the cycled groups showed that dentin subjected to 8 days of pH-cycling presented a deeper lesion, because, at the 180-µm depth, the hardness for this group was significantly lower than that found for the other groups (Figure 2). It is important to note that groups cycled for 2 or 4 days reached hardness numbers equal to values characteristic of deep sound dentin (Knoop hardness number > 60),[16] around the depth of 100 µm, and these values were statistically significantly higher than those found for the 8-day cycling group.

Generally, the presence of carious dentin decreases bond strengths,[17-19] and the higher the level of caries progression, the lower the bond strengths of adhesives to carious dentin.[20] The present results partially confirm this statement, since 8-day cycling group showed µTBS values lower than those found for the 2- and 4-day cycling groups. Further, in our study, lesion depth was found to be more relevant than mineral loss in determining bond strength, since the 2- and 4-day cycling groups presented both similar lesion depths and similar bond strengths. The 8-day cycling group showed the deepest lesions and reduced bond strength compared with the 2- and 4-day cycling groups. As previously suggested, deeper lesions that, like those in natural carious dentin, have a much higher water content may have compromised solvent and water evaporation before the adhesive cure, which impairs the polymerization of the adhesive, thus decreasing the bond strengths.[21] Nevertheless, no cause-effect relationship can be established between lesion depth and bond strength based on our results.

Surprisingly, sound dentin presented the lowest bond strength, suggesting that certain supplementary demineralization may have increased the initial bond strength, probably due to the mild acidity of the self-etch adhesive system used. The system may not be sufficiently acidic to dissolve the mineral casts and infiltrate the compact and thick smear layer, since the adhesive system used in this study was a mild self-etch system with a pH value of 2.2, and additional demineralization promoted higher μTBS values. The clinical relevance of our data is that mildly acidic self-etch adhesive systems may perform poorly when used with more mineralizing substrates.

Lesions created by *in vitro* pH-cycling have some limitations, because *in vivo* dentin is exposed not only to acid but also to bacterial and oral enzymes.[12] Thus, inasmuch as the demineralized and unprotected organic dentin matrix (collagen) was not degraded through bacterial and host-mediated enzymes, carious dentin obtained in the current research is not as similar to that found *in vivo* and presented bond strength higher than that of sound dentin.

Conclusion

Within the limitations of this *in vitro* study, the following conclusions may be drawn: the application of a self-etch adhesive system on CHX-treated sound dentin or artificially caries-affected dentin did not change the bond strengths, although additional dentin demineralization may increase the bond strength of a mildly acidic (pH ≥ 2) self-etch adhesive system to sound dentin.

Acknowledgements

This research received financial support from the National Council for Scientific and Technological Development (CNPq/Brazil, Grant # 477070/2008-0). The first and second authors received scholarships during this study from CNPq (564387/2008-8) and PIBIC/UFC, respectively.

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