Chlorhexidine does not Increase Immediate Bond Strength of Etch-and-Rinse Adhesive to Caries-Affected Dentin of Primary and Permanent Teeth

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The aim of this study was to evaluate the effect of 2% chlorhexidine digluconate (CHX) on immediate bond strength of etch-and-rinse adhesive to sound (SD) and caries-affected (CAD) primary dentin compared with permanent dentin. Flat dentin surfaces from 20 primary molars (Pri) and 20 permanent molars (Perm) were assigned to 8 experimental groups (n=5) according to tooth type (Pri or Perm), dentin condition (SD or CAD - pH-cycling for 14 days) and treatment (control - C or 60 s application of 2% CHX solution after acid etching - CHX). The bonding system (Adper Single Bond 2) was applied according to manufacturer's instructions followed by resin composite application (Filtek Z250). After 24 h water storage, specimens with cross-section area of 0.8 mm^2 were prepared for being tested under microtensile test (1 mm/min). Data were submitted to ANOVA and Tukey's post hoc test (α=0.05). Failure mode was evaluated using a stereomicroscope at ×400. Treatment with CHX did not result in higher bond strength values than no pre-treatment (C groups), independently of tooth type. Primary teeth and caries-affected dentin showed significantly lower ($p<0.05$) bond strength means compared with permanent teeth and sound dentin, respectively. Predominance of adhesive/mixed failure was observed for all groups. CHX did not influence the immediate bond strength to sound or caries-affected dentin of primary and permanent teeth.

Key Words: chlorhexidine, bond strength, caries-affected dentin, primary tooth, permanent tooth.

INTRODUCTION

According to the current concepts of restorative dentistry, ultraconservative treatment approaches are recommended for treating cavited dentin lesions. Therefore, the possibility of greater preservation of dental sound tissue, consequence of the partial removal of carious dentin (1) and of the use of adhesive restorative materials, have instigated the evaluation of bond strength to sound and caries-affected dentin in permanent (2,3) and primary (4-6) teeth.

Previous researches in permanent teeth have reported lower values of bond strength to caries-affected dentin compared with sound dentin (2,3), due to the presence of acid-resistant whitlockite minerals within the dentinal tubules (2,7) and intertubular dentin partially demineralized (3,8). Nevertheless, the results obtained in primary teeth are still controversial (4-6).

Questions also persist in the comparison of the adhesive system's performance between primary and permanent teeth, even in sound substrate. Some studies have shown lower bond strength values to primary dentin (8,9), while others have found similar values (10) or even superior performance in primary dentin (11).

Despite the adequate immediate bond strength, *in vivo* (12) and *in vitro* (13) studies have revealed that resin-dentin bonds deteriorate over time. It has been speculated that a decreasing concentration gradient of resin monomer diffusion within the acid-etched dentin, and a subsequent resin elution from hydrolytically unstable

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polymeric hydrogels within the hybrid layers (14) leave the exposed collagen fibrils susceptible to hydrolytic and enzymatic degradation, mediated by endogenous metalloproteinases (MMPs) (15). Researchers have demonstrated that chlorhexidine digluconate (CHX) acts as a potent inhibitor of these enzymes (13,16). In addition to minimizing the degradation of resin-dentin bonds, CHX application should not negatively influence the immediate bond strength, which has already been pointed out in literature (13,17). However, it is unclear in the literature the effect of CHX in caries-affected dentin (18-20), especially in primary teeth.

Furthermore, since primary and permanent dentin present differences in structure and composition (21), studies are necessary in order to compare the effect of CHX in caries-affected dentin on both substrates. To the best of our knowledge, this is a pioneering investigation of the influence of CHX application on adhesion of etch-and-rise system to caries-affected primary dentin compared with permanent dentin.

The aim of this *in vitro* study was to evaluate the effect of CHX on immediate bond strength to sound and caries-affected dentin of primary and permanent teeth. The null hypotheses were that: a) CHX has no effect on immediate bond strength to sound or caries-affected dentin; and b) adhesive performance with and without previous CHX application on primary and permanent dentin is not significantly different.

MATERIAL AND METHODS

Tooth Selection and Preparation

Ethical approval was obtained by the local Ethics Committee. Forty sound human teeth (20 primary second molars and 20 permanent third molars) were selected and stored in 0.5% chloramine T at 4ºC for 30 days before use. Teeth were assigned to 8 groups (n=5) according to the tooth type (primary - Pri or permanent - Perm), dentin condition (sound - SD or caries-affected - CAD) and treatment (no pretreatment/control - C or 2% CHX application - CHX).

The occlusal enamel was removed with a water-cooled diamond disc in a cutting machine (Labcut 1010; Extec Co., Enfield, CT, USA) to obtain flat dentin surfaces. Surrounding enamel was also removed with a diamond bur (#3195; KG Sorensen, Barueri, SP, Brazil) in a high-speed handpiece with water spray. Exposed occlusal dentin surfaces were then polished with 600-grit silicon carbide paper under running water to create a standardized smear layer.

Artificial Caries-Affected Dentin Induction

Half of the previously prepared teeth (n=20) were subjected to pH-cycling to create artificial caries-affected dentin. The roots and cervical portions were sealed with epoxy resin (Araldite Hobby; Ciba Especialidades Químicas Ltda, São Paulo, SP, Brazil) and received two layers of an acid-resistant nail polish (Colorama Maybelline Ltda, São Paulo, SP, Brazil).

Specimens were then immersed in 10 mL of demineralizing solution (2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 50 mM acetic acid adjusted pH of 4.8) for 8 h and in the same volume of remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 mM KCl adjusted pH of 7.0) for 16 h (22). After immersions, the teeth were rinsed with deionized water. Solutions were changed every day for 14 days (cycles).

Bonding Procedures

The flat dentin surfaces were etched with 35% phosphoric acid gel for 15 s, rinsed with water and dried with an air stream. Dentin surfaces were re-hydrated with 1.5 µL of distilled water (C groups) or 1.5 µL of 2% CHX (CHX groups). Solutions were left on the dentin surfaces for 60 s. Excess solutions was removed with absorbent paper and the dentin surfaces were keep moist. Two consecutive coats of the adhesive system (Adper Single Bond 2; 3M ESPE, St. Paul, MN, USA) were applied, gently air dried and light-cured for 10 s. Resin composite buildups (Filtek Z250; 3M ESPE) were placed on the bonded surfaces (increments of approximately 1.5 mm), which were individually light-cured with a LED unit with 800 mW/cm^2 light intensity (LED Olsen; Olsen Ind. e Com. S/A, Palhoça, SC, Brazil) for 20 s each.

Microtensile Bond Strength Test (µTBS)

After storage in distilled water at 37ºC for 24 h, each tooth was vertically sectioned with a low-speed diamond disc in a cutting machine (Labcut 1010; Extec Co.) to obtain sticks with a cross-sectional area of approximately 0.8 mm^2, according to microtensile ‘non-trimming’ technique.

Specimens were immediately subjected to a microtensile test on a universal testing machine (DL

2000; Emic, São José dos Pinhais, PR, Brazil) at a crosshead speed of 1 mm/min.

Failure Mode

Premature failure was considered as debonded specimens during its preparation that could not be tested.

After the test, all fractured specimens were observed on stereomicroscope with ×400 magnification (HMV II; Shimadzu, Kyoto, KYO, Japan) to determine failure mode: adhesive/mixed (failure at resin/dentin interface or mixed with cohesive failure of the neighboring substrate) or cohesive (failure exclusively within dentin or resin composite).

Statistical Analysis

The experimental unit in the current study was the tooth. Thus, the mean of μTBS values in MPa of all specimens from the same tooth were averaged for statistical analysis. Premature debonded specimens were included in the statistical analysis (23) and were assigned 4.0 MPa for value to these specimens.

Equality of variances and normal distribution of data were confirmed using the Bartlett and Kolmogorov-Smirnov tests respectively. Data were subjected to three-way ANOVA (tooth type x dentin condition x treatment) and Tukey's post hoc at 5% significance level. Statistical analysis was carried out with Minitab 16 software package (Minitab Inc., State College, PA, USA). Failure mode data were analyzed descriptively.

RESULTS

Microtensile bond strength means and standard deviations for all groups are summarized in Table 1. Only the main factors tooth type ($p<0.01$) and dentin condition ($p<0.01$) were statistically significant.

CHX application prior to the adhesive system did not influence the bond strength values, since there were no significant differences for CHX and Control groups. However, bond strength values to primary teeth and to caries-affected dentin were significantly lower than those obtained to permanent teeth and to sound dentin, respectively.

Failure Mode

Figures 1 and 2 present the percentage frequencies of failure modes for experimental groups according to tooth type (primary and permanent). Adhesive/mixed failure was predominant in all experimental groups.

DISCUSSION

Previous studies (13,24) have shown that

Table 1. Microtensile bond strength means (MPa) and standard deviations for all groups.

Tooth type	Control		Chorhexidine	
	SD[a]	CAD[b]	SD[a]	CAD[b]
Primary[A]	30.8±2.2	24.5±3.8	32.8±3.8	25.6±3.6
Permanent[B]	41.7±2.7	29.1±6.0	43.2±4.7	36.4±1.3

SD: sound dentin. CAD: caries-affected dentin. Different superscript uppercase letters indicate significant difference for tooth type. Different superscript lowercase letters indicate significant difference for dentin condition.

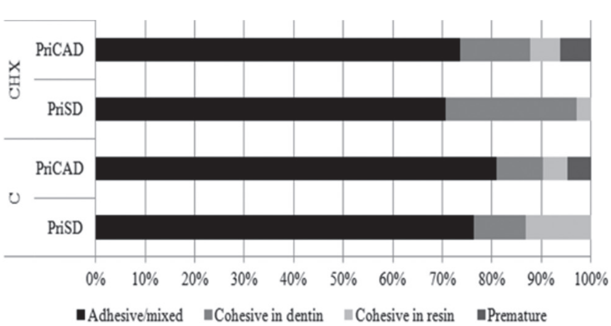

Figure 1. Percentage frequencies of failures for the control and experimental groups of primary teeth. SD: sound dentin. CAD: caries-affected dentin

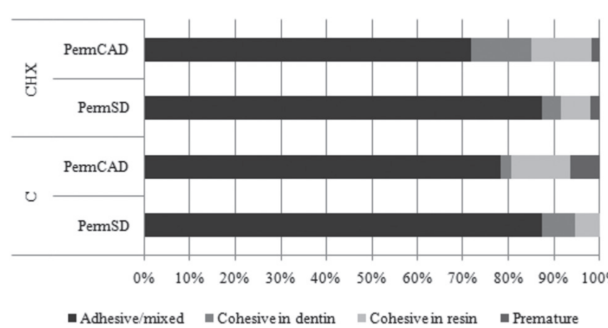

Figure 2. Percentage frequencies of failures for the control and experimental groups of permanent teeth. SD: sound dentin. CAD: caries-affected dentin

applying the 2% CHX after acid etching, in order to minimize the degradation of resin-dentin bonds, does not negatively influence the immediate bond strength to sound permanent dentin. Similar results were observed in the present study in primary teeth, corroborating the findings of Ricci et al. (17). The authors verified that CHX application increased the bond strength of Prime & Bond NT and Adper Single Bond to the acid-etched sound primary and permanent dentin, while no positive or negative effect was observed for Excite DSC.

Mobarak et al. (20) evaluated the effect of different CHX concentrations on bond strength of self-etch primer adhesive to sound and caries-affected permanent dentin and revealed insignificant differences between control, 2% and 5% CHX pretreatment for sound as well as for caries-affected dentin. Komori et al. (19) also observed that the CHX did not affect the immediate bond strength values for a simplified etch-and-rinse adhesive applied in sound or caries-affected dentin. Similar results were found in the present study. Since 2% CHX application did not affect the immediate bond strength of the tested etch-and-rinse adhesive to sound and caries-affected dentin, the primary null hypothesis was accepted.

However, although CHX application did not influence the bond strength values to sound dentin, it appears that this treatment favors the adhesion to caries-affected dentin, especially in permanent teeth, since there was a clear upward trend in bond strength values. Unluckily, there is no clear explanation for this issue. As caries-affected dentin has been reported to exhibit increased collagenolytic activity when compared with sound dentin (16), it could be speculated that CHX application could inhibit the degradation of caries-affected dentin-bonded interfaces in comparison with sound dentin (25). The higher porosity of caries-affected intertubular dentin may help in gaining the benefit of CHX pretreatment by allowing more penetration of CHX to deeper layers vulnerable to MMPs. Unfortunately, this phenomenon did not occur in the primary and permanent teeth of the present study, and future investigations can be addressed to test this hypothesis.

Adhesive performance in primary dentin was poor compared with permanent dentin, regardless to use of CHX pretreatment, resulting in the rejection of the second null hypothesis. Considering the great variability in shape, size and depth of natural carious lesions and the difficulty to obtain flat ideal carious dentin surfaces for testing bond strengths, especially in primary teeth, due to reduced thickness of enamel and dentin, often leading to pulp chamber involvement, in this study caries-affected dentin was induced using the protocol suggested by Marquezan et al. (22). It seems to promote a caries-affected dentin layer with superficial demineralization (until 50 μm).

Caries-affected dentin presents physics and chemicals characteristics different of the sound dentin (2,7). Carious intertubular dentin exhibits a higher degree of porosity, due to mineral loss. This will result in a deeper demineralized layer which, after penetration of the adhesive, allows the formation of hybrid layers that are much thicker than those produced in sound dentin (2). This increase in demineralized depth may contribute to the lower μTBS values to caries-affected primary and permanent dentin obtained, since resin monomers may not penetrate as deeply as acid in the intertubular dentin.

Differences in composition, tubular density, intrinsic moisture and dentinal permeability (21) between primary and permanent teeth may be responsible for the lower bond strength values obtained in sound primary dentin, which were similar to those of previous studies that also found worst results to primary dentin (8,9).

The results of the present study demonstrate the importance of the substrate on bonding to dentin because the dentin condition (sound and caries-affected) and tooth type (primary and permanent) significantly influenced the bond strength values. Furthermore, whereas pretreatment with CHX aims to minimize the deleterious action of MMPs on the collagen fibrils exposed at the adhesive interface and in caries-affected dentin, there is a greater exposure of demineralized dentin that is not infiltrated by resin monomers on the bottom of the hybrid layer, and thus further researches are needed to assess the effect of CHX application on the stability of resin-caries-affected dentin, mainly in primary teeth.

In conclusion, CHX did not influence the immediate bond strength to sound or caries-affected dentin of primary and permanent teeth. However, dentin condition and tooth type influenced on bond strength, whereas adhesion to caries-affected dentin was lower than that verified to sound dentin and primary teeth showed lower bond strength than permanent ones.

RESUMO

O objetivo deste estudo foi avaliar o efeito do digluconato de clorexidina (CHX) a 2% na resistência de união imediata de adesivo convencional à dentina decídua hígida (H) e afetada pela lesão de cárie (CA) comparada à dentina permanente.

Superfícies dentinárias planas de 20 molares decíduos (Dec) e 20 molares permanentes (Perm) foram divididas em 8 grupos experimentais (n=5) de acordo com o tipo de dente (Dec ou Perm), condição da dentina (H ou CA - ciclagem de pH por 14 dias) e tratamento (controle - C ou CHX - aplicação por 60 s de solução de digluconato de clorexidina a 2% após condicionamento ácido). O sistema adesivo (Adper Single Bond 2) foi aplicado de acordo com as instruções do fabricante, seguido da aplicação de resina composta (Filtek Z250). Após 24 h de armazenamento em água, espécimes com área de secção transversal de 0,8 mm^2 foram preparados para o teste de microtração (1 mm/min). Os dados foram submetidos à Análise de Variância e teste de Tukey para comparações múltiplas (α=0,05). Modo de falha foi avaliado usando estereomicroscópio com aumento de 400×. Tratamento com CHX não resultou em maiores valores de resistência de união do que sem pré-tratamento (C grupos), independente do tipo de dente. Dentes decíduos e dentina afetada apresentaram menores médias de resistência de união em comparação a dentes permanentes e dentina hígida, respectivamente ($p<0,05$). Predomínio de falha adesiva/mista foi observado para todos os grupos. CHX não influenciou a resistência de união imediata à dentina hígida ou afetada pela lesão de cárie de dentes decíduos e permanentes.

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