Original Article

Effect of chlorhexidine concentration on the bond strength to dentin in primary teeth

Efeito da concentração de clorexidina na resistência de união à dentina em dentes decíduos

Abstract

Purpose: To evaluate the effect of the use of 0.5% and 2% chlorhexidine digluconate on the immediate bond strength of a conventional adhesive system to dentin in primary teeth.

Methods: Twenty-one healthy primary molars were divided into three groups (n=7), being one control (A) and two experimental groups (B and C). After dentin exposure, in Group (A) the adhesive procedure was performed using 37% phosphoric acid gel (15 s); dentin was washed (15 s), air dried (30 s) and rehydrated with water. Groups B and C followed similar procedures but for re-hydration with 0.5% and 2% chlorhexidine, respectively, for 30 s. A resin composite block was built simulating a restoration, and the teeth were stored in distilled water at 37°C for 24 h before the microtensile bond strength test. The bond strength data were analyzed by analysis of variance.

Results: No statistically significant difference in bond strength was found among the tested groups ($P > 0.05$)

Conclusion: The 0.5% and 2% concentrations of chlorhexidine presented similar behavior and caused no adverse effects on the bond strength to dentin in primary teeth.

Key words: Chlorhexidine; bond strength; microtensile; primary teeth

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Resumo

Objetivo: Avaliar o efeito do uso de digluconato de clorexidina 0,5% e 2% na resistência de união imediata à dentina de dentes decíduos para um sistema adesivo convencional.

Metodologia: Vinte e um molares decíduos hígidos foram divididos em três grupos (n=7), sendo um controle e dois experimentais. Após a exposição da dentina, foi realizado no grupo controle (A) o procedimento adesivo utilizando ácido fosfórico gel a 37% (15 s); a superfície foi então lavada (15 s), seca com ar (30 s) e reidratada com água. Os grupos B e C foram idênticos ao grupo A, apenas com diferença no reumidecimento de clorexidina 0,5% e 2% respectivamente, por 30 s. Após a confecção do bloco de resina composta, os dentes foram armazenados em água destilada a 37°C por 24 h antes do teste de microtração. Os dados de resistência de união foram avaliados através de análise de variância.

Resultados: Os dados apresentaram distribuição homogênea, não havendo diferença estatisticamente significante entre os grupos ($P > 0,05$).

Conclusão: As concentrações de clorexidina a 0,5% e 2% apresentaram comportamentos similares e não causaram efeitos adversos na resistência de união em dentina de dentes decíduos, quando comparadas ao grupo controle.

Palavras-chave: Clorexidina; resistência de união; microtração; dentes decíduos

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Introduction

Bonding to the dentin substrate is much more complex than that to enamel. Dentin has less inorganic content and greater humidity content, these being the characteristics that make it difficult to achieve a lasting bond. Other important factors are the morphological and structural differences between primary and permanent teeth. The thinner dentinal layer of primary teeth may be responsible for the lower bond strength to these teeth (1). In addition to the heterogeneity of dental tissues, water plays an important role in obtaining adhesion. Water is believed to be related to the mechanisms of degradation at the bond interface and also with the reduction in mechanical properties of adhesive systems. The demineralized dentinal zone is not completely infiltrated by the resinous monomers (2), which would leave the exposed subjacent collagenous fibrils susceptible to hydrolytic degradation (3), causing weakening of the hybrid layer and possibly decreasing adhesion (4,5).

Chlorhexidine has been widely used for cavity cleaning and has been used after performing cavity preparation for dentinal disinfection to reduce the bacterial count (6). Another important aspect that has recently become the target of more in-depth studies is that in addition to its antimicrobial capacity, chlorhexidine has an inhibitory action on metaloproteinases (MMPs). The MMPs comprise a group of 23 enzymes that present the metabolic activity of remodeling and degradation of various types of collagens (7). Studies have revealed the contribution of the host's MMPs to the pathogenesis of dental caries (7). The authors have based themselves on the hypothesis that the degradation of collagen fibrils may be accelerated by the presence of these endogenous enzymes even in the absence of bacteria (5,8-10). As a result of the dentin mineralization process at the stage of dentinogenesis, these enzymes are retained in the extracellular matrix in a latent state, but can be activated if the dentin is demineralized (11). Therefore, it is expected that the MMPs will be released in the process of collagen exposure by acid etching and are related to the loss of bond strength over time (12,13).

Some studies demonstrated that the application of chlorhexidine has an inhibitory effect on the MMPs, significantly improving the integrity of the hybrid layer in the course of time (9). Chlorhexidine could be used as a complementary method for rehydrating dentin and therefore, preserve the humidity necessary to maintain the reactive collagen network, increasing the durability of polymeric restorations. Nevertheless, pre-treatment with chlorhexidine may become a problem should it interfere in the adhesive procedures to dentin. Although many studies have described the influence of chlorhexidine on bond strength to permanent teeth, its effect in primary dentition is still unclear. Considering the versatile use of chlorhexidine in Restorative Dentistry, it is opportune to investigate the influence of different concentrations of chlorhexidine on the immediate bond strength to primary teeth. Therefore, this study aimed to evaluate the effect of 0.5% and 2% chlorhexidine digluconate on the immediate bond strength to dentin of a simplified conventional adhesive system in primary teeth.

Methodology

Twenty-one healthy human primary molars were used in this research, which was approved by the local Research Ethics Committee (Protocol No. 2008/0110). The specimens were stored in a 0.5% chloramine solution and afterwards in distilled water at 4ºC. The sample (n=21) was divided into three experimental conditions (A, B, C; n=7), according to the dentin treatment performed.

The occlusal surface enamel of the teeth was worn with silicone carbide abrasive paper (grit 180) under irrigation, until a dentinal surface without any enamel remainder was obtained. To obtain a standardized smear layer, the surfaces were treated with silicone carbide abrasive paper (grit 600) for 60 s.

Bonding and restorative procedures

The dentinal surfaces were then etched with phosphoric acid for 15 s, followed by washing (15 s), drying (30 s), In Group A, the dentin was rehydrated with 1.5 µL water (14); while in Groups B and C the dentin was rehydrated with an aqueous solution of 0.5% and 2% chlorhexidine digluconate, respectively. After this the adhesive was applied in accordance with the specifications stated in Table 1, and light activated for 10 s with 600 mW/cm² (GNATUS, Optilight LD Max, Ribeirão Preto, São Paulo, Brazil). After the adhesive procedure, 3.0 mm high restorations were performed with resin composite Filtek™ Z250 (3M/ESPE, St Paul, USA), in three portions. Each of the portions was light activated for 40 s with a mean power of 600 mW/cm² (GNATUS, Optilight LD Max, Ribeirão Preto, São Paulo, Brazil). After this the teeth were stored in distilled water at 37ºC for 24 h. All the restorative procedures were performed by a single operator, at an ambient temperature of 24°C and 75% relative humidity of the air.

Microtensile Testing

The specimens were serially sectioned by a Labcut 1010 machine (Extec Corp., Enfield, CT, USA). The cuts were parallel and perpendicular o obtain specimens with a rectangular sectional area of approximately 0.8 mm². The specimens were stored in artificial saliva (0.70 mmol/L $CaCl_2$; 0.20 mmol/L $MgCl_2.6H_2O$; 4.00 mmol/L KH_2PO_4; 30.0 mmol/L KCL; 0.30 mmol/L NaN_3; 20.0 mmol/L HEPES) (5) and tested after 24 h.

Each specimen was fixed with cyanoacrylate adhesive (Zapit, Dental Ventures of North America, Carona, CA, USA) to a custom hook developed for microtensile tests, which was coupled to a test machine (EMIC, São José dos Pinhais, PR, Brazil). The microtensile test was conducted at a crosshead speed of 0.5 mm/min until rupture. The rupture force was recorded in N, and the cross-sectional area of the specimens was measured with a digital pachymeter in mm² to compute the bond strength in MPa. The data were submitted to analysis of variance at the significance level of 0.05.

The fractured test specimens were analyzed under a stereomicroscope (40×) and classified into the following fracture patterns: 1) cohesive in dentin; 2) cohesive in resin composite; 3) adhesive/mixed at the interface.

Table 1. Description of the adhesive procedures in the tested groups.

Groups	Procedures
A Control	Conventional acid etching at 37% (15 s); Washing with air and water spray (15 s); Air drying (30 s); Dentin rehydrated with 1 μL water; Two layers of Adper Single Bond 2 adhesive applied for 10 s; Air jet for 10 s at 20 cm; Light activation (10 s, 600 mW/cm²); Insertion of 3 increments of resin composite Filtek™ Z250 Light activation of each increment (40 s, 600 mW/cm²)
B 0.5% chlorhexidine	Conventional acid etching at 37% (15 s); Washing with air and water spray (15 s); Air drying (30 s); Dentin rehydrated with 1.5 μL chlorhexidine at 0.5% for 30 s; Two layers of Adper Single Bond 2 adhesive applied for 10 s; Air jet for 10 s at 20 cm; Light activation (10 s, 600 mW/cm²); Insertion of 3 increments of resin composite Filtek™ Z250 Light activation of each increment (40 s, 600 mW/cm²)
C 2% chlorhexidine	Conventional acid etching at 37% (15 s); Washing with air and water spray (15 s); Air drying (30 s); Dentin rehydrated with 1.5 μL chlorhexidine at 2% for 30 s; Two layers of Adper Single Bond 2 adhesive applied for 10 s; Air jet for 10 s at 20 cm; Light activation (10 s, 600 mW/cm²); Insertion of 3 increments of resin composite Filtek™ Z250 Light activation of each increment (40 s, 600 mW/cm²)

Table 2. Bond strength to dentin of the tested groups.

Groups (n=7)	Bond strength (Mean±SD)*
A: Control	50.8±12.8
B: 0.5% chlorhexidine	46.5±4.0
C: 2% chlorhexidine	44.0±8.7

* No significant difference between means (ANOVA, $P > 0.05$).

Results

The bond strength data presented a normal and homogeneous distribution. Table 2 shows the bond strength mean and standard deviation values of the tested groups. No statistically significant difference was found among the groups ($P = 0.403$).

Table 3 shows the percentage distribution of specimens according to the fracture mode after the microtensile test. The predominant fracture pattern was the adhesive/mixed type, irrespective from the tested group.

Discussion

Chlorhexidine is widely used as an antimicrobial agent to eliminate the harmful effects caused by bacteria, thus improving the prognosis of restorative treatment of dental caries (15). Therefore, many researchers have pointed out the importance of the use of chlorhexidine as a complement in bonding procedures (16).

Chlorhexidine digluconate is an organic compound known for its potent antiseptic and antibacterial action, that acts against a wide spectrum of microorganisms, among them gram positive, gram negative, fungi and some types of viruses (17). The action mechanism of chlorhexidine occurs due to the interactions between its cationic molecule and negative charge of the bacterial cell wall, altering the osmotic equilibrium of the microorganism and promoting cell death. Thus, the use of chlorhexidine provides a complementary treatment that contributes to suppressing the residual infection in restorative treatment.

The present results indicated that the use of 0.5% and 2% chlorhexidine digluconate, before the application of the adhesive system, did not result in a reduction in immediate bond strength when compared with the control group. Few studies have evaluated the influence of chlorhexidine on bond strength in primary teeth (9,19,20), but the results agrees with those of the present study, since the application of 2% chlorhexidine did not affect the resin-dentin bond strength. Previous *in vitro* and *in vivo* studies in permanent and primary teeth have shown that the application of chlorhexidine at concentrations ranging between 0.12% and 2%, before acid etching (16,19- 21) or after acid etching (10,13,21,22), did not result in adverse effects on the immediate bond strength between the dentinal substrate and polymeric material.

The use of the chlorhexidine solution after acid etching and before the application of the adhesive system is justified, because in addition to reducing bacteria, chlorhexidine has an inhibitory action on the metalloproteinases (MMPs), thus decelerating the process of adhesive interface degradation.

Table 3. Number (percentage %) of specimens according to the fracture patters for each group.

Groups	Adhesive or Mixed fracture	Cohesive fracture in dentin	Cohesive fracture in resin
A: Control	34 (97.1)	1 (2.9)	0 (0.0)
B: 0.5% chlorhexidine	27 (77.1)	5 (14.3)	3 (8.6)
C: 2% chlorhexidine	29 (82.9)	2 (5.7)	4 (11.4)

In the literature it is well established that the tooth-restoration bond deteriorates over the course of time (5). For conventional adhesive systems, an incomplete diffusion of the resinous monomers into the conditioned dentin results in incomplete hybridization, leaving exposed collagen fibrils that would be vulnerable to hydrolytic degradation (4). It has been speculated that activated forms of the metalloproteinases (MMPs) would be responsible for the self-degradation of unprotected collagen fibrils in the hybrid layer, even in the absence of bacteria (5). The use of chlorhexidine, even in low concentrations, strongly inhibits the colagenolytic activity of dentin. The authors believe that the inhibitory effect is owing to an action of calcium chelation and sequestration (9).

Few studies have evaluated the effect of chlorhexidine concentration on the capacity to preserve bond strength. Recently, Breschi et al. (23) demonstrated that the use of a 0.2% concentration of chlorhexidine digluconate was as efficient as a 2% concentration to preserve bond strength in permanent teeth after 12 months of evaluation. Moreover, the incorporation of chlorhexidine into phosphoric acid is also effective in the preservation of bond strength (13). These data suggest that low concentrations of chlorhexidine can be effective for retarding the degradation of adhesive interfaces.

From the clinical perspective, chlorhexidine appears to be an interesting substance, since it has been used for disinfecting cavity preparations, acting as an efficient method for inhibiting bacterial growth (6), while simultaneously preserving the integrity of the resin-dentin interface when used before application of the adhesive system. Nevertheless, further studies need to analyze the effects of different concentrations and application times of chlorhexidine on the durability of bond interfaces in primary tooth over time.

Conclusions

Within the limitations of this study, it was possible to conclude that the 0.5% and 2% concentrations of chlorhexidine presented similar behaviors and caused no adverse effects on the bond strength to dentin in primary teeth.

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