

Influence of chlorhexidine on longitudinal bond strength to dentin: in vitro study

Influência da clorexidina na resistência de união longitudinal à dentina: estudo in vitro

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ABSTRACT

Objective: This study evaluated the effect of 0.2% chlorhexidine gluconate solution used as an therapeutic primer on the long-term bond strength of etch-and-rinse adhesive to dentin. **Material and Methods:** Bovine incisors were worn to expose an area of dentin and were divided into 2 groups: Group C (Control) - acid etching with 37% phosphoric acid + Single Bond; Group CHX (0.2% CHX) - acid etching with 37% phosphoric acid + 0.2% CHX for 30 s + Single Bond. Blocks of composite were fabricated and stored for 24 h or 6 months, sectioned into beams and submitted to microtensile tests. Results were analyzed by two-way ANOVA and Tukey tests. **Results:** Mean (\pm SD) values (in MPa) were as follow: Group CHX/24h - 41.8(\pm 2.62)A; Group C/24h - 40.8(\pm 3.35)AB; Group CHX/6 months - 36.4(\pm 3.52)B; Group CHX/6 months - 26.1(\pm 1.54)C. **Conclusion:** CHX improve the imediatte bond strength of resin-dentin and significantly lowered the loss of bond strength after 6 months water storage as seen in the control bonds.

KEYWORDS

Tensile bond strength; Dentin; Total-etch adhesives; Chlorhexidine gluconate.

CLINICAL SIGNIFICANCE

The 0.2% chlorhexidine gluconate solution used as an therapeutic positively influencing the bond strength, and consequently, the durability of resin composite restorations.

RESUMO

Objetivo: Este estudo avaliou o efeito da solução de gluconato de clorexidina 0,2% (CHX) usado como primer terapêutico sobre a resistência de união longitudinal de adesivo convencional a dentina. **Material e Métodos:** dentes bovinos foram desgastados para obter uma área de dentina, e foram divididos em 2 grupos: Grupo C (Controle) - ácido fosfórico à 37% + 0,2% CHX por 30s + Single Bond. Blocos de resina composta form fabricados e armazenados por 24 h e 6 meses, seccionados e submetidos ao teste de resistência a microtração. Os resultados foram analisados por ANOVA dois-fatores seguido pelo teste de Tukey. Resultados: Valores de média (\pm Desvio-padrão) (MPa): Grupo CHX/24h - 41,8(\pm 2,62)A; Grupo C/24h - 40,8(\pm 3,35)AB; Grupo CHX/6 meses - 36,4(\pm 3,52)B; Grupo CHX/6 meses - 26,1(\pm 1,54)C. **Resultados:** Valores de média (\pm Desvio-padrão) (MPa): CHX (24 h) - 41,76 (\pm 2,62)a; C (24h) - 40,81 (\pm 3,35)ab; GT (24h): 37,38(2,98)abc; CHX (6 meses) - 36,04 (\pm 3,52)bcd; EGCG (24h) - 35,91 (\pm 4,82)cd; EGCG (6 meses) - 35,75 (\pm 4,44)cd; GT (6 meses) - 31,95 (\pm 3,40)de; C (6 meses): 30,05 (\pm 1,54)e. **Conclusão:** CHX aumentou a resistência de união imediata da interface dentina-resina e significativamente reduziu a perda de resistência de união após 6 meses de armazenagem em água quando comparado ao grupo controle.

PALAVRAS-CHAVE

Resistencia de união; Adesivos convencionais; Gluconato de clorexidina.

SIGNIFICÂNCIA CLÍNICA

A solução de gluconato de clorexidina 0,2% usada como primer terapêutico influenciou positivamente a resistência de união e, consequentemente, a durabilidade das restaurações de resina composta.

INTRODUCTION

After nearly 4 decades of research, advances in the adhesive systems and bond techniques allow less wear of sound tooth structure and contributed to the increase of bond strength between tooth and restorative material. [1] However, the longevity of hybrid layer has been still widely questioned as a consequence of hydrolysis of the adhesive interface over time. [2-4]

Degradation of the hybrid layer may be triggered by various factors. The degradation of the collagen fibers present in the dentin organic matrix which were left unprotected after the process of demineralization by acid etching and not infiltrated by the adhesive monomers is the main factor related to decreases in bond strength. [5,6] Dentine has proteolytic enzymes, also called matrix metalloproteinases (MMPs) that can be activated by acid etching during adhesive procedure. [7,8] These MMPs are responsible for rapidly degrade denatured collagen fibers. Among the MMPs identified in the organic matrix dentin are gelatinases (MMP-2 and -9) collagenase (MMP-8) and enamelysin (MMP-20) [9]. It is believed that these MMPs are related to degradation of the adhesive interface [10].

Thus, inhibition of MMPs could be effective in the preservation of the hybrid layer [10]. Studies have shown that the use of MMP inhibitors improves the integrity of the hybrid layer [7]. Thus, the use of chlorhexidine digluconate (CHX) disinfectant solution prior to the use of a dentin bonding agent have been investigated in order reduce the degradation of the hybrid layer [11,12] due to their MMP inhibitory properties [13]. Previous studies have shown that the degradation of the organic matrix infiltrated with resin can be delayed through the use of CHX [7,12,14,15].

Therefore, longitudinal evaluation of the bond strength using this disinfectant solution has become of primordial importance. The

aim of this study was to evaluate in vitro, the influence of CHX on the microtensile bond strength to dentin of the two-step total-etch adhesive at time intervals of 24 h and after a 6 months period of storage in water at 37 °C.

This study tested two null hypotheses: 1) CHX applied prior to the adhesive system does not affect bond strength to dentin; 3) the storage period does not affect the bonding effectiveness of two-step total-etch adhesive to dentin.

MATERIAL AND METHODS

Forty freshly extracted bovine incisor teeth bovine were used in this study. The roots were sectioned with a steel diamond disc (KG Sorensen, Rio de Janeiro, Brazil) at the cement-enamel junction. The buccal surfaces were worn using abrasive papers (granulation 600) coupled to a circular polishing machine (PA-10, Panambra, São Paulo, Brazil) under water cooling, to obtain a 5mm² area of flat dentin.

The dental surface was worn to standardize the remaining dentin thickness at 2 mm, using a caliper thickness gauge (Golgran, São Caetano do Sul, Brazil) to measure it. The smear layer was standardized using 600 grit abrasive papers coupled to a circular polishing machine.

Bonding Procedures

The teeth were divided into 2 groups (n = 20), according to the dentin treatment performed:

Group C (Control technique): The surfaces were etched for 15 s with 37% phosphoric acid gel, rinsed and the excess moisture was removed with absorbent paper. Two layers of Single Bond total-etch adhesive (3M ESPE, St. Paul, MN, USA) were applied on the surface actively for 15 s and gently air dried for 10 s. The adhesive was light activated for 10 s with a LED light unit (Emitter A, Schuster, Santa Maria, RS, Brazil) with power density of 600 mW/cm².

Group CHX (0.2% CHX technique): The surfaces were etched for 15 s with 37% phosphoric acid gel, rinsed and the excess

moisture was removed with absorbent paper. An aliquot of 1.5 μ l of 0.2% chlorhexidine digluconate disinfectant solution (Byofórmula, São José dos Campos, SP, Brazil) was applied prior to adhesive actively for 30s with mini brushes, and excess of CHX was removed with absorbent paper, leaving a moist substrate surface. The Single Bond total-etch adhesive (3M ESPE) was applied following the same protocol applied for Group C.

Restoration placement

Composite resin blocks (Amelogen Plus, Ultradent, Indaiatuba, SP, Brasil), approximately 4 mm high were built on the treated surfaces. Each 2 mm portion was light activated for 40 s. Materials used in this study, chemical composition and manufacturers are presented in Table 1.

The bonded teeth were stored in distilled water at 37 °C, for time intervals of 24 h or 6 months. The water was changed every week during the course of 6 months. [16]

Microtensile Bond Strength Test

The test specimens were cut into parallel sections measuring approximately 1 mm, made from the mesial to the distal, and from the cervical to the occlusal surface, using a diamond disc attached to a Labcut 1010 (Ext

Technologies Inc., USA) cutting machine to obtain sticks, producing a minimum of 7 sticks per tooth. The sections were made at low speed under water cooling to prevent stress induction at the bond interface.

The sticks were attached to a microtensile device in a universal testing machine (DL-1000, EMIC, São José dos Pinhais, PR, Brazil), with a 10 kg load cell, at a cross-head speed of 0.5 mm/min, in accordance with the ISO 11405 Standard. The bond strength data were expressed in Megapascals (MPa).

Statistical Analysis

Pre-test failures (PTFs) were not included in statistical analyses. To the cohesive failures (dentin or composite), the specimens were discarded. The mean value for the sticks originating from each tooth was calculated and used for the statistical analysis. [17]

Data, expressed in megapascal (MPa), were analyzed by two-way ANOVA (technique X storage time) followed by Tukey test (α 5%).

Scanning electron microscopy (SEM) examination

Two teeth from each group were prepared for SEM analysis. The specimens were sectioned perpendicularly to the bonding interface. The sections were polished with 2000 and 4000 mesh sheets. Phosphoric acid etchant was applied for 15 s, rinsed off with water for 10 s. Specimens were dehydrated, sputter-coated with gold-palladium and examined by SEM (high vacuum, 15KV, Everhart-Thornley Detector/ETD, 5000X magnification).

RESULTS

Table 2 shows the results of the Tukey test for the interaction between the independent variables of “technique” and “storage time” ($p=0.00$) and type of fracture detected under microscopy for each group.

The Group CHX assessed after 24 h showed the highest tensile bond strength mean values. The Group C with storage interval of 6 months presented the worst tensile bond strength values.

Table 1 - Materials, composition and material's manufacturers

Materials	Manufacturers	Composition
Condac 37	FGM, SC, Brazil	Phosphoric acid 37%.
Single Bond 2	3M ESPE St.Paul, MN, USA	Bis-GMA, HEMA, dimethacrylate, methacrylate functional copolymer of polyacrylic and polytaconic acid, water, alcohol, photoinitiator.
0.2% chlorhexidine gluconate	Byofórmula, São José dos Campos, SP, Brazil	0.2% chlorhexidine gluconate solution, pH 6.8
Amelogen Plus Cor A2	Ultradent, Indaiatuba, SP, Brazil	Bis-GMA, TEGDMA, microparticles barium, amine, silicate, borate

* Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, bisphenol A glycidyl methacrylate.

The Group CHX with storage interval of 6 months presented significantly lower mean bond strength values when compared with Group CHX with storage interval of 24 h, and higher mean bond strength values when compared with Group C with storage interval of 6 months.

Table 3 presents the fracture type results.

The adhesive and mixed fractures were the predominately detected types of fracture.

Figures 1A and 1B show SEM images obtained of the interfaces created by Group C (Control), respectively storage for 24 h and 6 month of storage. Figures 2A and 2B show SEM images obtained of the interfaces created by

Table 2 - Mean (\pm standard-deviation) bond strengths of Group C versus Group CHX tested 24 h or after 6 months of aging

Group	Technique	Storage Time	Mean(\pm SD)	Homogeneous sets*
CHX	0.2% CHX	24 h	41.8 \pm 2.62	A
C	Control	24 h	40.8 \pm 3.35	A B
CHX	0.2% CHX	6 months	36.4 \pm 3.52	B
C	Control	6 months	26.1 \pm 1.54	C

*Groups identified by different letters are significantly different ($p < 0.05$).

Table 3 - Results of fracture types

Group	Technique	Storage time	PTF/IST	Failure mode (%)			
				A	CD	CC	M
CHX	0.2% CHX	24h	0/90	40	5	3	52
C	Control	24h	0/90	42	3	5	50
CHX	0.2% CHX	6 months	32/78	45	3	2	50
C	Control	6 months	8/82	40	3	2	55

Legends: PTF/IS = pre-test failures/intact sticks tested. A = adhesive; CD = cohesive failure in dentin; CC = cohesive failure in resin composite; M = mixed failure

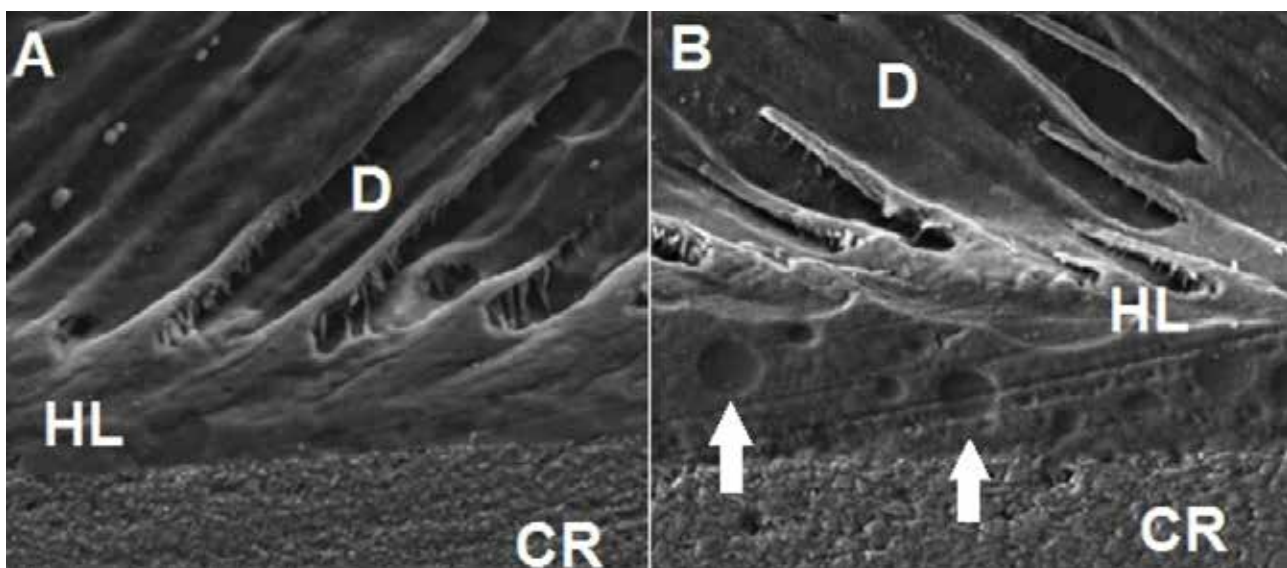


Figure 1 - A and B – Hybrid layer formed by Group C (Control), respectively storage for 24 h and 6 month of storage. A typical hybrid layer was created (Figure 1A). Adhesive resin with bubbles (arrows), which can indicate degradation due hydrolysis (Figure 1B). (Legends: CR – Composite resin; HL – Hybrid Layer; D – Dentin).

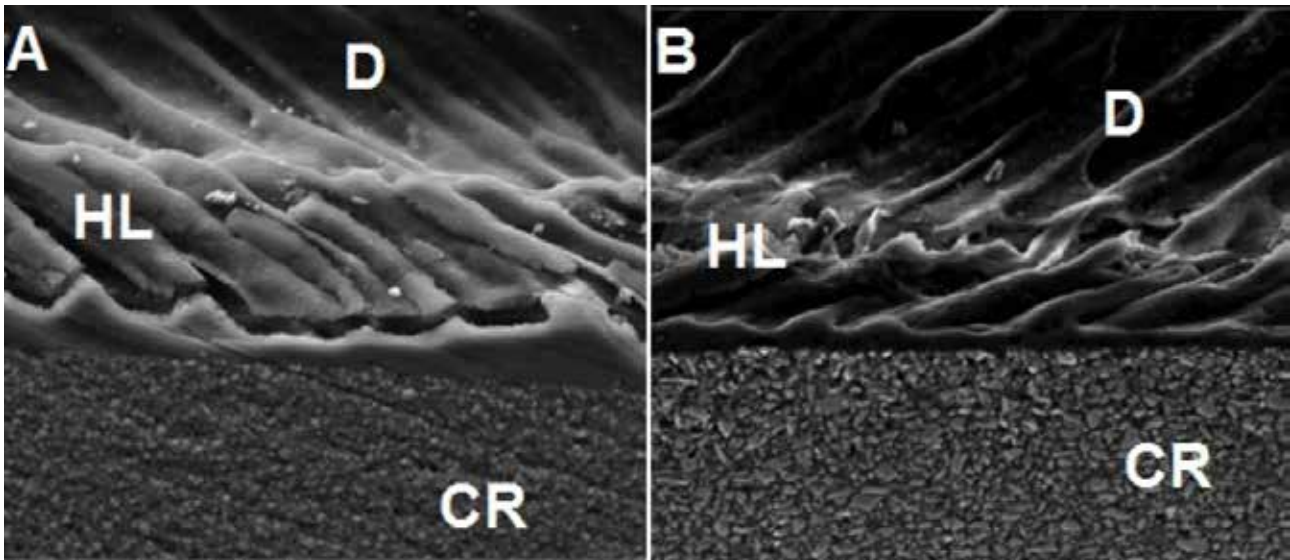


Figure 2 - A and B – Hybrid layer formed by Group CHX, respectively storage for 24 h and 6 month of storage. CHX used as a therapeutic primer did not interfere the formation of hybrid layer. No areas of degradation of hybrid layer, over time, were observed (Figure 2B). (Legends: CR – Composite resin; HL – Hybrid Layer; D – Dentin.)

Group CHX, respectively storage for 24 h and 6 month of storage.

DISCUSSION

The first null hypothesis was rejected, because the 0.2% CHX technique presented higher bond strength values compared with the Control technique. The second null hypothesis was rejected, because the storage time of 24 h presented higher bond strength values in comparison with the storage time of 6 months.

Adhesive systems have been modified over the years mainly as a result of technological advances, however, the possibility of failures in hybrid layer formation can be observed. Degradation of the hybrid layer may be triggered by various factors. When excessive acid etching is performed, it enables a very deep etched dentin surface to be formed; the adhesive is unable to infiltrate into it completely, giving rise to a region where the exposed collagen fibers are weakened and more susceptible to hydrolysis. [18]

The MMPs present in dentin matrix are zinc- and calcium-dependent enzymes, which are responsible for physiology regulation and

pathogenic metabolism of collagen-based tissues [19,20]. Previous studies have detected the presence of MMP-2 and -9 in the dentin matrix [9,21], and when released and activated, play an important role in the degradation of collagen fibers exposed within the hybrid layer. Collagenolytic and gelatinolytic activity of these enzymes are likely responsible for rapidly degrade denatured collagen which were left unprotected after the process of demineralization by acid etching and not infiltrated by the adhesive monomers [21], resulting in gaps in the adhesive interface over time. [10]

Several in vitro and in vivo studies have been accomplished with the aim of improving the adhesive interface using inhibitors of the MMPs in the adhesive protocol [12,15,22]. CHX is one of the main substances used as potential MMP inhibitor, which has been introduced as a complementary step in the adhesive protocol, with good results in in vitro studies [7,14,15,18,22,23].

CHX is an antiseptic and antimicrobial solution [24], which inhibits bacterial adhesion to dental surfaces by competing with calcium or retention sites. Therefore, this solution can

prevent the formation of calcium-link between bacteria-dental surfaces or between bacteria-bacteria [25]. This mechanism may be effective in the inhibition of MMPs, since these are zinc and calcium-dependent [10]. Recent studies have shown that the use of CHX prior to adhesive system, without rinsing, significantly inhibited the MMPs activity in the hybrid layer [7,15,23] even at low concentrations [13], which is in agreement with the results of our study.

When CHX is ionized, it is characterized with a strong base, with cationic properties. And this cation portion is able to bind to negatively charged surfaces / target sites of the substrate. Thus, CHX is able to remain on the substrate and re-mineralized dentin [26] independent of incubation time and its concentration. [27] This prevents that proteases bind collagen fibers not infiltrated by monomers, preserving the hybrid layer after long periods of exposure to water. [28] According to Gendron et al. [13], two mechanisms of action are involved in the inhibition of MMP; chelation inhibiting MMP-2 and -9 and the interaction with cysteines and/or sulfhydryl groups present in the active site of MMP-8.

It can be observed in this study that the use of CHX, as an adjuvant in dentin bonding, did not chemically interfere with the immediate adhesion of resin materials (Figure 2A), because there is no difference in bond strength between 0.2% CHX technique and control technique after 24 h (Figure 1A). Also, 0.2% CHX technique after 6 months of storage presented lower bond strength when compared with the same technique at 24 h, but higher bond strength when compared with control technique with storage interval of 6 months. Therefore, one noted that the storage time did not interfere so strongly in the bonding of groups that used 0.2% CHX, when compared with those that did not use 0.2% CHX (Figure 1B), which can demonstrate a relative maintenance of the bond strength of the 0.2% CHX technique (Figure 2B) when compared to control technique.

According to Carrilho et al. [29] and Brackett et al. [30], long-term action of CHX can be explained by their confinement in the adhesive interface, since their removal through the dentinal fluid flow is reduced by the formation of resin tags that obliterate the dentinal tubules. Thus, CHX may remain trapped between the collagen fibers during the impregnation of interfibrillar spaces for fluid resin during application of the adhesive. [31]

CHX is a large molecule (molecular mass = 897.8 g mol⁻¹) compared to some monomers adhesive systems, such as Bis-GMA (molecular mass = 512.0 g mol⁻¹) and HEMA (molecular mass = 130.0 g mol⁻¹) [32], however, in this study, CHX did not influence the resin monomers infiltration in dentin after acid etching, as observed in other studies. [29,33-35]

After 6 months of storage, both techniques showed decrease in bond strength values, however, the control technique showed the highest decrease, due to degradation of the hybrid layer. However, the 0.2% CHX technique was not also able to maintain the stability of the resin-dentin bond strength values over time. This decrease in bond strength may have occurred not only by the presence of MMPs but also by the incomplete polymerization of infiltrated monomers, or presence of free water in the dentine due to incomplete evaporation of residual solvents in the adhesive, as well as the presence of water in the saliva and in the intrinsic humidity of dentin resulting in a high osmolarity of the mixture of hydrophilic adhesive, creating water-filled channels within the adhesive, as observed by Stanislawczuk et al. [28]

However, the better bond strength result of 0.2% CHX technique within 6 months of storage compared to control technique may be due to the ability of CHX to inactivate MMPs exposed in the hybrid layer, and their ability to be retained in the dentin matrix and their antimicrobial substantivity [27], allowed the formation of a hybrid layer which remained stable over time. The term substantivity is present in the literature

much more with the residual antibacterial activity of CHX in human dentin [36,37] than for the inhibitory effect of CHX on MMP activity.

The antimicrobial substantivity of CHX is associated with the release of positively charged molecules from the substances treated with CHX and its ability to adsorb substances from the oral cavity. According to Loguercio et al. [23], it can also occur in the exposed collagen fibers within the hybrid layer, which can reduce degradation of the adhesive interface after exposure to water in the long term.

The use of 0.2% CHX and the storage time did not influence the type of fracture, and fractures were predominantly adhesive or mixed fractures.

Our study suggests that a low concentration of CHX (0.2%) applied for 30 s, is sufficient to preserve the adhesive interface for at least 6 months under in vitro conditions. Furthermore, in vivo studies are needed to clarify whether 0.2% CHX for 30 s is sufficiently able to preserve the adhesive interface after long time.

Therefore, within the limitations of this study, 0.2% CHX has a considerable potential in maintaining the stability of bond interface to dentin after 6 months of water storage. These results are in agreement with those of various studies [7,22,29,31], that observed a real potential MMP inhibitor of CHX, which can increase the time durability of adhesive restorations. However, new researches are necessary to finding an effective way of maintaining the adhesive interface in the long term.

CONCLUSION

Within the limitations of this study it can be concluded that:

- CHX used as a therapeutic primer improve the immediate bond strength of resin-dentin and significantly lowered the loss of bond strength after 6 months water storage as seen in the control bonds.

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