INVESTIGATION

Effect of chlorhexidine pretreatment on demineralized dentin bond strength

Efecto del pretratamiento con clorhexidina en la resistencia de unión a dentina desmineralizada

Efeito do pré-tratamento de clorexidina na resistência de união à dentina desmineralizada

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Abstract

Objective: To analyze the bond strength to healthy and demineralized dentin, immediately and after 6 months, using a 2% chlorhexidine (CHX) pretreatment. **Method:** 40 healthy third molars with incomplete root development were abraded exposing dentin. The pieces were subjected to pH cycling. They were randomly divided into 2 groups: with and without CHX. In dentin, 4 resin buttons were created using universal adhesive in self-etching mode. The samples were stored in distilled water at 37ºC until analysis. Micro shearing was carried out at 24 hours and at 6 months of aging. **Results:** Healthy dentin group, without immediate CHX presented higher bond strength (23.37±1.84). (Demineralized dentin group, without CHX, aged) presented the lowest bond strength (8.87±1.51). **Conclusions:** CHX prior to adhesive application doesn't improve bond strength values to healthy or demineralized dentin in short nor long term.

Keywords: Demineralized dentin, Universal adhesives, Chlorhexidine, Young permanent teeth.

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Resumen

Objetivo: Analizar la resistencia de unión a dentina sana y desmineralizada, en forma inmediata y a los 6 meses, utilizando un pretratamiento de clorhexidina (CHX) 2%. **Método**: 40 terceros molares sanos con desarrollo radicular incompleto se desgastaron exponiendo dentina. Las piezas fueron sometidas a ciclado de pH. Se dividieron aleatoriamente en 2 grupos: con y sin CHX. En dentina se crearon 4 botones de resina utilizando adhesivo universal mediante autoacondicionamiento. Las muestras se almacenaron en agua destilada a 37ºC hasta su análisis. El microcizallamiento se ejecutó a las 24 horas y a los 6 meses de envejecimiento. **Resultados**: El grupo de dentina sana, sin CHX inmediato presentó mayor resistencia adhesiva (23,37±1,84). El grupo de dentina desmineralizada, sin CHX, envejecido presentó la menor resistencia adhesiva (8,87±1,51). **Conclusiones**: La CHX al 2% previo a la aplicación del adhesivo no mejora los valores de resistencia de unión a dentina sana ni desmineralizada a corto o largo plazo.

Palabras clave: Dentina desmineralizada, Adhesivos universales, Clorhexidina, Dientes permanentes jóvenes.

Resumo

Objetivo: Analisar a resistência de união à dentina hígida e desmineralizada, imediatamente e após 6 meses, utilizando um pré-tratamento com (CHX) a 2%. **Método:** 40 terceiros molares hígidos com desenvolvimento radicular incompleto foram desgastados expondo a dentina. As peças foram submetidas a ciclagem de pH. Eles foram divididos aleatoriamente em 2 grupos: com e sem CHX. Em dentina, foram criados 4 botões de resina utilizando adesivo universal em modo autocondicionante. As amostras foram armazenadas em água destilada a 37ºC até a análise. O microcisalhamento foi realizado às 24 horas e aos 6 meses de envelhecimento. **Resultados:** O grupo de dentina saudável, sem CHX imediata apresentou maior resistência adesiva (23,37±1,84). O grupo de dentina desmineralizada, sem CHX , envelhecida apresentou a menor resistência adesiva (8,87±1,51). **Conclusões**: A CHX antes da aplicação do adesivo não melhoraria os valores de resistência de união em dentina saudável ou desmineralizada a curto ou longo prazo.

Palavras-chave: Dentina desmineralizada, Adesivos universais, Clorexidina, Dentes permanentes jovens.

Introduction

Restorative dentistry has made progress in the selective removal of carious tissue (RSTC) in young adults. Currently, it is a recommended treatment for controlling the progression of carious lesions [1].

Schwendicke (2016) et al. [2] state that maintaining a reduced bacterial remnant in the cavity floor, deprived of nutrients, becomes irrelevant, as microorganisms sealed under a satisfactory restoration remain viable but inactive, preventing lesion progression. RSTC is recognized as a valid option for minimally invasive treatments that combine adhesive dentistry and restorative biomaterials. Current adhesive systems have not only improved their performance but also involve fewer handling steps, often offering advantages in bond strength to dentin substrates [3,4,5].

Adhesion to dentin poses a challenge beyond scientific advances, as its predominant organic component and moisture create an unfavorable terrain for hydrophobic adhesives. The hybrid layer formed between the adhesive system and dentin undergoes degradative processes of the resin and collagen components, as the monomers do not completely infiltrate the exposed collagen fibers (6, 7). To overcome these challenges, universal adhesives have emerged, capable of bonding to various substrates such as enamel, dentin, metals, and ceramics (8,9). The main feature of universal adhesives is the inclusion of the 10-MDP molecule, a bifunctional acidic monomer (dehydrogenated methacryloyloxydecyl-phosphate) with the ability to bind to the calcium in hydroxyapatite, forming a less soluble and more stable salt - Ca-10MDP (10, 11, 12). Additionally, universal adhesives enable proper priming and interaction with dentin tissue, which is naturally wet; they exhibit a higher degree of polymerization, reducing residual free monomers and repelling water to prevent hydrolysis, thereby providing enhanced stability of the hybrid layer (5, 11). Unprotected collagen fibers can be degraded by endogenous proteolytic enzymes found in dentin, known as matrix metalloproteinases (MMPs). CHX is an inhibitor of proteolytic activity. Scientific evidence shows (13,14) that CHX inhibits MMPs by its zinc and calcium chelating action preventing MMPs from performing their catalytic action. CHX at 2% has an extrinsic inhibitory effect on MMPs, especially MMP-2, MMP-8 and MMP-9 (15). In this study, the use of 2% CHX within the adhesive protocol has been proposed as a way to prevent the degradation of the exposed collagen, thus delaying the degradation of the hybrid layer, which will be responsible for successful adhesion.

Objectives

To analyze the effect of a 2% CHX pretreatment on healthy and demineralized dentin through the bond strength of a universal adhesive, immediately and after 6 months.

Method:

An in vitro, experimental, and longitudinal study was conducted at the Laboratory of Analysis and Development of Biomaterials (LADBio) at the Faculty of Dentistry (FO), **University of the Republic (Udelar).** The sample size was calculated using the SigmaPlot 12.0 program, considering a power of 80%, a type I error of 5%, and taking into account results reported in the literature (16). The calculation determined that a minimum of 8 specimens per group was needed to detect differences. The sample comprised 40 healthy, intact third molars that had not completed root development. These teeth were collected from the Surgical Block of the FO (all extractions were indicated for reasons beyond the scope of this research). Patients provided written consent for donation to the research.

Laboratory procedures

Once extracted, the teeth were stored in 0.5% Chloramine T for seven days and then in distilled water at a temperature of 3° to 5°C until the time of the study, for no more than three months. Each tooth was transversely abraded using a refrigerated trimmer, removing the enamel from the occlusal face, exposing a coronal dentin surface without pulp exposure. The abraded pieces were embedded in polypropylene (PPL) tubes using acrylic resin, leaving the dentin surface exposed. After being embedded, the exposed dentin surfaces were sequentially polished with silicon carbide sandpaper of 220, 400, and 600 grit size to standardize them (Fig. 1).

Once the standardization process was completed, the sample was randomly divided into two main groups of 20 molars each. One group was healthy dentin (DS) and the other group was demineralized dentin (DD). In the DS group, the smear layer was standardized by underwater sanding using 600g sandpaper. For the DD group, a protocol previously established in the literature was used. (17) The pieces included in this group were subjected to pH cycling. Initially, they were immersed in 10 mL of a demineralizing solution (2.2 mM $CaCl^2$ + 2.2 mM $KH2PO^4$ + 50 mM acetic acid at pH 4.8)

Fig. 1. Standardized pieces embedded in PPL tubes.

for 8 hours. They were then immersed in a remineralizing solution (1.5 mM $CaCl^2$ + 0.9 mM $KH2PO^4$ + 0.15M KCl at pH 7) for 16 hours. This cycling was carried out for 14 days at room temperature and under agitation. Once this period was over, the samples were washed using distilled water. Finally, the DD surface was sanded with 600g sandpaper for 30 seconds creating a demineralized surface with smear.

Then, the specimens were randomly divided into two subgroups (10 pieces per group) according to the application or not of a 2% CHX pretreatment. In the CHX pretreatment subgroups, a 2% aqueous solution of CHX (Laboratorio Abarly S.A. Lot 67162. Reg. MSP 38840) was applied with a microbrush for 15 seconds. After removing the excess water, the Single Bond Universal adhesive system (3M ESPE, USA) was applied to all groups using the self-etching adhesive technique, strictly following the manufacturer's instructions. After the adhesive strategy was completed in all groups, a cylindrical silicone matrix with four holes of 1.4 mm internal diameter was placed on the dentin surface. Each of the holes was filled with composite resin (Z250xt, 3M ESPE, USA). The resin was handled according to the manufacturer's instructions: light-cured for 20 seconds using a light-curing unit (Optilight MAX, Gnatus, Brazil) with an intensity of 1000mW/cm2 which was previously tested with a radiometer (Bluelight Metter, Ivoclar Vivadent, Liechtenstein). Immediately after photopolymerization, the silicone matrix was removed to expose the four resin cylinders.

Once this process was completed, eight groups were created, which are described below; the first four groups correspond to healthy dentin (DS) and the remaining four to demineralized dentin (DD). Also, the 2% chlorhexidine pretreatment is expressed in each group and whether the group was subjected to the immediate test or after six months.

- Group 1: Healthy dentin, with 2% chlorhexidine pretreatment, immediate shear test.
- Group 2: Healthy dentin, with 2% chlorhexidine pretreatment, shear test after six months.
- Group 3: Healthy dentin, without 2% chlorhexidine pretreatment, immediate shear test.
- Group 4: Healthy dentin, without 2% chlorhexidine pretreatment, shear test after six months.
- Group 5: Demineralized dentin, with 2% chlorhexidine pretreatment, immediate shear test.
- Group 6: Demineralized dentin, with 2% chlorhexidine pretreatment, shear test after six months.
- Group 7: Demineralized dentin, without 2% chlorhexidine pretreatment, immediate shear test.
- Group 8: Demineralized dentin, without 2% chlorhexidine pretreatment, shear test after six months.

All specimens were immersed in distilled water at 37°C for 24 hours. Subsequently, two out of the four resin cylinders from each specimen underwent the micro-shear test. After the initial testing, the specimens with the remaining two resin cylinders were stored in distilled water at 37°C for six months. Following the aging period, the remaining two cylinders in each specimen underwent the micro-shear test. Shear bond strength testing was conducted in accordance with ISO 29022 [18] using a universal mechanical testing machine (CMT 2000, MTS SANS, China). A 0.5 mm diameter stainless steel wire loop was precisely positioned at the resin-dentin adhesive interface at a crosshead speed of 1.0 mm/min (Fig. 2). The bond strength (in MPa) was calculated by dividing the load (in Newtons) by the bond interface area (mm^2).

Fig. 2: Universal testing machine performing micro-shear mechanical test.

Statistical analysis

The bond strength values are reported descriptively by averages and standard deviation in each group. Comparisons were made by means of a mixed ANOVA model, taking the following as fixed factors: pretreatment (with and without CHX), time (immediate and after 6 months) and dentin condition (DS and DD). Meanwhile, the intra-individual variation of each tooth was considered as a random factor. A statistical significance of 5% was established for all tests. All analyses were performed with R software for Windows (R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

Results

The average values obtained during the micro-shear test (MPa) are shown in Fig. 3. It can be seen that group 3 (DS, without immediate CHX pretreatment) had the highest average bond strength values (23.37±1.84), while group 8 (DD, without aged CHX pretreatment) had the lowest average bond strength values (8.87±1.51).

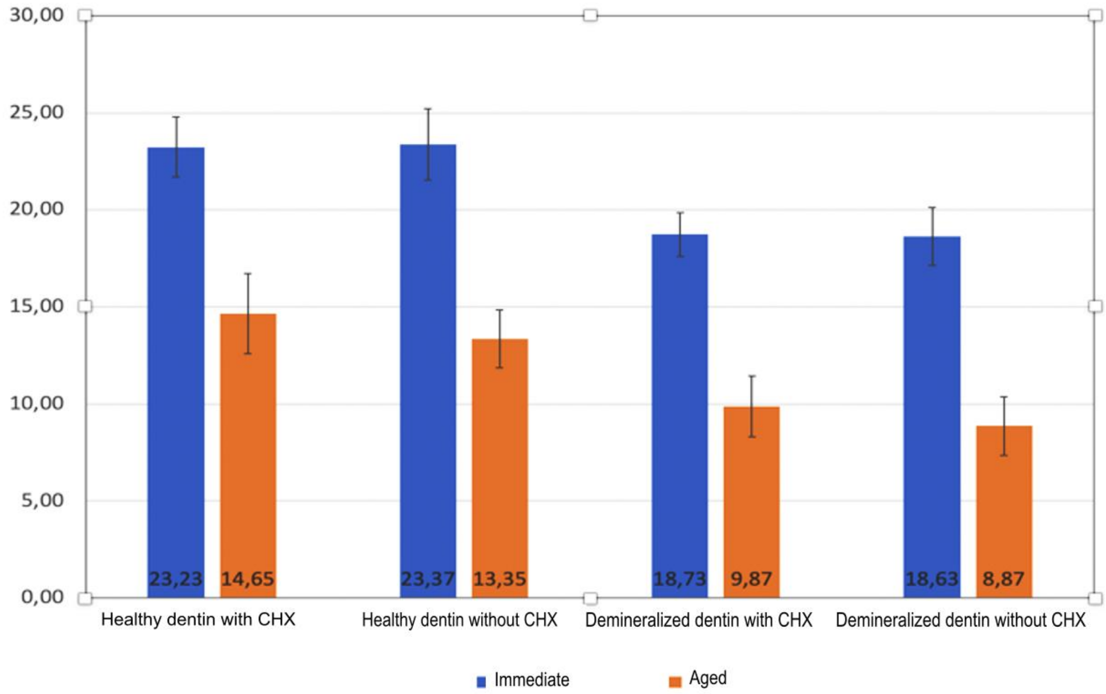

Fig. 3 Micro-shear bond strength values (MPa) of all analyzed groups.

The variations in bond strength values among different specimens were explained through a marginal effects model, taking into account three factors: pretreatment (with and without CHX), time (immediate and six months), and dentin status (DS and DD) (Table 1). A significant interaction of pretreatment with CHX over time (immediate and six months) in bond strength values was observed with a likelihood ratio test LRT=33.93, p≤0.001. However, there was no significant interaction of CHX pretreatment concerning dentin status (DS and DD) on bond strength, as shown by LRT=0.02, p=0.88.

Model	df	LRT	p-value
Marginal Effects	6		
+ CHX Interaction: Time	7	33,93	<0,001
+ CHX Interaction: State	8	0,02	0,882

Table 1. Interaction between CHX and time/state.

A significant effect of CHX pretreatment was observed concerning time ($p < 0.001$), but not regarding the demineralization state ($p = 0.882$). Multiple comparisons (Table 2) indicated that bond strength in the immediate test between CHX-pretreated cylinders did not significantly differ from untreated ones ($p = 0.89$). However, after six months, a statistically significant difference of 1.2 MPa was observed between the CHX-treated group and the non-CHX group ($p \leq 0.001$). Both comparisons were adjusted based on their demineralization state.

	Estimation	Standard deviation	p-value
Immediate			
with CHX	21,0	0,34	0,89
without CHX	21,0	0,34	
Aged			
with CHX	12,3	0,34	<0,001
without CHX	11,1	0,34	

Table 2. Multiple comparisons

Fig. 4 shows that the distribution of bond strength values did not differ when comparing the immediate CHX-treated and untreated cylinders. However, after six months, the bond strength decreased compared to the initial levels. The demineralized samples exhibited lower bond strength (irrespective of pretreatment). As indicated in Table 2, at six months, there appears to be a slightly higher bond strength in the CHX-pretreated cylinders (irrespective of the demineralization state).

Discussion

In our study, we were able to confirm that 2% CHX prior to adhesive application does not improve bond strength values to healthy or demineralized dentin neither in the short nor long term. Bond strength values did not show immediate differences when comparing groups with or without CHX pretreatment. At six months, a slightly higher bond strength seems to be evident in the CHX-pretreated cylinders (irrespective of the dentin state).

Regarding the generation of demineralized dentin in the laboratory, scientific evidence has shown that there are different protocols to achieve this. Marquezan et al. (17) proposed a protocol with pH cycling as they considered it more appropriate to simulate a substrate resembling the dentin layer affected by caries.

Moreover, Koyuturk et al. (19) reported that the primary cause of reduced bond strength in carious human dentin could be attributed to the components of the biomaterials used, rather than the acidity of the monomers included in self-adhesive systems. Additionally, Hosoya et al. (20) proposed that the altered mineral in the interfibrillar space of demineralized dentin might influence the formation of the hybrid layer and the chemical bonding with carboxylic and phosphate derivatives of methacrylates. Shen et al. (21) concluded that the 10-MDP monomer reduces both MMP activation and nanofiltration through a mechanism involving the formation of Ca-MDP salts. While CHX may interfere with the formation of these salts when applied in conjunction with 10-MDP, it does not have a detrimental effect. Furthermore, the bonding performance is enhanced by the application of 10-MDP.

As indicated in the literature, adhesives containing 10-MDP exhibit prolonged adhesion (22). Lima (23) demonstrated the presence of MMPs beneath the hybrid layer of exposed and non-infiltrated collagen. These enzymes can be activated by the presence of weak acids in adhesive systems. High dentin bond strength was achieved when the adhesive effectively infiltrated acid-exposed collagen or inhibited MMPs in the demineralized zone, responsible for degrading proteins like collagen and elastin (24).

In summary, while there is evidence suggesting that CHX can inhibit MMP activity, its impact

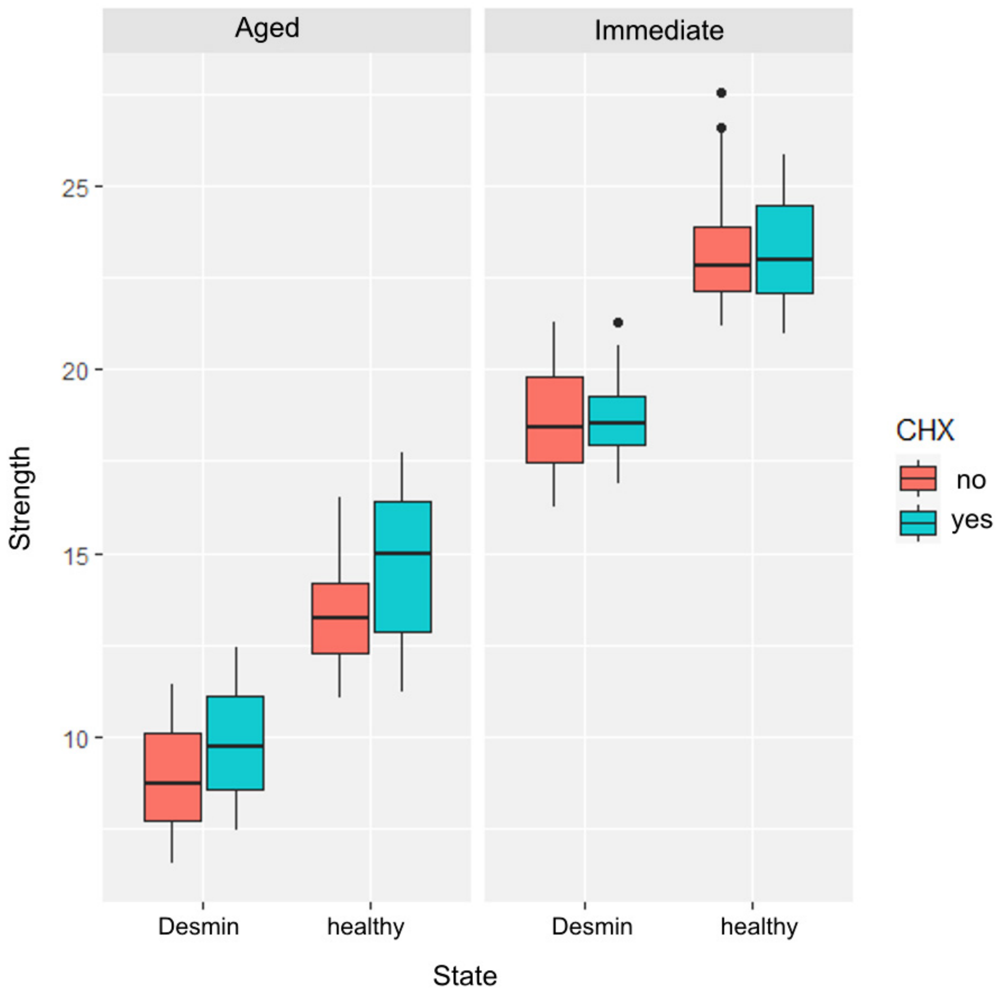

Fig. 4 Distribution of bond strength according to time, pretreatment, and state.

on bond strength in universal adhesive systems remains unclear due to contradictory results[23]. Regarding adhesive strategies, the use of CHX as an antiseptic before adhesive application does not directly impact the bond strength to demineralized dentin. Conversely, according to de Breschi et al. [25], the use of CHX has a direct influence on inhibiting the MMPs present in dentin. In line with the findings of Breschi et al. [25], the results from the study by Tessore R et al. [26], which examined the adhesive efficacy of two universal adhesive systems—one of them containing CHX in its composition—demonstrated that the use of CHX does not influence bond strength to dentin. Although MMPs' ability to degrade the extracellular matrix was recognized decades ago, the correlation between nanofiltration and hybrid layer degradation was not established until 1999 when Sano [27] and his research team demonstrated the hydrolytic degradation of collagen in the hybrid layer. The water within the hybrid layer serves as a functional medium for the hydrolysis of the resin matrix. This adhesive hydrolysis is considered the main reason for the degradation of the hybrid layer, consequently affecting bond strength over time [28].

In the systematic review and meta-analysis conducted by Kiuru et al. [29], a total of 43 articles were analyzed, and 21 articles involving CHX treatments were included for meta-analysis. The results clearly demonstrate the benefits of inhibiting collagen-degrading enzymes in preserving dentin bond strength. Given that CHX exhibits no adverse effects on immediate bond strength, its clinical use to enhance the longevity of resin-dentin bonds can be recommended. One limitation of this study is that the demineralized dentin achieved in the laboratory may not precisely replicate human carious dentin. However, it can be considered highly analogous, especially for the evaluation of pretreatment. It is important to acknowledge that, in clinical application, certain biases might influence the results.

Conclusions

One of the challenges posed by adhesive strategies is the degradation of the hybrid layer. In this sense, it can be concluded that the use of a 2% CHX pretreatment in adhesive procedures is beneficial over time. Specifically, immediate 2% CHX pretreatment does not improve bond strength values for healthy nor demineralized dentin. However, over time, it exhibits a favorable effect, potentially linked to its role as an MMP inhibitor. MMP inhibitors show promise for future applications, particularly in targeting the prevention of dentin proteolysis. Under the conditions of this study, the application of a 2% CHX pretreatment in adhesive procedures on both healthy and demineralized dentin did not reveal any discernible difference in bond strength.

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Conflict of Interest Statement

The authors have no conflict of interest in the publication of the article.

Authorship contribution note

1. Study concept and design
2. Data acquisition
3. Data analysis
4. Discussion of results
5. Manuscript drafting
6. Approval of the final version of the manuscript

AG has contributed in 1, 2, 3, 4, 5, and 6.
MCLJ has contributed in 1, 3, 4, 5 and 6
AF has contributed in 3, 6
JL has contributed in 1, 3, 4, 5 and 6

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