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SELF-ETCHING AND ETCH-AND-RINSE ADHESIVE TO BLEACHED DENTIN**

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SELF-ETCHING AND ETCH-AND-RINSE ADHESIVE TO BLEACHED DENTIN**

Trabalho de conclusão de curso apresentado à
comissão de estágio de Graduação em Odontologia
da Universidade Federal de Sergipe.

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Aracaju
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Dentin pre-treatment on bond strength stability of self-etching and etch-and-rinse adhesive
to bleached dentin

Short-title: Bonding stability to bleached dentin

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SUMMARY

This study aimed to evaluate the effect of using chlorhexidine or glutaraldehyde on bonding stability of self-etching and etch-and-rinse adhesives to bleached dentin. The occlusal surface of 80 third molars was abraded to exposed a flat medium dentin surface, which submitted to bleaching procedure using 20% hydrogen peroxide and sodium perborate for 14 days. Afterwards the dentin remained the same time in distilled water prior to adhesive procedures that was carried-out with a 3-step etch-and-rinse (Scotchbond Multipurpose Plus – SBMP) or a 2-step self-etching (Clearfil SE. Bond – CSE) adhesive. For each approach, the bleached dentin was treated with chlorhexidine, glutaraldehyde or non-treated prior to adhesive procedure. Non-bleached dentin was used as control. Composite cylinders were built-up over the adhesive covered dentin followed by parallel section of specimens to obtain 1-mm slices. The slices were trimmed to obtain hour-glass shape specimens with around 1.0 mm² of bonding interface. Half of slices were submitted to microtensile bond strength test after 24h, while the remaining was stored in distilled water for 3 months prior to the same test. Failure mode was analyzed under optic microscopy. For SBMP, glutaraldehyde-treated dentin resulted in highest bond strength, maintaining the values after 3 months. For CSE, the highest bond strength was obtained on chlorhexidine-treated dentin. In general, it was observed bond strength reduction within water storage for both approaches. In conclusion, glutaraldehyde and chlorhexidine can be available treatments to improve the bond strength to bleached dentin.

Keywords: bond strength, adhesive systems, tooth bleaching.

INTRODUCTION

Endodontic treatment is usually followed by tooth discoloration due to iatrogenic procedures, such as pulp tissue or filling material remaining in pulpal chamber; or for factors related pulp necrosis as mechanical trauma (1). The internal bleaching through the placement of bleaching agent directly over the dentin from pulp chamber is the most indicate conservative treatment for discoloration of endodontically-treated teeth (2). Despite the risk to external root resorption, the internal bleaching present high successful rate and it is safe when the procedure is well-performed (3). The bleaching agent is usually the hydrogen peroxide (H_2O_2) which is applied directly or produced in a chemical reaction from sodium perborate or carbamide peroxide (1,2).

The bleaching ability has been associated with the oxidative effect of free radicals, released by the breakdown of H_2O_2 . Thus, bleaching effect is due to oxidation of organic matrix of dentin tissue (4). Further to possible structural alteration on dentin matrix due its oxidation, the bleaching agent also increase the matrix metalloproteinase (MMP)-mediated collagen degradation in dentin (5). MMPs are structural endopeptidases that contribute to dentin matrix organization and mineralization; while their releasing after bonding procedures has been associated to degradation of bonding interface. It has been reported that this endogenous MMPs degrade the exposed and unprotected collagen fibrils in the bottom of hybrid layer (6,7). This degradation occurs even for self-etching adhesives that etch and infiltrate simultaneously (8).

Several approaches have been advocated in order to extend the longevity of bonding interfaces in dentin. The use of 2% chlorhexidine digluconate that is an unspecific MMPs inhibitor has demonstrated be effective through *in-vitro* and *in-vivo* studies (8-10). Further to MMPs inhibition, the stabilization of organic matrix also can help to prolong the longevity of bonding interface (12). Cross-linkers agents as glutaraldehyde are able to o enhance the ultimate strength of dentin (13-15) and dentin-resin interfaces (16,17); resulting in more stable interfaces. Despite the known advantageous on stability of bonding interfaces; limited information is available about the effects of MMPs inhibitors and cross-linkers agents on bonding to bleached dentin, while the organic matrix is modified by action of peroxide. Thus, the aim of this study was to evaluate the effect of chlorhexidine or glutaraldehyde on bonding stability of self-etching and etch-and-rinse adhesives to bleached dentin. The null hypothesis was that using chlorhexidine or glutaraldehyde does not affect the bonding stability.

MATERIAL AND METHODS

Experimental design:

This *in vitro* investigation was conducted using a $2 \times 3 \times 3$ factorial study design to evaluate the factors ‘adhesive system’ in two levels (self-etching or etch-and-rinse), ‘dentin treatment’ in three levels (non-treatment, chlorhexidine or glutaraldehyde); and the sub-parcel ‘storage time’ in two-levels (24 h or 3 months in distilled water). The bond strength of adhesives systems applied over the bleached dentin, treated or non-treated, was evaluated by micro-tensile bond strength test followed by failure mode analysis ($n = 10$). Half of specimens from each tooth was tested after 24 h of water storage and the specimens remaining after 3 months. This study was approved by the local Research Ethics Committee (CAAE # 45156115.8.0000.5546).

Substrate preparation:

Eighty non-carious human third molars stored in 0.05% thymol saline solution at 4°C for no more than 3 months were used in this study. The occlusal surfaces were ground flat with a plaster trimmer under running water, followed by the use of 100 grit SiC papers to remove the enamel and expose a flat medium dentin surface. A sectioning was performed parallel to the occlusal surface 2 mm below the cementum–enamel junction using a water-cooled slow-speed diamond saw (#7020; KG Sorensen, Barueri, Brazil) and the roots were discarded. Surfaces were inspected with an optical stereomicroscope at 40× magnification to ensure the absence of enamel.

Bleaching procedures:

A bleaching agent (Whiteness Perborato, FGM, Joinville, SC, Brazil) consisting of 20% hydrogen peroxide and sodium perborate, indicated for internal bleaching, was mixed and placed over the occlusal dentin of sixty teeth. The bleaching agent remained for 14 days under absolute humidity at 37°C, while the agent was replaced after the 7th day. After the bleaching period, the agent was removed, the dentin surface rinsed with water-stream and the specimens stored in distilled water at 37°C for 14 days.

Bonding procedures:

Prior to bonding procedures, the dentin surfaces were wet-polished with 600 grit SiC paper for 1 min to standardize the smear layer. The bonding procedures were performed with

a three-step etch-and-rinse adhesive Scotch bond Multipurpose Plus (3M ESPE, St. Paul, MN, USA) or a two-step self-etching adhesive Clearfil SE Bond (Kuraray Co., Osaka, Japan). The bonding procedures are described at Table 1 ($n = 10$). The bonding protocol control was used for both bleached and non-bleached dentin surfaces. Following, increments of composite resin Z-350 XT (3M ESPE, St. Paul, MN, USA) were inserted over the hybridized dentin to build-up crowns with around 4 mm of height.

Micro-tensile bond strength test:

The restored specimens were mounted in plastic plates and hold in cutting machine (Isomet low-speed, Buehler Ltd, Lago Bluff, IL, USA), where parallel sections were done with diamond saw (Exttec® Technologies Inc., Enfield, CA - USA) to obtain 1-mm slices. These slices were then trimmed with fine cylindrical diamond bur in a high-speed hand piece, under a water spray coolant, to obtain hour-glass shape specimens with around 1.0 mm² of bonding interface. Half of the specimens were stored for 24 h in distilled water at 37°C prior to micro-tensile bond strength test, while the remaining specimens were aged for 3 months in the same conditions.

After storage, the specimens were attached to the flat grips of a micro-tensile testing device using ethyl cyanoacrylate glue (IC-Gel, BSI, Atascadero, CA, USA) tested under tension (Microtensile OM100, Odeme, Joaçaba, SC, Brazil) with a crosshead speed of 0.7 mm/minute until failure. Data from KgF were converted to MPa considering the transversal area of bonding interface for each specimen. Fractured specimens were observed under magnification of up to 50× on an optical microscope to classify the failure mode: Ad – Adhesive failure; Mi – Mixed failure; CR – cohesive failure in resin; or CD – cohesive failure in dentin.

Statistical analysis:

Data analysis was performed using the SigmaStat v.3.5 statistical software package (Systat Software Inc., Chicago, IL, USA). For each adhesive system, bond strength data were submitted to 2-way repeated-measured analysis of variance (ANOVA), while the ‘moment of evaluation’ was used as sub-parcel. Pairwise multiple comparison procedures were performed by Tukey’s test. Data on failure mode ratio were submitted to chi-square to assess the effect of each factor on distribution of failures. The significance level was set at $\alpha = 0.05$ for all analyses.

RESULTS

The results for micro-tensile bond strength are displayed at Table 2. For the etch-and-rinse adhesive, 2-way Repeated Measures ANOVA showed significant effect of ‘dentin treatment’ ($p < 0.001$), ‘moment of evaluation’ ($p < 0.001$) and for the interaction between factors ($p < 0.001$). Except for dentin treated with Glutaraldehyde, the specimen storage reduced the bond strength values. At both moments of evaluation, dentin treatment with Glutaraldehyde resulted in highest values of bond strength, while no difference was observed among the other treatments. Regarding the use of self-etching adhesive, 2-way Repeated Measures ANOVA showed significant effect of dentin treatment ($p < 0.001$) and moment of evaluation ($p < 0.001$) on bond strength values, but there was observed significant interaction between these factors ($p = 0.083$). At both moments of evaluation, the dentin treated with Chlorhexidine resulted in highest values of bond strength, while no difference was observed among the other treatments. Highest values of bond strength were observed for specimens evaluated immediately irrespective the treatment performed in dentin.

The results of failure mode analysis are illustrated at Figure 1. There was a predominance of adhesive failure for all experimental conditions. Increased amount of cohesive failure in dentin was observed for etch-and-rinse adhesive bonded dentin treated with Chlorhexidine and tested after 3 months; and for self-etching adhesive in dentin treated with Glutaraldehyde and evaluated immediately. However, chi-square test showed that the ‘adhesive type’ ($p = 0.780$), the ‘moment of evaluation’ ($P = 0.564$), and the ‘dentin treatment’ ($P = 0.330$) did not affect the failure mode.

DISCUSSION

The tooth bleaching is achieved by oxidation of organic matrix of dentin by hydrogen peroxide and its products (4), when this oxidative process might impair further bonding procedures due to possible alterations on collagen structure. However, in opposite to expectation; the bond strength values obtained to bleached dentin was similar to those observed to control non-bleached, irrespective the adhesive protocol used (etch-and-rinse or self-etching). Moreover, the bond strength values observed to these substrates demonstrated similar degradation under water storage for 3 months. The reduction observed on bond strength values within storage is mainly due to degradation of exposed collagen non-covered by adhesive resin on base of hybrid layer and to hydrolysis of resin adhesive (6,9,10,18).

In the present study, two approaches were evaluated seeking out to reduce the degradation of bonding interface. Glutaraldehyde is a cross-linker agent used in order to

enhance the ultimate cohesive strength of collagen fibrils (13-15,19). In fact, applying Glutaraldehyde solution prior to bonding procedure was able to improve the bond strength when etch-and-rinse adhesive was used. Furthermore, no alteration on values of bond strength were observed after water storage of specimens for Glutaraldehyde-treated dentin, demonstrating improved stability of bond strength. Interestingly, using Glutaraldehyde did not affect the bond strength (immediate and after storage) of self-etching adhesive to bleached dentin. One reasonable explanation is that using approach the adhesive etches and infiltrates simultaneously the dentin substrate reducing the amount of exposed collagen non-covered by adhesive resin and the possible advantageous of using cross-linkers agents (20).

In other hand, using Chlorhexidine increased the bond strength values obtained with self-etching adhesive, whereas this solution did not affect the bond strength of etch-and-rinse adhesive to bleached dentin. Chlorhexidine is an unspecific MMPs inhibitor and several studies has been demonstrated its effectiveness on bond strength stability improvement (21). It has been demonstrated that bleaching techniques using 35% hydrogen peroxide increase the dentinal proteases activity, including MMPs (5). In the present study, the dentin was bleached using an association between 20% hydrogen peroxide and sodium perborate, while this technique is advocated for internal bleaching by placement of bleaching agent inside of pulpal chamber (22). Despite differences regarding the bleaching agent, increased activity of MMPs with reduction on bond strength values after storage due to collagen degradation can be expected with bleached dentin. However, similar reduction on bond strength values was observed when bleached and non-bleached dentin were used as substrate, regardless the bonding approach. Furthermore, using a MMPs inhibitor did not alter the bond strength reduction.

Even when used in non-bleached dentin, prior studies have been demonstrated the ability of 2% chlorhexidine digluconate to prevent bonding interfaces degradation demonstrated by bond strength reduction after storage (9-11,21). The most of these study used water storage as artificial aging technique, similarly to storage medium used in the present study (21). However, it has been shown that the use of water instead of Ca- and Zn-containing artificial saliva may underestimate the hydrolytic activity of endogenous dentin MMPs (22). Thus, a possible reduced action of MMPs on bonding interface degradation might hide any benefit of using chlorhexidine during the adhesive procedure. In opposite, chlorhexidine application increased the bond strength values for the self-etching adhesive. Self-etching adhesives are applied commonly over dried dentin (23,24), whereas the dentin treated with

chlorhexidine remained moist prior to primer application. Self-etching primers present functional acidic monomers that required water to ionize and etch the tooth substrate (23,24). Despite the presence of water on primer composition of Clearfil SE Bond, the presence of dentin moist can enhance the acidic monomers ionization improving their dentin etching ability (25). A possible more efficient dentin etching due to moist substrate can be responsible by increased bond strength values observed when chlorhexidine was used.

In conclusion, the findings of present study demonstrated that bleaching procedures using 20% hydrogen peroxide associated to sodium perborate did not affect the bonding interface stability; demonstrating similar degradation to those observed when the dentin was not bleached. Applying glutaraldehyde over the etched dentin was able to enhance the bond strength values, while no significant reduction was observed within water storage when this cross-linker agent was used. Finally, applying chlorhexidine prior to self-etching primer application improved the bond strength to dentin.

RESUMO

Este estudo teve como objetivo avaliar o efeito do uso da clorexidina ou glutaraldeído sobre a estabilidade de ligação dos adesivos auto-condicionante e convencional à dentina clareada. A superfície oclusal de 80 terceiros molares foi desgastada para expor uma área plana de dentina, a qual foi submetida a processo de clareamento utilizando-se de peróxido de hidrogénio 20% + perborato de sódio durante 14 dias. Após esse período a dentina manteve-se pelo mesmo tempo em água destilada antes dos procedimentos adesivos convencional de 3 passos (Scotchbond Multipurpose Plus - SBMP) ou auto-condicionante de 2 passos (Clearfil SE Bond - CSE). Para cada uma das abordagens, a dentina clareada foi tratada com clorexidina, glutaraldeído ou não-tratada antes do procedimento adesivo. A dentina não clareada foi utilizada como controle. O compósito foi aplicado em incrementos sobre a dentina coberta de adesivo, seguido por secção paralela dos espécimes em fatias de 1 mm. As fatias foram cortadas para obter amostras na forma de ampulheta, com cerca de 1,0 mm² na interface de união. Metade das fatias foram submetidas ao teste de microtração de força união após 24 horas, enquanto o restante foi armazenado em água destilada durante 3 meses antecedentes ao mesmo teste. O modo de falha foi analisado sob microscopia óptica. Para SBMP, a dentina tratada com glutaraldeído resultou em maior resistência de união, mantendo os valores após 3 meses. Para CSE, a maior resistência de união foi obtida em dentina tratada

com clorexidina. Em geral, observou-se redução da resistência de união após armazenamento em água para ambas as abordagens. Em conclusão, glutaraldeído e clorexidina podem ser tratamentos disponíveis para melhorar a resistência de união à dentina clareada.

ACKNOWLEDGMENTS

C.S.A. and I.M.B.S. are grateful to CNPq and COPES/UFS for their scholarships.

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TABLES

Table 1. Bonding protocols used in the study.

Adhesive	Dentin treatments	Protocols
Etch-and-rinse	Control	1. Acid etching with 35% phosphoric acid for 15 s; 2. Water rising for 15 s and drying with absorbent paper; 3. Application of primer followed by slight air-stream to evaporate the solvents; 4. Application and light-curing (20 s) of adhesive resin.
	Glutaraldehyde	1. Acid etching with 35% phosphoric acid for 15 s; 2. Water rising for 15 s and drying with absorbent paper; 3. Application of 5% of glutaraldehyde solution for 2 min; 4. Water rising for 15 s and drying with absorbent paper; 5. Application of primer followed by slight air-stream to evaporate the solvents; 6. Application and light-curing (20 s) of adhesive resin.
	Chlorhexidine	1. Acid etching with 35% phosphoric acid for 15 s; 2. Water rising for 15 s and drying with absorbent paper; 3. Application of 2% chlorhexidine digluconate for 30 s; 4. Water rising for 15 s and drying with absorbent paper; 5. Application of primer followed by slight air-stream to evaporate the solvents; 6. Application and light-curing (20 s) of adhesive resin.
Self-etching	Control	1. Primer was vigorous spread over dentin for 20s followed by slight air-stream application; 2. Application and light-curing (20 s) of adhesive resin.
	Glutaraldehyde	1. Application of 5% of glutaraldehyde solution for 2 min; 2. Water rising for 15 s and drying with air-stream; 3. Primer was vigorous spread over the dried dentin for 20s followed by slight air-stream application; 4. Application and light-curing (20 s) of adhesive resin.
	Chlorhexidine	1. A 2% chlorhexidine digluconatesolution was spread for 30 s; 2. Primer was vigorous spread over the moist dentin for 20s followed by slight air-stream application; 3. Application and light-curing (20 s) of adhesive resin.

Table 2. Means (standard deviation) of bond strength in MPa.

Adhesive	Treatment	Moment of evaluation	
		Immediate	After 3 months
Etch-and-rinse	Control – non-bleached	25.6 (4.6) ^{Ab}	15.8 (5.4) ^{Bb}
	Control – bleached	25.8 (3.3) ^{Ab}	17.4 (3.6) ^{Bb}
	Glutaraldehyde	35.9 (3.6) ^{Aa}	35.4 (6.1) ^{Aa}
	Chlorhexidine	26.3 (5.4) ^{Ab}	15.2 (5.0) ^{Bb}
Self-etching	Control – non-bleached	23.9 (1.6) ^{Ab}	16.0 (3.4) ^{Bb}
	Control – bleached	24.3 (4.1) ^{Ab}	14.2 (3.8) ^{Bb}
	Glutaraldehyde	22.3 (4.1) ^{Ab}	14.6 (3.6) ^{Bb}
	Chlorhexidine	33.3 (3.6) ^{Aa}	20.6 (3.5) ^{Ba}

For each adhesive, distinct letters (lowercase for column; uppercase for line) indicate statistical difference ($p < 0.05$).

FIGURES

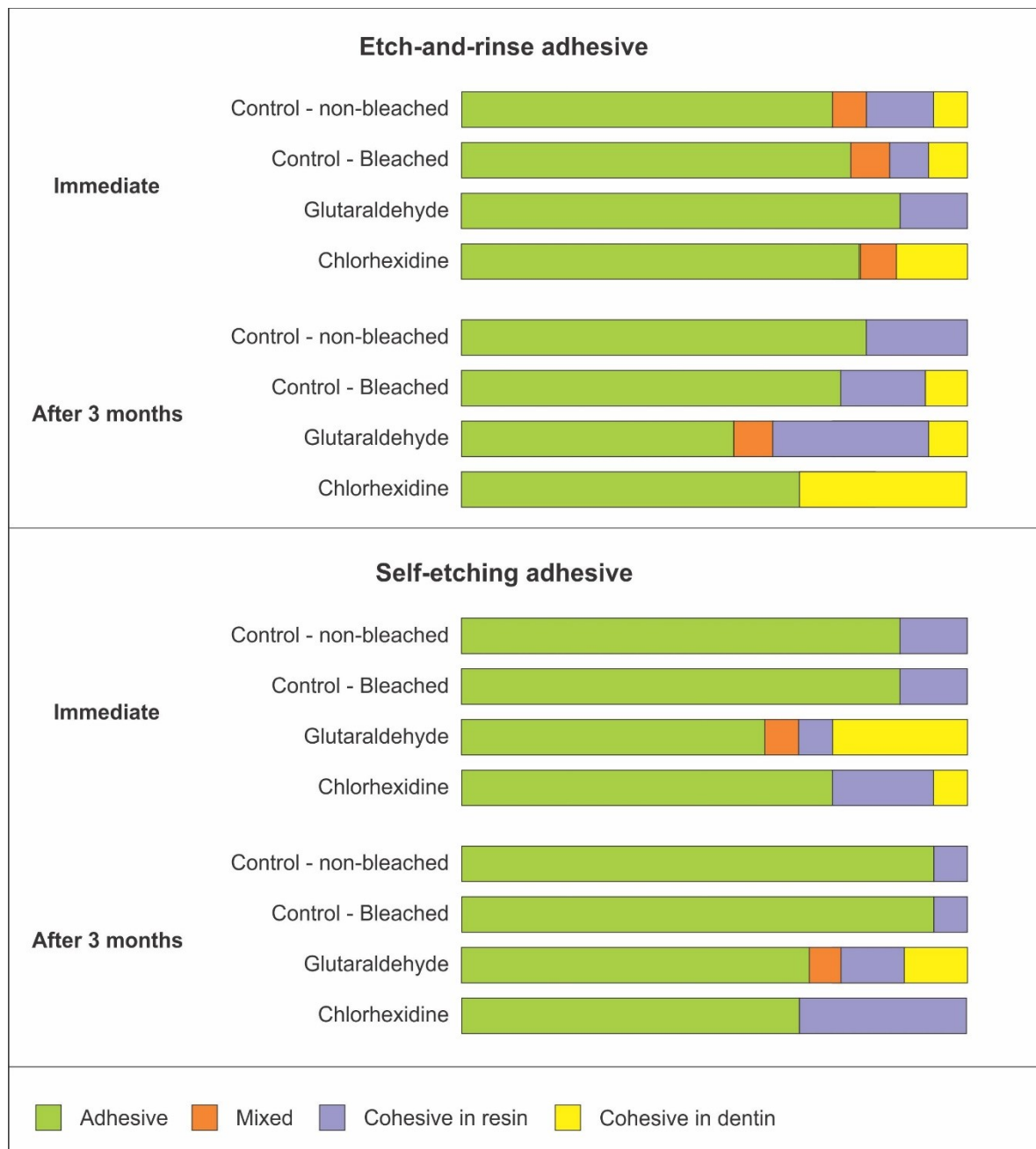


Figure 1. Distribution of failure modes by experimental conditions