

# New trends in dentin bonding: treatment with Chlorhexidine, Hyaluronic acid, vitamin C and green tea

Novas tendências em adesão dentinária: tratamento com clorexidina, ácido hialurônico, vitamina C e chá verde

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## ABSTRACT

**Objective:** The aim of this study was to evaluate the bond strength of dentin treated with chlorhexidine, hyaluronic acid, vitamin C and green tea. **Material and Methods:** The roots of 50 bovine teeth were removed and buccal coronal dentin was exposed. After acid-etching, the specimens were divided into 5 groups (n = 10), according to the dentin treatment strategy: CO - untreated dentin; CHX - treated with 2 wt% chlorhexidine for 30 s; HA - treated with 1 wt% hyaluronic acid for 30 s; VC - treated with 10 wt% vitamin C for 30 s; GT - treated a 1% green tea extract for 30 s. Adper Single Bond was then applied to the treated according to the manufacturer's recommendations. The specimens were restored with a 4 mm thick layer of the resin composite, which was polymerized for 40 s. The specimens were stored in distilled water at 37°C for 24 h and sectioned into 1 x 1 mm<sup>2</sup> sticks containing the adhesive interface. Microtensile bond strength testing was performed with a universal testing machine at a cross-head speed of 1.0 mm/min. **Results:** The results were analyzed with one-factor ANOVA and Tukey's multiple comparison tests. GT group presented the highest values bond strength (29.4 ± 3.1) a, but no significant difference compared to the other experimental groups HA (26.7 ± 3.1) ab, CHX (25.4 ± 2.6) ab and VC (22.4 ± 6.0) b. Bond strengths of experimental groups were not significantly different from the CO. **Conclusion:** Immediate bond strength was preserved after acid-etched dentin was treated.

## KEYWORDS

Chlorhexidine; Hyaluronic acid; Vitamin C; Green tea; Bond strength.

## RESUMO

**Objetivo:** O objetivo deste estudo foi avaliar a resistência de união dentinária tratada com clorexidina, ácido hialurônico, vitamina C e chá verde. **Material e Métodos:** As raízes de 50 dentes bovinos foram removidas e a superfície de dentina vestibular exposta. Após o condicionamento ácido, os espécimes foram divididos em 5 grupos (n = 10), de acordo com as estratégias adesivas na dentina: CO - dentina não tratada; CHX - tratamento com clorexidina 2% por 30 s; HA - tratamento com ácido hialurônico 1% por 30 s; VC - tratamento com vitamina C 10% por 30 s; GT - tratamento com extrato de chá verde 1% por 30 s. Sistema adesivo Adper Single Bond II foi aplicado segundo recomendações do fabricante. Os espécimes foram restaurados com uma camada de 4 mm de espessura de resina composta, que foi polimerizada por 40 s. As amostras foram armazenadas em água destilada a 37 ° C durante 24 h, e seccionada em palitos de 1 x 1 mm<sup>2</sup> contendo a interface adesiva. Teste de microtração foi realizado com uma máquina de ensaios universal a uma velocidade de 0,5 mm/min. **Resultados:** Os resultados foram analisados com teste de variância ANOVA um-fator e testes de múltipla comparação de Tukey (p < 0,05). Grupo GT apresentou os maiores valores de resistência de união (29,4 ± 3,1) a, mas sem diferença significativa em relação aos demais grupos experimentais HA (26,7 ± 3,1) ab, CHX (25,4 ± 2,6) ab e VC (22,4 ± 6,0) b. Resistência de união dos grupos experimentais não foi significativamente diferente do grupo CO. **Conclusão:** a resistência de união imediata foi mantida mesmo após os diferentes tratamentos dentinários.

## PALAVRAS-CHAVE

Clorexidina; Ácido hialurônico; Vitamina C; Chá verde; Resistência adesiva.

## INTRODUCTION

The main objective of adhesive dentistry is to create an effective, durable union between the tooth structure and the restorative material. However, degradation of the adhesive-dentin interface remains largely responsible for the relatively short lifetime of tooth-colored resin restorations [1,2]. Although most adhesives bond satisfactorily to enamel, problems are still encountered when bonds are created in dentin. For this reason, many studies have been performed on materials and treatments that promote increased bond strength between the substrate and the restoration, as well as reduce the undesirable effects associated with microleakage, such as dentin hypersensitivity, marginal staining and secondary caries.

The durability of the dentin adhesive interface is directly related to the hydrophilic characteristics of the adhesive and the quality of the hybrid layer. In the case of etch-and-rinse adhesives, adhesive resin monomers must penetrate through the dentin collagen fibrils that are exposed by acid-etching. Several studies have shown that this goal is seldom achieved. This results in the loss of adhesion over time, as polymerized hydrophilic adhesives containing incompletely polymerized hydrophilic monomers with ester linkages that are slowly hydrolyzed by salivary esterases [3,4]. In addition, denuded collagen fibrils within the hybrid layer are progressively degraded when endogenous collagenolytic pro-enzymes within the mineralized dentin matrix are activated into active enzymes during the process of acid demineralization [5,6].

Adjunctive collagen pre-treatment strategies have been proposed to improve dentin adhesion, via the use of agents that maintain the stability of fibrillar collagen toward enzymatic degradation. These agents include the use of substances that are considered to be inhibitors of matrix metalloproteinases (MMPs) and cysteine cathepsins. Different types of MMPs have been identified from human dentin, including MMP-

2, MMP-3, MMP-8, MMP-9, MMP-20 [7-9]. More recently, different types of cysteine cathepsins, in particular cathepsin B, have also been identified from sound and carious dentin [10-12]. Together, these proteases are thought to contribute to degradation of denuded collagen within the hybrid layer [13].

Chlorhexidine has been widely studied in adhesive dentistry as an inhibitor of both MMPs and cysteine cathepsins [11,14]. Studies have shown promising results for the use of chlorhexidine in promoting the longevity of resin-dentin bonds and preventing the degradation of the dentin hybrid overtime [15,16]. In recent years, the potential for the inhibition of MMPs by substances derived from natural products has gained increasing attention; among these natural products is green tea extract. This substance contains epigallocatechin-3-gallate, which has the ability to inhibit certain MMPs [17,18].

In the field of skin cosmetics, hyaluronic acid and vitamin C (ascorbic acid) have been widely used for topical application. The hyaluronic acid is used to provide a moisturizing effect and act as an antioxidant. The vitamin C is used to increase the level of messenger RNA of collagen I and III, as a co-factor for the conversion of some enzymes, and as an inhibitor of MMPs [19-21].

In order to use these agents for extending the longevity of resin-dentin bonds, it is necessary to first determine whether they interfere with dentin bond strength following the use of these agents to pre-treat acid-etched dentin. This motivated us to evaluate the effects of different dentin collagen pre-treatment agents, which are considered potentially useful in preventing collagen degradation, on dentin bond strength. The null hypothesis tested was that the use of chlorhexidine, green tea extract, hyaluronic acid or vitamin C as pre-treatment agents of acid-demineralized dentin collagen has no adverse effect on the microtensile immediate bond strength of a 2-step etch-and-rinse adhesive to dentin.

## MATERIALS AND METHOD

### Tooth Preparation:

The research protocol was approved by the Research Ethics Committee of São José School of Dentistry, Sao Paulo State University, Brazil. Fifty bovine incisors were cleaned and stored in thymol solution 0.1 wt% at a temperature of -18 °C until they were used [22]. The selected teeth were sectioned in the apical direction, 1.0 mm away from the cemento-enamel junction, with a carborundum disk, at high speed, under water cooling. The roots were discarded and pulp tissues were removed with endodontic files. The pulp chambers were copiously irrigated with distilled water and dried with air-spray. The buccal enamel of each tooth was removed using wet 400-grit silicon carbide paper coupled to a circular polisher (DP-10, Panambra, São Paulo-SP, Brazil), under water cooling, until an area of approximately 8 mm<sup>2</sup> in diameter of the dentin was exposed and the dentin thickness was standardized at 2 mm. The pulp chamber was filled with utility wax to avoid penetration of embedding media. The teeth were embedded in self-curing acrylic resin, so that the exposed buccal was parallel to the horizontal plane and polished on a wet 600-grit silicon carbide paper for 30 s to create a realistic smear layer on the buccal surface.

### Bonding Procedures:

Specimens were randomly assigned into five groups (n = 10). Control group (CO): Acid-etching of the exposed dentin buccal was performed for 15 s with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE, St. Paul, MN, USA). The acid was rinsed off with an air/water spray for 30 s, and the acid-etched dentin was briefly air-dried for 2 s and bonded with adhesive system, Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA), to the etched dentin according to the manufacturer's instruction (application of two consecutive coats of adhesive, exciting the product with a brush applicator (Microbrush international,

WI, USA) 15 s, gently drying with air/water spray for solvent evaporation and light curing 10 s). Chlorhexidine (CHX): The procedures described for CO group were followed, but the acid-etched dentin was pre-treated with 2% chlorhexidine (Byofórmula, São José dos Campos, SP, Brazil) for 30 s, active application with a brush applicator and the excess removed with cotton pellet prior to the application of Adper Single Bond 2. Green Tea (GT): The procedures described for the CO group were followed; the acid-etched dentin was pre-treated with 1% green tea extract in aqueous solution (Byofórmula, São José dos Campos, SP, Brazil) for 30 s, active application with a brush applicator and the excess removed with cotton pellet prior to the application of Adper Single Bond 2. Hyaluronic acid (HA): The procedures described for the CO group were as followed; the acid-etched dentin was pre-treated with 1% hyaluronic acid in gel solution (Byofórmula, São José dos Campos, SP, Brazil) for 30 s, active application with a brush applicator and the excess removed with cotton pellet prior to the application of Adper Single Bond 2. Vitamin C (VC): The procedures described for the CO group were as followed; the acid-etched dentin was pre-treated with 10% vitamin C in aqueous solution prepared immediately before used (Byofórmula, São José dos Campos, SP, Brazil) for 30 s, active application with a brush applicator and the excess removed with cotton pellet prior to the application of Adper Single Bond 2. Five or six increments of resin composite (Filtek Z350, 3M ESPE, St. Paul, MN, USA) were added to the bonded surfaces and individually light-cured for 40 s using LED light-curing unit with an output of 600 mW/cm<sup>2</sup>. The teeth were then stored in distilled water at 37 °C for 24 h.

### Microtensile Bond Testing:

The teeth were longitudinally sectioned across the bonded interface in sections perpendicular to the bonded surfaces with a diamond saw (Isomet 1000, Buhler Ltda., Lake

Bhuff, IL, USA) in a Labcut 1010 machine (Extec Technologies Inc., Enfield, CT, USA) under water cooling at 300 rpm to obtain bonded sticks with a cross-sectional area of approximately 0.8 mm<sup>2</sup>. From 7 to 9 beams were obtained from each preparation. The cross-sectioned area of each stick was measured with a digital caliper to the nearest 0.01 mm and recorded for subsequent calculation of the microtensile bond strength. Each specimen was individually fixed to a custom-made testing jig with a cyanoacrylate resin (Super-Bonder Gel, Loctite, São Paulo, SP, Brazil) and subjected a tensile force in a universal testing machine (EMIC, São José dos Pinhais, PR, Brazil), and subjected to tensile load at a crosshead speed of 0.5 mm/min until failure.

### Debonds pathway determination:

Both surfaces of each fractured specimen were observed under a stereomicroscope with 80x magnification to record the failure modes. The fracture modes were classified as cohesive in dentin (D), cohesive in composite (C), adhesive (failure at resin-dentin interface – A), or mixed failure (M).

### Statistical Analysis:

The experimental unit in the current study was the tooth. The values  $\mu$ TSB and percentage of failure mode of all sticks from the same tooth were averaged for statistical purposes. The pre-test failures were included in the tooth mean for  $\mu$ TSB. The specimens with cohesive failure were excluded from the data analysis. The  $\mu$ TSB (MPa) means for every testing group resulted from the average of the ten teeth used per group. The results were expressed in MPa and subjected to one-way analysis of variance (ANOVA) and Tukey's test at the 5% level of significance.

## RESULTS

### Microtensile Bond Strength:

The means and standard deviations of the microtensile bond strengths ( $\mu$ TBS) are

summarized in Table 1. Chlorhexidine, green tea, hyaluronic acid, and vitamin C did not affect in vitro bond strength of specimens tested after 24 h in every group ( $p = 0.013$ ).

**Table 1** – In vitro microtensile bond strength values and the respective standard deviations (MPa) obtained in each experimental conditions, as well as, statistical significance

Conditions	$\mu$ TSB Mean ( $\pm$ SD) in MPa*
Control	26.7( $\pm$ 8.9)ab
Chlorhexidine	25.4( $\pm$ 2.6)ab
Green Tea	29.4( $\pm$ 3.1)a
Hyaluronic Acid	26.7( $\pm$ 3.1)ab
Vitamin C	22.4( $\pm$ 6.0)b

\*Groups accompanied by the same letters are not significantly different (Tukey's test,  $p > 0.05$ ).

### Distribution of the failure made:

Approximately 7 – 9 sticks could be obtained per tooth including those with pre-test failures. Table 2 summarizes the distribution of the failure modes. The most failure modes were adhesive and mixed failures. A low percentage of cohesive in dentin and composite occurred for all the groups after 24 h. All mixed failure were the combination of cohesive in resin and failures in adhesive joint. The mean cross-sectional area ranged from 0.81 to 1.01 mm<sup>2</sup> and no difference amongst groups was detected ( $p > 0.05$ ).

**Table 2** – Number and percentage of specimens (%) according to fracture pattern mode and the premature debonded specimens for each experimental conditions\*

Conditions	A/M	C/D	P
Control	53(58.8)	25(27.8)	12(13.4)
Chlorhexidine	58(64.4)	22(24.4)	10(11.2)
Green Tea	63(70)	19(21.2)	8(8.8)
Hyaluronic Acid	51(56.7)	24(26.6)	15(16.7)
Vitamin C	49(54.4)	28(31.2)	13(14.4)

\*A/M – adhesive / mixed failure mode; C/D – cohesive fracture mode in composite and dentin; P - premature debonded specimens.

## DISCUSSION

The success of a resin composite restorative procedure depends on the hybrid layer quality. The formation of this layer is related to many factors, which include: dentin permeability, the conditioning agents, the conditioning time, the quality of dentin, the humidity, and the adhesive system, the diffusivity of the monomers, the depth of cure and the structural characteristics of the collagen network [23].

Studies have revealed defects along the adhesive interface. These defects occur as a result of incomplete infiltration of adhesive resin monomers into the demineralized collagen matrix. Thus, micropores are formed that promote the permeation of fluid, ions, molecules and bacteria. The presence of these porosities expedites degradation of the adhesive-dentin interface and results in decreases in bond strength overtime [2,24].

Degradation of exposed collagen fibrils within hybrid layers has been accepted as a key factor in the deterioration of the adhesive-dentin interface [15]. Recent studies reported that degradation is expedited by the presence of endogenous proteases present in dentin such as MMPs [7,15,25]. Dentin MMP preforms are activated during acid-etching, being activated by low pH and the presence of metal ions such as zinc and calcium [14,26]. Pre-treatment of acid-etched dentin with MMP inhibitors has been advocated to improve the durability of restorations. Studies have shown that chlorhexidine may be used for such a purpose [15]. This agent inhibits at least three types of MMPs: MMP-2, MMP-8 and MMP-9 [27]. These authors stated that the MMP inhibition capacity of chlorhexidine is attributed to its chelating action, scavenging calcium and zinc ions. Application of chlorhexidine to acid-etched dentin does not adversely affect the bond strength of dentin [28].

The present study was conducted on agents derived from natural products that have been reported to possess anti-MMP potential. Among

them, we selected green tea, because it contains polyphenols, especially epigallocatechin-3-gallate (EGCG), which has inhibitory activity against MMPs [17,18]. The EGCG appears to exhibit hydrogen bonding and hydrophobic interactions with the collagenases, changing its structure and consequently its enzymatic activity. Thus, the application of green tea to acid-etched dentin may improve the longevity of resin-dentin bonds by inhibiting MMPs [18].

Hyaluronic acid is a polysaccharide belonging to the group of glycosaminoglycans, and is present in the extracellular matrix of connective tissue. It interacts with various macromolecules, including collagen fibrils, and others small molecules, to control their permeability to water [29]. The wide applicability of HA in skin cosmetology is attributed to its excellent hygroscopic character, optimal rheological behavior, viscoelastic property and moisturizing effect, which may promote infiltration of adhesive resin monomers in the exposed collagen network of acid-etched dentin. According to Bogovic et al. (2011) [19], hyaluronic acid may be applied in direct pulp capping to stimulate vascularization, creating favorable conditions for mineralization and stimulating the proliferation and differentiation of odontoblasts. This agent also possesses antioxidant characteristics, neutralizing the harmful effects of free radicals, and preserves the structural integrity of tissues [31,32]. This is important because the presence of oxygen can expedite the degradation of the collagen matrix of the hybrid layer, and inhibit the polymerization of resin monomers, thereby impairing the longevity of the restoration [33,34].

Vitamin C or ascorbic acid is a water soluble vitamin and humans are unable to synthesize it. This vitamin plays an important role in the formation of collagen, because it is a cofactor for hydroxyproline and hydroxylysine. These amino acids are required for stabilizing the helical configuration of collagen [20,35]. Type I collagen is the major organic constituent of dentin. Vitamin C, as well as green tea extract

and chlorhexidine, also act as MMP inhibitors [36,37].

The purpose of the modification protocol adhesive system etch-and-rinse is the introduction of products with possible potential for inhibition of MMPs in order to maintain the dentin adhesive interface. However, as some of these products are not used for this purpose in dentistry, but widely used in other medical fields, such as hyaluronic acid, the aim of this study was to evaluate the use of green tea, hyaluronic acid and vitamin C concentrations used would be able to act on the dentin bond strength without an immediate reduction or even inhibiting the formation of the adhesive layer. The parameters compared were the control groups (negative) and chlorhexidine (positive control). Thus, the test was performed only after 24 h of storage as it aimed to determine whether certain concentrations these products used would be really effective. And thereafter conduct longitudinal studies.

Although the use of green tea, vitamin C after hyaluronidase on acid-etched dentin may stabilize collagen and improve the longevity of composite restorations, it is imperative that they do not adversely affect adhesive bonding to dentin. The results of the present study show that pre-treatment of acid-etched dentin with GT, VC or HA did affect the immediate bond strength of an etch-and-rinse adhesive to dentin. In particular, the green tea extract had the highest mean bond strength compared with the other groups. As these pre-treatments were able to maintain immediate dentin bond strength, there is not enough information to reject the null hypothesis. This provides the justification for further studies that are designed to investigate the effects of these agents on the longevity of resin-dentin bonds. The ability of these agents to improve the durability of resin-dentin should be evaluated in future studies.

## CONCLUSION

The use of chlorhexidine, green tea, hyaluronic acid or vitamin C, as pre-treatment

agents of acid-demineralized dentin collagen has no adverse effect on the microtensile immediate bond strength of a 2-step etch-and-rinse adhesive to dentin.

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