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Influence of desensitizers agents on the dentin bond strength after one-year water storage

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Aim: Evaluate the effect of adhesives systems combined with desensitizer agents on the microtensile bond strength (μTBS) of a composite resin to dentin. **Methods:** Cervical dentin of thirty-two human molars were used to simulate hypersensitivity areas. The teeth were divided into four groups (n=8), according to the type of adhesive system and desensitizer agents. No desensitizer was used in the control (Clearfil SE Bond – CS). Two experimental groups were pretreated with either MS Coat Bond (MS) or Biofluorid 12 (BF) immediately prior to bonding with CS. The last group corresponded to Gluma Comfort Bond + Desensitizer (GC) application. After dentin treatments, a composite block was built-up on dentin surface and after 24 hours teeth were serially sectioned to obtain bonded bean specimens. Beams were stored in water for 24 hours or one year. Subsequently, the specimens were submitted to the μTBS test. Data were analyzed by two-way mixed ANOVA and Bonferroni's test (α = 0.05). **Results:** At 24 hours, there was no significant difference in μTBS among groups. However, at one year, dentin treated with MS or BF demonstrated significantly lower μTBS of CS to dentin compared to control and GC, which kept their μTBS stable. **Conclusion:** The effect of MS and BF desensitizer agents on the μTBS of CS to dentin did not reduce the μTBS at 24 hours, but it decreases significantly after one year.

Keywords: Dentin desensitizing. Dentin sensitivity. Calcium Fluoride. Oxalic acid.

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Introduction

A common problem in dental practice is dentin hypersensibility, either on exposed root surfaces or under restorations. Dentin hypersensibility is characterized by pain that could be presented from mild to intense sensitivity[1-4]. Some conditions like periodontal disease, gingival recession, abrasion, caries, and the development of non-carious cervical lesions may result in exposed dentin[4,5]. Dentin hypersensitivity arises from the application of an exogenous stimulus (chemical, mechanical, evaporative or thermal) on the exposed dentin surface[1,2,6,7].

Several theories have been suggested to explain the mechanism of dentinal hypersensitivity. The Brännström's hydrodynamic theory, the most widely accepted, assumes that the application of stimuli to the exposed dentin surface causes movement of the dentinal fluid that stimulates the mechanical receptors, causing pain[8].

Numerous methods have been proposed to treat or manage dentin hypersensitivity[1,4,5,9]. These methods include the occlusion of dentinal tubules through the application of sedative agents[3-5,10], cavity varnishes, dentin bonding agents[11], resin composite restorations, lasers[12,13], and dentin remineralization strategies[14,15]. However, the most frequent treatment consists in occluding the dentinal tubule through the use of desensitizer agents[2,9], which are based on fluoride, oxalate, potassium nitrate, and calcium phosphate[6,9,16].

The desensitizing agents could reduce the dentin hypersensibility using two mechanisms: by the depolarization of nerve fibers with an immediate effect and by the gradual occlusion of the tubules reducing the pain after several applications[7]. In recent years, desensitizers have been used in combination with adhesive systems in most adhesive restorative procedures. Although desensitizers have demonstrated that effectively reduce the sensibility, little is known about the possible adverse effects on the adhesive performance by the use of desensitizers, and the results are still controversial. Several studies have reported that the use of a desensitizer previous to the adhesive application did not reduce the dentin bond strength[5,17]. On the other hand, other studies have shown that the bond strength to dentin is reduced after using desensitizing agents[7,10,18]. However, most previous studies have evaluated the bond strength either immediately or in short periods of time after restoration placement[17,18]. Thus, the long-term effect of desensitizers on the dentin bond strength is unclear and needs to be clarified, to better understand the effects of this therapy on the longevity of composite restorations.

This study aimed to evaluate the effect of the previous application of desensitizer agents on the micro-tensile bond strength of a two-step self-etching adhesive to dentin, comparing to the same self-etching adhesive without desensitizing treatment and to a two-step, etch-and-rinse adhesive containing a desensitizer in its composition, after 24 hours and one year of water storage. The null hypotheses investigated were that 1- application of desensitizer agents would not affect the μTBS of the self-etch adhesive, regardless the evaluation time and 2- no μTBS reduction would be observed after one year.

Materials and Methods

Tooth Preparation

This research was approved by the Ethics Committee of Piracicaba Dental School, University of Campinas (2.099.16). Thirty-two extracted, non-carious permanent human molars were collected and stored in aqueous solution containing thymol crystals at 4ºC. The crowns were separated from the roots with a slow-speed diamond saw in a precision low-speed cutting machine (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA). Buccal cervical surfaces were abraded with 180 grit SiC sandpaper on a polishing machine (Aropol-E, Arotec, Cotia, SP, Brazil), under water-cooling, to remove the enamel and to expose a flat cervical dentin. This cervical area was polished with 600-grit SiC for 20 seconds.

Bonding procedure

The teeth were immersed in 0,5M ethylenediamine tetra-acetic acid (EDTA) for 5 min to remove the smear layer and smear plug, simulating the morphological characteristics of hypersensitivity areas. Afterwards, teeth were washed with distilled water for one minute and placed in an ultrasonic cleaner (USC 1400; Unique, Indaiatuba, SP, Brazil) for three minutes. Then, the teeth were randomly divided into four groups, according to the following treatments:

- **Group CS:** Dentin surfaces were treated with Clearfil SE Bond. The self-etch primer was applied, left for 20 s and air-dried. Bonding resin was applied over priming, gently air-dried and polymerized for 10 s.
- **Group BF+CS:** Biofluorid 12 was applied to dentin and after 2 min, Clearfil SE Bond the adhesive was applied over desensitizing dentin as previously described.
- **Group MS+CS:** MS Coat One was applied for 30 s, followed by water rinsing and air-dried. The adhesive Clearfil SE Bond was applied over desensitizing dentin as previously described.
- **Group GC:** 37% phosphoric acid was applied for 10 s followed by water rising. Dentin surface was left visibly moist, the adhesive Gluma Comfort Bond + Desensitizer was applied, and light-cured for 10 s.

The adhesives were polymerized using a polywave LED curing light (Bluephase G2, Ivoclar Vivadent, Schaan, Liechtenstein) using the high power mode. The composite resin (Filtek Supreme Ultra, shade A2E, 3M Oral Care, St Paul, MN, USA) block was built-up incrementally on the bonded cervical dentin surface. Each 2 mm-increment was light cured for 20 seconds and teeth were stored in distilled water at 37°C for 24h. The composition of the materials (adhesives and desensitizing agents) used is presented in Table 1.

Table 1. Description of the materials used in the study

Material (batch number)	Manufacturer	Composition
Clearfil SE Bond (CS)	Kuraray Medical Inc., Tokyo, Japan	Primer: MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water. Bond: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, photoinitiator, silanated colloidal silica.
MS Coat One (MS)	Sun Medical, Shiga, Japan	3% MS Polymer (Copolymer of methyl methacrylate and styrene sulfonic acid), 1% oxalic acid, water.
Bifluorid 12 (BF)	Voco Cuxhaven, Germany	Sodium and calcium fluoride, ethyl acetate, pyroxylin, isoamylpropionate, fumed silica
Gluma Comfort Bond +Desensitizer (GC)	Heraeus Kulzer, Hanau, Germany	10-25% HEMA, 5% glutaraldehyde, 25-50% ethanol, 5-10% polyacrilic acid, 5% 4-META, maleic acid, UDMA, photoinitiator.

* Abbreviations: Bis-GMA: Bisphenol A diglycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogenphosphate; UDMA: Urethane dimethacrylate; 4-META: 4-methacrylolyloxethyl trimellitate anhydride.

Water storage

After water storage for 24 h, the restored teeth were serially sectioned with a diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) under water cooling, in both *X* and *Y* directions across the adhesive interface to obtain bonded beams with a cross-sectional area of approximately 1 mm^2. The beams of each tooth were randomly divided into two groups, as follows: immediately tested (24 h) and stored in water for one year at 37°C before testing (one year). Water was changed monthly.

Micro-tensile bond strength evaluation (μTBS)

After storage period (24 h or one year), beams were fixed by the ends to the micro-tensile device with a cyanoacrylate adhesive (Zapit, DVA, Anaheim, CA, USA) and tested in tension at a crosshead speed of 0.5 mm/min, until fractured (EZ-test, Shimadzu Co., Kyoto, Japan). The cross-sectional area of the tested was measured with a digital caliper (Mitutoyo Corp, Tokyo, Japan) after removing them from the micro-tensile device. The μTBS of each beam was expressed in MPa.

Energy-dispersive X-ray spectrometry

After μTBS test the dentin-side from fractured specimens were used to analyze the chemical elements present at the resin-dentin interface, using energy-dispersive X-ray spectroscopy (EDS). The fractured samples were fixed on metal stubs and sputter-coated with carbon (MED 010; Balzers Union, Balzer, Liechtenstein) prior to EDS. The X-ray detector (X-Act; Oxford, Tubney Woods, UK) was coupled to a scanning electron microscope (JSM IT 300; JEOL, Tokyo, Japan) and images were obtained for each material.

Statistical analysis

All beams were tested for each tooth, and the mean bond strength value was considered for each group (n=8). Statistical Analysis was performed with IBM SPSS 21 (SPSS Inc.; Chicago, IL, USA) software for macOS. The normality of data distribution was tested using Shapiro-Wilk test. The μTBS data were analyzed by two-way mixed ANOVA, followed by the Bonferroni's post hoc test ($\alpha = 0.05$).

Results

The μTBS means and standard deviation for experimental groups are described in Table 2. Shapiro-Wilk test indicated that the data were normally distributed (p= 0.20), Consequently parametric statistical analyses were applied. The two-way mixed ANOVA revealed that there are a significant effect for the "storage time" factor (F(1, 27) = 8,704; p=0.006) and for the interaction Treatment*Storage time (F(3,27)=3,304; p=0.035).

Table 2. Means and standard deviations (MPa) of μTBS for the experimental groups at 24 hours and after one year of water storage.

Groups	Storage Time	
	24 horas	1 Year
Clearfil SE Bond	33.0 ± 9.7 Aa	32.1 ± 11.8 Aa
MS Coat One + Clearfil SE Bond	33.4 ± 9.0 Aa	20.4 ± 7.4 Bb
Bifluorid 12 + Clearfil SE Bond	36.1 ± 9.0 Aa	20.9 ± 8.8 Bb
Gluma Comfort Bond +Desensitizer	33.7 ± 7.7 Aa	33.4 ± 4.2 Aa

Upper case letters compare storage times within the same group, while lower case letters compare groups within the same storage time.

At 24 hours no difference among groups was found. However, at one year, BF+CS (20.9 ± 8.8 MPa) and MS+CS (20.4 ± 7.4 MPa) showed lower μTBS than those obtained by CS and GC, because of the μTBS reduction after water storage for one year. The μTBS of CS and GC kept stable following the long-term water storage.

The EDS analysis identified the presence of calcium, phosphorus, and silicon for all groups.(Fig.1) For the group BF+CS, sodium, and fluorine were detected. (Fig. 1B)

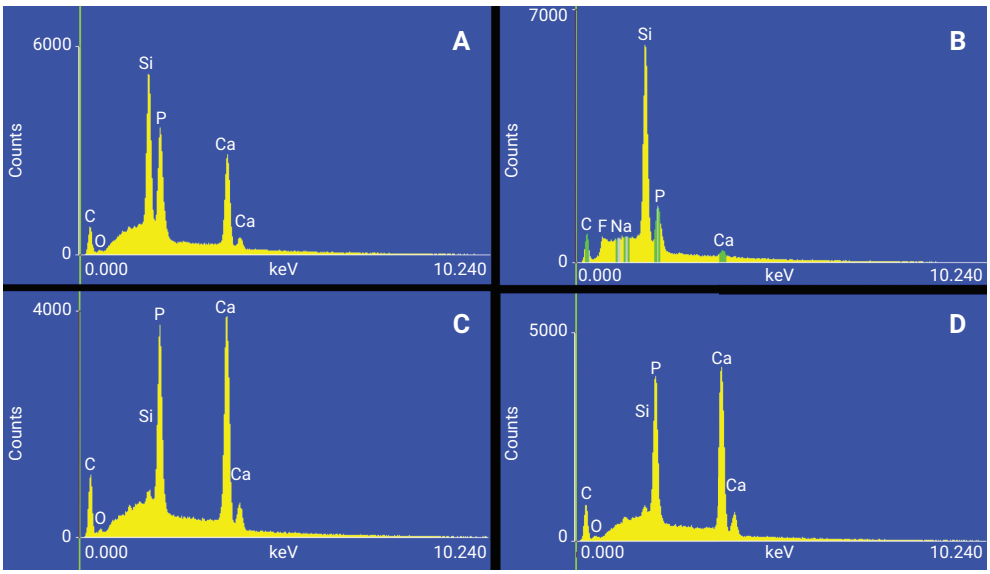

Figure 1. Identified elements by EDS analyses (A) Clearfil SE Bond, (B) Biofluorid 12 + Clearfil SE Bond, (C) MS Coat One + Clearfil SE Bond, and (D) Gluma Confort Bond + Desensitizer.

Discussion

This study demonstrated that the previous treatment of dentin with desensitizer agents did not compromise the early dentin μTBS of self-etch adhesive. However, a significant reduction in μTBS after one-year water storage was noted when the dentin was treated with Bifluorid 12 or MS Coat One followed by the application of Clearfil SE Bond. Thus, both null hypotheses were rejected because the using of desensitizer agents (Bifluorid 12 and MS Coat One) affected the μTBS of self-etching adhesive to dentin at one year, which were lower than CSEB and GCBD groups.

The μTBS reduction after one year might have been the result of incompatibility between the desensitizer agents and the self-etch adhesive system or remnant of them on the dentin surface that impaired the bonding. Clearfil SE Bond is able to form a thin hybrid layer as well as to react chemically with calcium from hydroxyapatite and both bonding mechanisms might be compromised, thus the use of desensitizer agents must be avoided when using this self-etch adhesive[19,20]. On the other hand, the μTBS of Gluma Comfort Bond + Desensitizer kept stable after one year.

The desensitizer agents used in this study present different compositions and are able to occlude dentinal tubules by different mechanisms of actions. MS Coat One is a single-bottle tubule sealant, based on oxalic acid and a moisture of polymethyl methacrylate and a copolymer of polystyrene sulfonic acid (Table 1)[15]. When the acid oxalic is applied on the dentin surface, it liberates calcium ions from the dissolution of hydroxyapatite to form calcium oxalate crystals and polymer-Ca complex[6,7,15,21,22]. The efficacy of oxalate desensitizer in permeability reduction has been demonstrated[16,18,22]. However, previous studies have shown that the desensitizing effect promotes by the oxalic acid may not be permanent. The permeability of the tubules can be re-established by partial crystal dissolution in oral fluids or because the removal it by toothbrushing[1,18,22]. The use of this desensitizer agent did not influence the μTBS of Clearfil SE Bond to dentin at 24 hours, but it was reduced after one year probably because the crystal solubilization from the dentinal fluids and storage water, which also affected the bonding and decreased the μTBS[18,23].

Bifluorid 12 is a fluoride varnish based on sodium and calcium fluoride (Table1). The EDS analysis detected fluorine and sodium that confirm the interaction of these components with dentin surface (Fig. 1B). These compounds work in two different ways: (1) occluding the dentinal tubules by the crystallization of sodium fluoride and (2) formation of a calcium fluoride precipitation on the dentin surface[1,6,17]. It has been demonstrated that precipitation of calcium fluoride is slowly solubilized in saliva, which may explain the transitory action of this barrier[1]. Other studies have shown the loss of adhesion between dentin and the varnish in short periods of time by dissolution or for fluoride release[11]. According to our results, the application of Bifluorid 12 also compromised the μTBS after one-year storage. This μTBS reduction may be attributed to the same reasons as the MS Coat One. Gluma Comfort Bond + Desensitizer is a commercial product that combines an adhesive system and a desensitizer agent. It is an alcoholic solution that contains three resin monomers (HEMA, UDMA, and 4-META), two organic acids (polyacrylic and maleic) and 5% glutaraldehyde as desensitizer agent[24]. In dentistry, besides desensitizer agent, glutaraldehyde has been

used in many different ways such as antibacterial, cross-linker agent, and MMPs inhibitor in order to increase the resistance of collagen fibrils against enzymatic degradation[24-26]. Its ability as MMPs inhibitor and cross-linker agent may have played an important role to maintain the stability of the dentin μTBS after one year[27,28].

Desensitizing mechanism of Gluma Comfort Bond + Desensitizer occurs by means of two reactions[1], the glutaraldehyde reacts with some proteins of the dentin fluid forming precipitates, and[2] the HEMA aggregates these precipitated proteins that occludes the dentinal tubules[29,30]. However, little is known about the stability and durability of these precipitates that are occluding the dentinal tubules. Some studies have shown that saliva and dentinal fluid contain esterases that could degrade ester and peptide bonds of this precipitates[17,31]. If the dentinal fluid attacked the precipitates created by Gluma, the desensitizing activity and the bond strength should be affected. However, in this in vitro study, the bonding procedures were performed in the absence of pulpal pressure with minimum dentinal fluid. Thus, it did not influence the bond strength values, especially in the one-year storage group. Obtained data also suggested that the hybridization mechanism, the chemical interaction of the adhesive system that was promoted by 4-META and the desensitizer are the key factor to guarantee the durability of the adhesion.

The results of the EDS analysis detected calcium and phosphorus, which are chemical elements from hydroxyapatite. Regarding silicon, this chemical element is present in the composition of Bonding Resin from CS. However, for GC adhesive the presence of silicon might be from composite resin, representing remnant of composite over adhesive, such as a cohesive failure within the composite.

Therefore, despite Bifluorid 12 and MS Coat One desensitizers not having affected the initial μTBS of Clearfil SE Bond to dentin, their application reduced the bond strength over time. Clearfil SE Bond used alone and Gluma Comfort Bond + Desensitizer adhesives promoted stable μTBS to dentin after one year and besides their desensitizing mechanism, they could provide long-lasting restorations.

In conclusion, the effect of desensitizer agents based on fluoride varnish and oxalic acid on the μTBS to dentin appears to be affected over time. A careful selection of the desensitizing agent must be performed.

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