

# Microtensile Bond Strength of Self-Etch Adhesives on Dentin Treated with Chlorhexidine

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## ABSTRACT

*Aim:* To evaluate the microtensile bond strength of self-etching adhesive systems in dentin treated with 2% chlorhexidine digluconate (CHX). *Materials and Methods:* The occlusal surfaces of 20 human molars were removed and divided into eight groups: 1A, Clearfil SE adhesive (SE) with self-etching technique (TS); 1B, SE with TS and previous application of CHX; 2A, Scotchbond Universal (SBU) adhesive with TS; 2B, SBU adhesive with TS and previous application of CHX; 1C, SE with total-etching technique (TT); 1D, SE with TT and previous application of CHX; 2C, SBU with TT; 2D SBU with TT and previous application of CHX. Composite resin (5mm) was applied on the hybridized surface. Samples were subjected to microtensile test and evaluated on Scanning Electron Microscope (SEM) and with energy-dispersive X-ray (EDX). *Results:* Low values of bond strength were observed in groups 1A (39,77±11,56) and 2A (40,84±12,49) comparing with 1B (22,86±5,18) and 2B (27,02±5,58). TS group presented adhesive type remover fracture while TT groups presented cohesive at the top of hybrid layer. EDX revealed the presence of CHX crystals for TS, which was not found in the TT. *Conclusion:* The previous application of chlorhexidine on dentin decreased the bond strength of adhesive systems on self-etching technique.

## INTRODUCTION

Active bacteria can remain in enamel and dentin after preparation procedures, due to incomplete removal of infected tissue. Adverse effects induced by the presence of microorganisms can be eliminated by antibacterial agents.<sup>1-4</sup>

Chlorhexidine (CHX) has a large spectrum being effective against Gram-positive and Gram-negative microorganisms. Because of the antibacterial action, CHX is used as a disinfectant in the cavities before applying restorative material and, more recently, as a root canal irrigant.<sup>1</sup> Furthermore, in the adhesive system bonding process, the use of CHX seems to provide increased bond strength between the restorative material and the dentin, possibly because the fact that promote stabilization of the hybrid layer and inhibition of the action of matrix metalloproteinase (MMP), thus enabling the preservation of this interface and the extension of the degradation process of the adhesive restorations.<sup>5-8</sup> In this way, the substantively of CHX, in other words, the ability of CHX to be retained in the dentinal matrix could be the reason for the dentin to form more stable hybrids layers after the treatment with CHX followed by acid etching.<sup>9</sup> This property is also extremely important in dentin remineralization process, allowing a greater inhibition of MMPs for a longer period.<sup>10</sup>

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The bond between restorative materials and dentin is still a major challenge due to the heterogeneous character of the structure, depth and surface morphology of dentin.<sup>11-14</sup> The longevity of restorations is directly affected by the integrity of the hybrid layer. Degradation of polymer chains and collagen fibrils could compromise the adhesive system bond strength in the long term.<sup>15,16</sup> The MMP inhibitory action of CHX, has been demonstrated *in vivo* and *in vitro* studies by CHX application on the dentin surface after application of the phosphoric acid and before applying the conventional adhesive system.<sup>5</sup>

Universal adhesives are classified as “universal” or “multi-mode”, because of the possibility to be use it both in self-etching technique, as in conventional.<sup>17</sup> This multi-approach capability allows the clinician to apply the adhesive with the so-called selective etching technique of the enamel, which can combine the advantages of enamel etching technique, with the self-etching approach in dentin and its additional chemical bond to the remaining dentin.<sup>18</sup>

Differently from the conventional technique, in which contact with the dentin occurs only by micromechanical retention, the self-etching adhesives has the characteristic of penetrate and demineralize the tooth surface simultaneously with the same depth. This fact means that there is a series of secondary reactions between the monomers and the hydroxyapatite resin, featuring a chemical bond, being essential in this case, a close contact with the dentin. In this way, changes in the dentin surface would interfere in the adhesion process.<sup>19,20</sup>

When applied to dentin, CHX may form birefringent needle shaped crystals due to the reaction with phosphate ions which slowly precipitated out of solution, acting as a physical barrier and therefore limiting the chemical interaction of the adhesive system with the surface and minimizing the adhesion potential.<sup>1</sup>

Thus, the aim of this study was to evaluate the influence of dentin pretreatment with CHX on bond strength to self-etching and universal adhesive systems. The experimental hypothesis was that application of CHX on dentin would not affect microtensile bond strength to dentin.

## MATERIALS AND METHODS

For this study, 20 healthy human third molars were obtained from the Bank of Teeth of Dentistry School of Federal University of Juiz de Fora. The teeth were kept immersed in a solution of chloramine T to 0.05% at 4 °C until use. The study was approved by on the Research Ethics Committee in Human of the Federal University of Juiz de Fora (no.226.747).

### SAMPLE PREPARATION

The teeth underwent coronary prophylaxis with pumice paste and water, aided by a rotatory brush, and then washed with air-water spray and dried with air jets. The occlusal enamel was removed using diamond blade (Isomet, Buehler Ltd., Lake Bluff, IL) under water cooling to expose dentin surface average. Then,

a second cut for removal of the root region of the teeth was carried out. These cuts resulted in dentin disks on average 5 mm in height. The space left by the pulp chamber was subjected to wear with spherical carbide bur carbide of compatible size, and then restored with adhesive system Single Bond II (3M/ESPE, St Paul, MN, USA), and Filtek Z350 resin composite (Color A3; 3M/ESPE, St Paul, MN, USA) on the incremental technique and following the manufacturer’s instructions. To standardize the smear layer, the dentin top surfaces were grounded on sandpaper with granulation 400 and 600, manually refrigerated with water for one minute, in circular movements.

### MICROTENSILE BOND STRENGTH EVALUATION AND FRACTURE ANALYSIS BY SCANNING ELECTRON MICROSCOPY (SEM)

These dentine disks were treated in two different modes: self-etching and total-etching modes, thereby forming eight groups distributed according to the text described below:

#### Self-Etching Mode:

SEself Group – the acidic primer of the Clearfil SE Bond (Kuraray) adhesive system was applied on dentin for 10 s with light pressure, followed by drying and subsequent application of bonding agent of the same system, which was light-cured for 10 s operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein).

SEself + CHX Group – it was initially applied chlorhexidine digluconate solution at 2% during 20 s on the dentin surface with the aid of microbrush, followed by air drying. Then, the acidic primer of the Clearfil SE Bond (Kuraray) adhesive system was applied on dentin for 10 s with light pressure, followed by drying and subsequent application of bonding agent of the same system, which was light-cured for 10 s operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein).

SBself Group – on middle dentin, Scotchbond Universal Adhesive (3MESPE) was applied for 20 s exerting light pressure. Then, it was dried for 5 s and light-cured for 10 s operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein).

SBself + CHX Group – it was initially applied chlorhexidine digluconate solution at 2% during 20 s on the dentin with the aid of microbrush, followed by drying. Then, the Scotchbond Universal Adhesive was applied as indicated by manufacturer and as realized in the preceding group.

#### Total Etching Mode:

SEtotal Group - the dentin was etched with phosphoric acid 35% for 15 s, then lightly washed with air / water spray and dried with absorbent paper for humidity reduction. After, the acidic primer of the Clearfil SE Bond (Kuraray) adhesive system was applied on dentin for 10 s with light pressure, followed by drying and subsequent application of bonding agent of the same system, which was light-cured for 10 s operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein).

SEtotal + CHX Group - the dentin was etched with phosphoric acid 35% for 15 s, then lightly washed with air / water spray and dried with absorbent paper for humidity reduction. In this moment, it was applied chlorhexidine digluconate solution at 2% during 20 s on the dentin with the aid of microbrush, followed by drying. After, the acidic primer of the Clearfil SE Bond (Kuraray) adhesive system was applied on dentin for 10 s with light pressure, followed by drying and subsequent application of bonding agent of the same system, which was light-cured for 10 s operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein).

SBUtotal Group - the dentin was etched with phosphoric acid 35% for 15 s, then lightly washed with air / water spray and dried with absorbent paper for humidity reduction. Then, Scotchbond Universal Adhesive (3MESPE) was applied for 20 s exerting light pressure and it was dried for 5 s and light-cured for 10 s operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein).

SBUtotal + CHX Group - the dentin was etched with phosphoric acid 35% for 15 s, then lightly washed with air / water spray and dried with absorbent paper for humidity reduction. In this moment, it was applied chlorhexidine digluconate solution at 2% during 20 s on the dentin with the aid of microbrush, followed by drying. Then, Scotchbond Universal Adhesive (3MESPE) was applied for 20 s exerting light pressure and it was dried for 5 s and light-cured for 10 s operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein).

Filtek Z350 (Color A3; 3MESPE) resin blocks were made with 5 mm of height on hybridized dentin surface through incremental technique. Every 2 mm layer, resin was light-cured with light emitting diodes operating at 1000 mW/cm<sup>2</sup> (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) for 20 s. Composition, batch and manufacturers are shown in Table 1. After obtaining the specimens (n = 30) groups were stored in deionized water at 37°C for 24 hours. After the storage period, the specimens were fixed with cyanoacrylate glue (Super Bonder gel - Loctite) and sticky wax in a standard device and brought to digital cutter (Isomet, Buehler Ltd., Lake Bluff, IL) to be cut into two mutually perpendicular planes, resulting in non-trimmed bar-shaped specimens with dimensions of 1x1x10 mm. Only the bars obtained from the central region of each disc were selected. Then, obtained samples were stored in deionized water at 37°C for 24 hours prior to mechanical testing.

After the storage period, bar-shaped specimens were attached by both ends to a 2-part device with cyanoacrylate glue (Super Bonder gel - Loctite) and subjected to microtensile test in universal testing machine (EMIC DL 2000) at a cross-head speed of 1 mm/min until fracture. The values obtained in the microtensile bond strength test for the different groups were recorded and microtensile bond strength ( $\sigma$ ) was calculated by  $\sigma = L/A$ , where 'L' is the maximum load applied (N) and A is the interface area of each specimen (mm<sup>2</sup>). Data were subjected to statistical analysis by two-way ANOVA (type of adhesive systems and application of CHX), followed by Tukey test for mean comparison ( $\alpha = 0.05$ ). To compare the self-etching and total-etching modes, t test was performed for independent samples ( $\alpha = 0.05$ ).

**Table 1. Materials used in the study with the presentation of the composition, lot number and manufacturer.**

Materials	Composition	Batch number	Manufacturer
Clearfil SE Bond	Primer: MDP 10; HEMA; dimethacrylates hydrophilic; camphorquinone; N, N-diethanol-p-toluidine; water. (pH=2.0) Adhesive: 10 MDP; Bis-GMA; HEMA; hydrophobic dimethacrylates; camphorquinone; N, N-diethanol-p-toluidine; silanized colloidal silica.	01147A 01713A	Kuraray, Osaka, Japan
Scotchbond Universal	10-MDP, HEMA, Vitrebond™ copolymer, inorganic particles, ethanol, water, initiators and silabo (pH=2.7)	488913	3MESPE Dental Products St. Paul MN, USA
Filtek Z350	TEGDMA; UDMA; BisGMA; BisEMA; silica particles 20nm; Particle zirc4,1-phenyleneoxy (2-hydroxy-3,1-propanediol)	N421309	3MESPE Dental Products St. Paul MN, USA
35% phosphoric acid	Phosphoric acid 35%	L69798	3MESPE Dental Products St. Paul MN, USA

10-MDP (metacriloxidecil dihydrogenphosphate), HEMA (hydroxyethylmethacrylate) copolymer Vitrebond™ (copolymers of acrylic and itaconic acids), BisGMA (bisphenol A glycidyl methacrylate), TEGDMA (triethyleneglycol dimethacrylate), UDMA (urethane dimethacrylate), BisEMA (bisfenilglicidil dimethacrylate).

The fractured samples were stored in a dry environment and subsequently mounted on aluminum stubs, coated with gold and observed by Scanning Electron Microscopy (JEOL, JSM-5800 LV model, Tokyo, Japan), operating at 20 kV in order to check the areas of fractures and surface morphology. The fractures were classified as Type I - cohesive failure in the hybrid layer; Type II - cohesive failure in resin composite; Type III - cohesive failure in the adhesive layer; Type IV - mixed fracture between composite and hybrid layer; and Type V - mixed fracture between adhesive layer and hybrid layer.

### ANALYSIS BY ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDS)

Flat dentin surfaces were prepared as described above. The dentin surfaces were standardized with sandpaper of granulation silicon carbide 400 and 600 under refrigeration for 60 s to standardize the smear layer (n=5). The teeth were divided into four groups according to the surface treatment: (1) healthy dentin (control) (2) application of chlorhexidine digluconate solution at 2% for 20 s followed by drying (3) over the healthy dentin and phosphoric acid etching to 35% for 15 s (4) on sound dentin followed by applying the solution of chlorhexidine digluconate 2% for 20 s. The teeth were dehydrated in silica gel for 2 h. After, the samples were subjected to evaporation carbon (SCD 050, Balzer Union AG, Balzers, Lichtenstein) for EDS analysis using a SEM (JSM 5600LV; LEOL, Tokyo, Japan). The EDS was equipped with an ultra-thin window Norvar and operated with a digital microanalysis system based on Windows NT (Vantage). Analysis of chemical elements present on the samples were performed in secondary electron mode operating at 15 kV at a working distance (WD) of 20 mm.

## RESULTS

Previous application of CHX on dentin negatively affected the bond strength (MPa) of self-etching adhesives on dentin. SEself and SBself groups presented the highest bond strength values, without statistical difference between them. SEself + CHX and SBself + CHX presented the lowest bond strength results and were similar between them (Table 2).

When adhesives were applied in total etching mode, the presence of CHX did not affect bond strength values. SBtotal, SBtotal + CHX and SEtotal + CHX presented the highest values of bond strength and were similar between them (p > 0.05). SEtotal group presented the lowest bond strength values (Table 3). All groups presented statistical difference when self-etching and total etching modes were compared (Table 4).

The morphological analysis by scanning electron microscope (SEM) was performed in two magnifications (80x and 200x). Through this evaluation, it was possible to identify three surface patterns. In the areas where the adhesive was identified, it was possible to observe a smooth surface with the presence of splinters. Already on other surfaces, it was possible to observe many orifices, the dentinal tubules; characterizing the hybrid

**Table 2. Means (standard deviations) for microtensile bond strength to dentin (MPa) for different adhesive systems on self-etching mode.**

Adhesive Systems	Means for microtensile bond strength (SD)	
SEself	39.77 (±11.56)	A
SBUsself	40.84 (±12.49)	A
SEself+CHX	22.86 (±5.18)	B
SBUsself+CHX	27.02 (±5.58)	B

Different letters for each adhesive system differ by Tukey test (p<0.05).

**Table 3. Means (standard deviations) for microtensile bond strength to dentin (MPa) for different adhesive systems on total etching mode.**

Adhesive Systems	Means for microtensile bond strength (SD)	
SEtotal	25.95 (±5.43)	B
SBUstotal	31.53 (±9.22)	Ab
SEtotal+CHX	28.09 (±4.20)	Ab
SBUstotal+CHX	37.81 (±11.69)	A

Different letters for each adhesive system differ by Tukey test (p<0.05).

**Table 4. Means (standard deviations) for microtensile bond strength to dentin (MPa) among self-etching and total etching modes.**

	SE	SE+CHX	SBU	SBU+CHX
Self-Etching	39.77 (±11.56)a	22.86 (±5.18)b	40.84 (±12.49)a	27.02 (± 5.58)b
Total Etching	25.95 (± 5.43)b	28.09 (±4.20)a	31.53 (± 9.22)b	37.81 (±11.69)a
	p=0.0002	p=0.0010	p=0.0160	p=0.0016

Different letters for each adhesive system differ by test t (p =0.05).

layer. In the areas where the composite was present it was possible to notice a irregular surface, with the absence of the dentinal tubules. Some fractures had more than one superficial standard, what has characterized as mixed fractures. Thus, after

evaluating fractures in SEM, it was possible to classify them into five types: (1) cohesive failure in hybrid layer; (2) cohesive failure in composite; (3) cohesive failure in the adhesive layer; (4) mixed failure between the adhesive layer and hybrid layer; (5) mixed failure between composite and hybrid layer.

The most common fractures found were Type III, presented in most groups where the adhesive system was used in self-etching mode; and type V, in most groups where total-etching technique was used. In other words, for the groups SEself, SEself + CHX and SBUsef; the most common fracture type found was type III. In SBUsef + CHX; SEtotal, SEtotal + CHX and SBUtotal + CHX groups; type V was the most common. In SBUtotal group was observed fracture type I as most frequently type (Figure 1).

EDS analysis revealed the presence of chlorine on the dentin surface after use of CHX (Figures 2 to 5).

## DISCUSSION

According to several studies, the adhesive systems containing functional monomer 10-MDP showed excellent results on bonding to dentin.<sup>14</sup> The Clearfil SE Bond (Kuraray) adhesive, specifically, has been considered the gold standard among the other self-etching systems in recent years, due to its better chemical interaction with calcium present in the hydroxyapatite when compared to other functional monomers TEM-4 (4-methacryloxyethyl-trimellitic acid) and P-Phenyl (2-methacryloxyethyl phosphate phenylhydrogen). In order to further reduce the clinical steps, the SBU, a universal adhesive which also contains 10-MDP, was introduced in the market to be used both in the total-etching technique as in self-etching. The present study compared the microtensile bond strength of two adhesive systems in human dentin in the two different techniques, adding the pretreatment of dentin with a solution of chlorhexidine digluconate 2%.<sup>14</sup>

For the results showed in the self-etching technique, both adhesives (SEself 39.77 MPa and SBUsef 40.84 Mpa) behaved in an appropriate manner presenting high mean values of bond strength, showing that a good performance of both, probably due to the presence of the monomer 10-MDP.<sup>18,21</sup> In the total-etching technique, both adhesives showed lower values of bond strength (SEtotal 25.95 MPa and SBtotal 31.53 MPa), being statistically different from the SEself and SBself (Table 4). This is because preconditioning the dentin by 37% phosphoric acid, removes the superficial mineral content, lowering the 10-MDP connections with the calcium of hydroxyapatite. Moreover, the dentin etching forms a low quality of hybrid layer and susceptible to nanodegradation.<sup>17,22</sup> As the SE system is presented separately in two bottles, acidic primer and adhesive, the latter being more hydrophobic failed to adequately infiltrate in the demineralized dentin. This fact confirms the predominant type of fracture found in these groups, the mixed fracture between the adhesive layer and the hybrid layer (Figure 1).

It is known that the application of CHX, after completion of the cavity preparation by clinicians as a method of cleaning the cavity, acts as a potent antimicrobial agent which is still recognized by minimizing the effects of MMPs along the demineralized layer of collagen that is more exposed in the total-etching mode, in order to interact with these. Thus, over time, prevents collagen degradation, preserving the hybrid layer.<sup>23</sup> These proteases are secreted by odontoblasts during dentinogenesis, remaining inactive within the dentin extracellular matrix. On contact with an acid environment, from the adhesive systems or from biological dental caries process, different dentin MMPs (-2, -8 and -9) can be activated.<sup>7</sup> Therefore, the prior application of an antimicrobial agent having the function of inhibiting MMPs contributes to the adhesive bonding process.<sup>5,6,24</sup> But when the CHX was applied associated to the self-etching technique, there was significant reduction in bond strength (Table 2) with prevalence of the fractures in the adhesive layer (Figure 1).

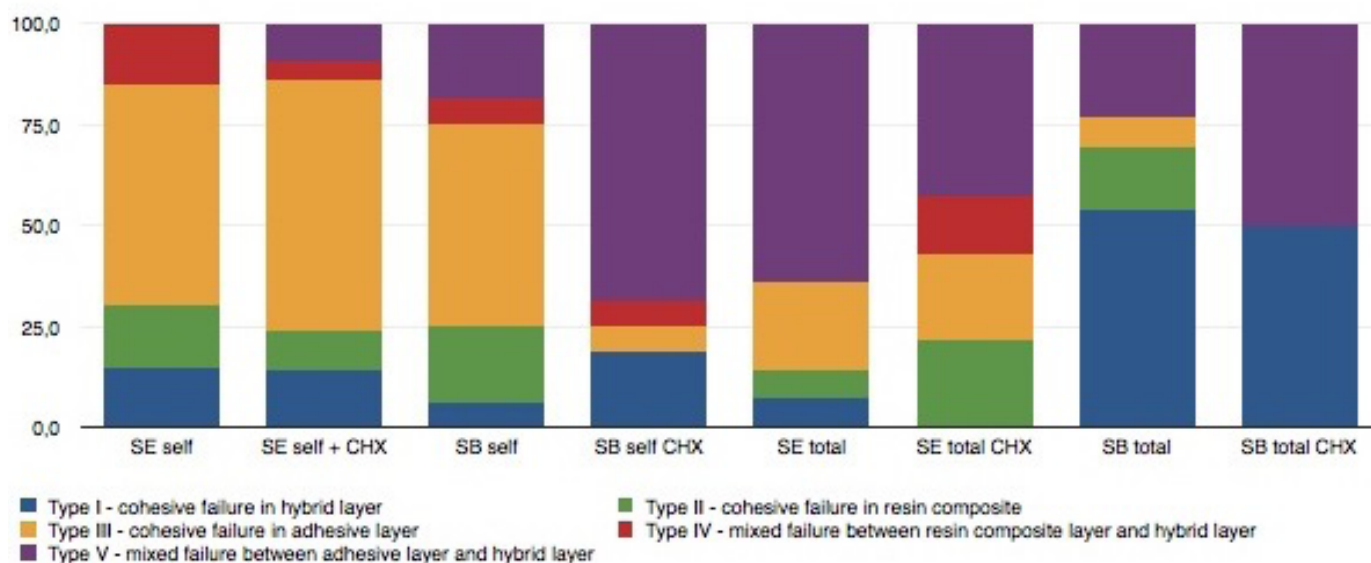
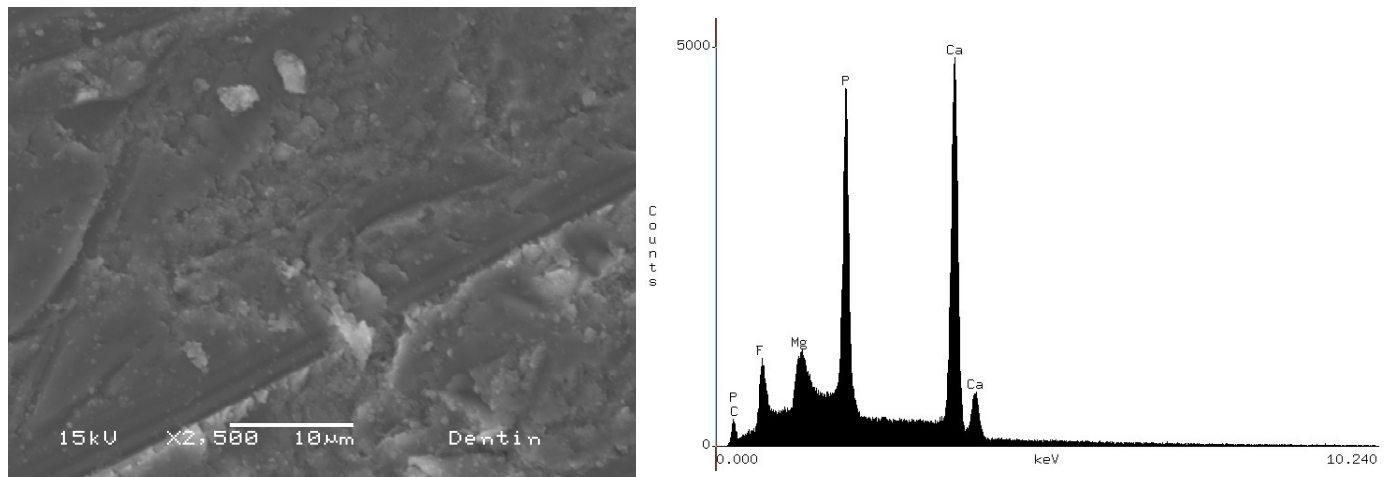
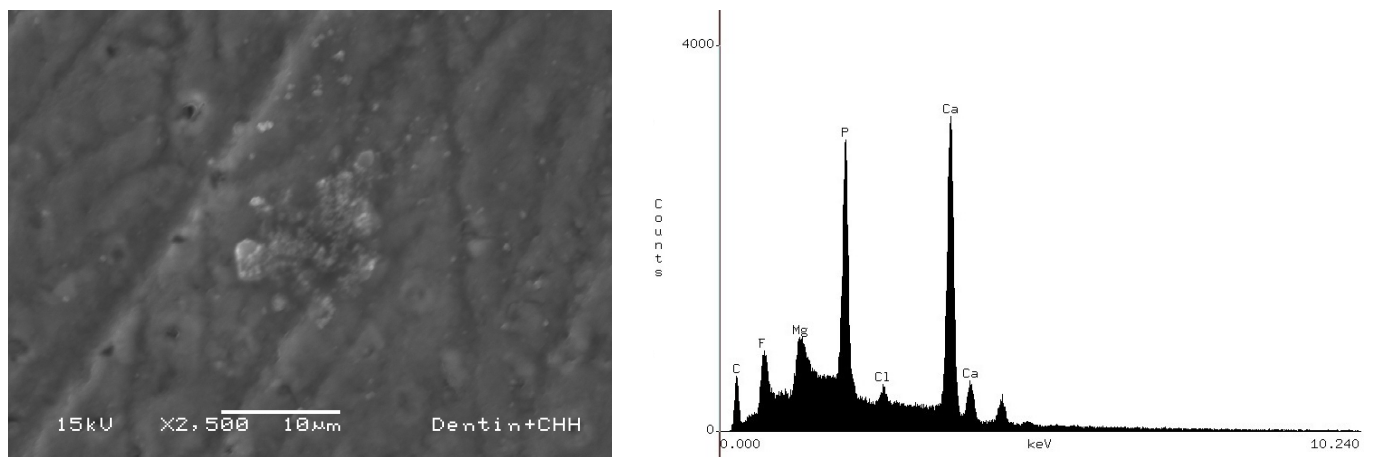


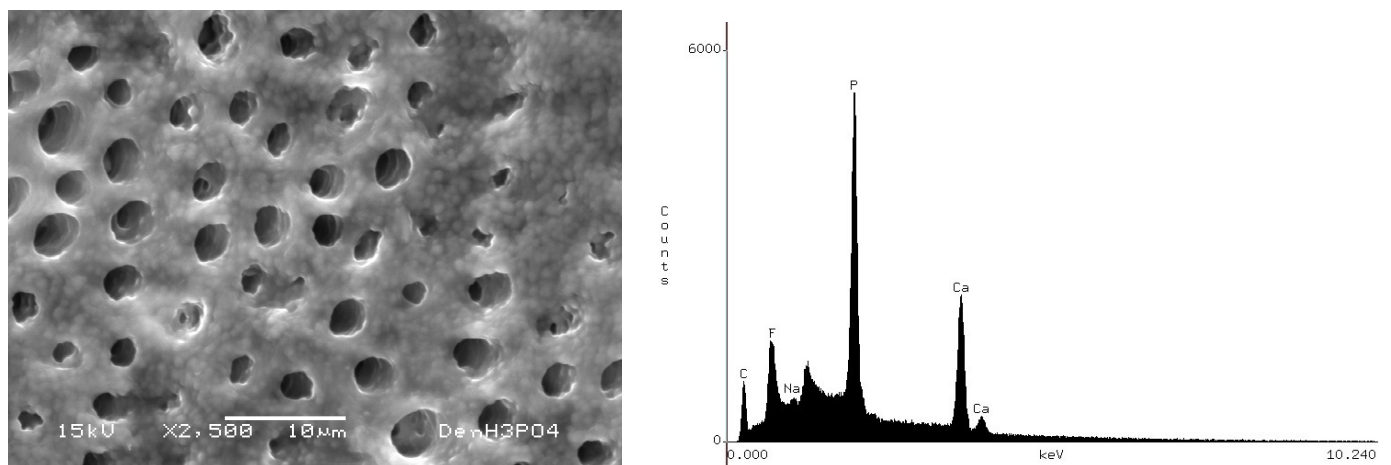
Figure 1: Graphic illustration of classification and percentage distribution of fracture types (%).



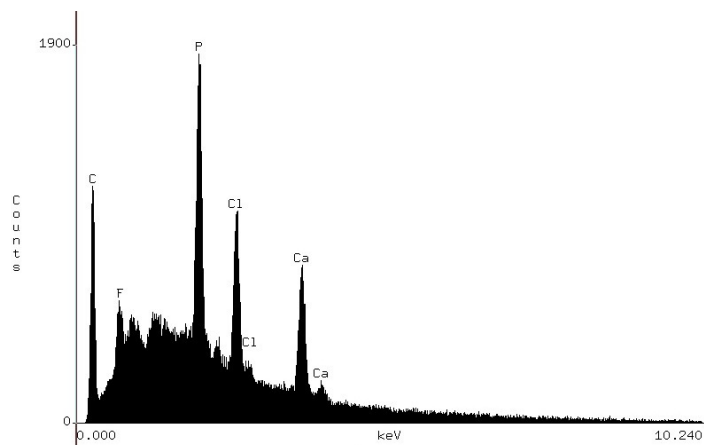
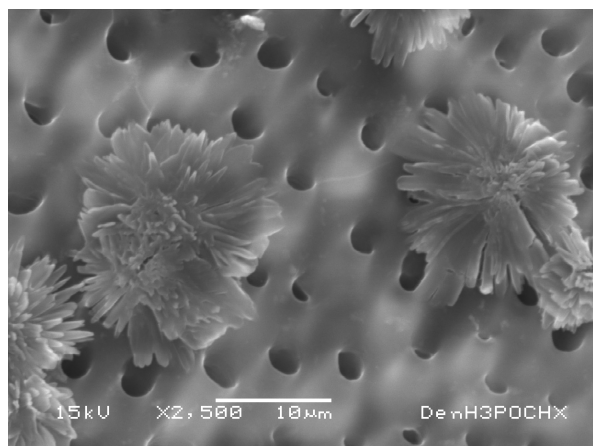
**Figure 2:** SEM images of the morphological aspect of healthy dentin (control) through 2500 magnifications and on the right hand side the representative map of the chemical elements presented on this surface. Morphological aspect of smear layer that covers the entire dentin surface where it is possible to observe some dentin tubules and irregularities. The chemical elements analysis map shows the presence of phosphorus, calcium and magnesium.



**Figure 3:** SEM images of the morphological aspect of the dentin after the application of chlorhexidine through 2500 magnifications and on the right hand side the representative map of the chemical elements presented on this surface. Morphological aspect of the smear layer covering the entire dentin surface where it is possible to observe the presence of minimally identified chlorine crystals (asterisks). The chemical elements analysis map shows the presence of phosphorus, calcium, magnesium and chlorine.



**Figure 4:** SEM images of the morphological aspect of dentin after etching with 35% phosphoric acid through 2500 magnifications and on the right hand side the representative map of the chemical elements presented on this surface. It is possible to observe the presence of open dentinal tubules and exposed collagen fibrils inside them. The chemical elements analysis map shows the presence of sodium, magnesium, phosphorus, sulfur and calcium.



**Figure 5:** SEM images of the morphological aspect of dentin after etching with 35% phosphoric acid and application of chlorhexidine through 2500 magnifications and on the right hand side the representative map of the chemical elements presented on this surface. It is also possible observe the presence of open dentinal tubules due to the demineralization process caused by phosphoric acid. Note micrometric structures in the form of crystals (crystallites) distributed along the entire dentin surface. The chemical elements analysis map predominantly shows the presence of chlorine in addition to magnesium, phosphorus, sulfur and calcium. Carbon used to cover the samples was detected in all analyses.

The interaction between the dentin surface and the restorative material depends on the balance between the surface energy and wettability. Although the application of CHX provide an increase in surface energy of dentin and not change the morphology, decreased of bond strength and adhesive failure in self-etching systems may be due to chemical interference of chloro crystals.<sup>1</sup> EDS analysis revealed the presence of chlorine on the dentin surface, indicating that these residues are only remaining after the use of CHX and may have interacted with the adhesive systems (*Figures 2 to 5*).

When the dentin is treated with CHX 2% solution, there is the formation of these precipitates in the form of birefringent needles, according to Hipólito *et al.* (2012).<sup>1</sup> These structures are the result of the reaction between the CHX and phosphate ions. These precipitates initially, acted as a physical barrier, limiting the interaction with the surface and minimizing the potential for union (*Figure 5*). Also, the application of CHX promoted an ionic dissociation which released  $\text{Ca}^{2+}$  ions of the dental surface.<sup>10</sup>

As CHX is a symmetrical molecule with two positive charges; these charges may have been electrostatically attracted to the anion of this phosphate in the mineral hydroxyapatite of the dentin involving them and forming crystals.<sup>5</sup> This fact is evidenced by the decrease of  $\text{Ca}^{2+}$  after the analysis in EDS (*Figures 2 to 5*). Therefore, it is suggested that the decrease of Ca ions due to the application of CHX may have reduced the potential for chemical bonding with the adhesives in self-etching technique.

On the other hand, when applying the solution of CHX on the total etching technique, the bond strength was not affected (*Table 3*). The SBUtotal, SBUtotal + CHX and SEtotal + CHX groups had the highest bond strength values and showed no statistically significant difference between them. Their fractures were predominantly in hybrid layer. In this study, adhesive systems were compared based on the analysis of the

influence of prior administration of CHX on both hybridization techniques; however, only the SBU adhesive manufacturer states that this system can be used with / without preconditioning. The SBU has the 10-MDP monomer and Vitebond TM copolymer which have chemical affinity with calcium hydroxyapatite.<sup>21,25</sup> The presence of these components probably promoted greater bond and made that present higher mean values of bond strength and the CHX application to this technique did not interfere. The concept that the bond is primarily based on micromechanical retention is giving way to a new concept of chemical interaction called “Adhesion-Decalcification Concept”, proposed by Yoshida *et al.*, 2012 and Van Meerbeek *et al.*, 2020.<sup>21,26</sup> For the SE system was expected lower bond strength values in this technique, since the mineral content has been removed, lowering the connections of 10-MDP with calcium hydroxyapatite.

In the same way, Carrilho *et al.* 2007 evaluated the effect of protease inhibition in the resin-dentin bonding and described that the most prevalent standard of fracture for control samples, regardless of time or storage solution, it occurred in the hybrid layer in any top or lower, which could be justified not only by the action of metalloproteinases (MMPs) in the dentin matrix, but also by demineralization promoted by applying phosphoric acid with the opening of dentinal tubules. The authors also stated that there was an improvement with the use of CHX, both in bond and in durability of the hybrid layer in the samples stored for six months.<sup>16</sup>

The endogenous proteases can degrade the collagen fibrils because demineralized dentin contains MMPs and cathepsins that, once activated by etching, can slowly degrade the collagen fibrils in the hybrid layer.<sup>23</sup> According Pashley *et al.* 2011 a way to inactivate these enzymes and, consequently, reduce the degradation of the collagen in the dentine and increase the durability of the restorations can be by application of CHX in the dentin surface after application of the phosphoric acid

and before system application adhesive since CHX have the ability to inhibit the action of these MMPs.<sup>27</sup> However, this property of CHX may not be immediately evident, but for samples which have undergone aging, where the substantivity of CHX appears to contribute to bond restorative material to the tooth surface.<sup>28</sup>

According to the presented study, it can be stated that residues left by CHX on dentine surface may be responsible for commitment on the bond strength of the self-etching technique tested adhesives. This commitment can have both chemical character, explained by the presence of chlorine that may have affected the chemical bond characteristic of etch; as well as physical, due to the presence of crystals formed after application of CHX that may have acted as a mechanical barrier to the flow of adhesive on inter- and peritubular dentin, compromising their adhesion. However, more studies are needed in this regard.

Considering the limitations of this study, further studies with a larger sample size and different bonding systems are required to investigate the effect of previous application of chlorhexidine on dentin on the bond strength of adhesive systems.

## CONCLUSION

The previous application of CHX did not affect the bond strength of self-etch and universal adhesives used with total-etching technique. For self-etching technique, CHX acted negatively on the bond strength of adhesives. Thus, the use of CHX prior to the adhesive system must not be recommended for self-etching adhesives.

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