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# Long-term bond strength of a self-adhesive resin cement to intraradicular dentin pretreated with chlorhexidine and ethanol

*Resistência de união ao longo do tempo de um cimento resinoso autoadesivo à dentina intrarradicular pré tratadas com clorexidina e etanol* 

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#### Resumo

Rev Odontol UNESP. 2017 Mar-Apr; 46(2): 97-103

Doi: http://dx.doi.org/10.1590/1807-2577.01716

**Introdução:** Cimentos resinosos autoadesivos não necessitam de tratamento prévio da superfície dental, por esta razão o pré tratamento da dentina pode influenciar a longevidade da resistência adesiva. **Objetivo:** Avaliar a influência do tratamento dentinário com etanol (ET) 100% e clorexidina (CL) 2% na resistência de união (RU) de um cimento resinoso autoadesivo (CRA) à dentina intrarradicular. **Material e método:** 80 raízes bovinas restauradas com pino de fibra de vidro e CRA (U200 3M/ESPE) foram distribuídas em 4 grupos, de acordo com o tratamento prévio da dentina intrarradicular: Grupo 1 – nenhum tratamento; Grupo 2 – CL2% por 1 minuto; Grupo 3 – ET100% por 1 minuto; Grupo 4 – CL2% seguido pelo ET100%. As amostras foram seccionadas no sentido radial para obtenção de duas secções de aproximadamente 0,7 mm de espessura em cada terço – cervical, médio e apical. Após 48 horas e 180 dias foi realizado o teste push-out. **Resultado:** A ANOVA a três critérios para blocos casualizados demostrou que não houve diferença entre os valores de resistência de união nos tempos 48 h e 180 dias, independentemente do tratamento e do terço (p>0,05). A interação tratamento-terço foi significativa (p = 0,041) sendo que o tratamento com CL promoveu menor RU no terço cervical e o tratamento com ET promoveu melhor RU no terço apical. **Conclusão:** Os tratamentos com CL e ET individualmente ou associados não promoveram diferenças entre os valores de RU do CRA à dentina intrarradicular ao longo do tempo.

Descritores: Etanol; clorexidina; técnica para retentor intrarradicular; cimentos de resina.

#### Abstract

**Introduction:** Self-adhesive resin cements do not require prior preparation of the tooth surface, therefore dentin pretreatments may influence long-term bond strength. **Objective:** To evaluate the influence of 100% ethanol (ET) and 2% chlorhexidine (CL) treatment of intraradicular dentin on the long-term bond strength (BS) of a self-adhesive resin cement (SRC). **Material and method:** 80 bovine roots were restored with fiber posts and SRC (U200 3M/ESPE) and distributed into 4 groups according to dentin treatment: Group 1 – without treatment; Group 2 – 2% CL for 1 minute; Group 3 – 100% ET for 1 minute; Group 4 – 2% CL, followed by 100% ET. The samples were cross-sectioned to obtain two sections (0.7 mm) thick for each root third: coronal, middle and apical. The immediate push-out test was carried out after 48 hours, and the long-term push-out test, after 180 days. **Result:** The three-way ANOVA test for randomized blocks showed no difference between the BS values at 48 hours and 80 days, irrespective of the treatment with CL promoted lower BS in the coronal third, while treatment with ET promoted better BS in the apical third. **Conclusion:** Treatment with CL and ET, separately or combined, promoted no differences between the BS values of the SRC to root dentin over time.

Descriptors: Ethanol; chlorhexidine; post and core technique; resin cements.

# INTRODUCTION

Extensive tooth loss may require root retention to ensure long-term retention and stability of coronal restorations, which can be achieved using intraradicular fiber posts<sup>1,2</sup>. Traditionally, prefabricated posts are cemented with adhesive systems combined with dual-curing or chemically cured resin cements<sup>1</sup>. Dual-curing resin cements may act as luting agents with different polymerization processes: either a physical process activated by using light from a light-curing unit on photoinitiators (camphorquinone); or by a chemical reaction between benzoyl peroxide and tertiary amines, determining polymerization conditions and conversion of the most suitable monomers, mainly in the most apical portions of root canals, where it is difficult for light to reach<sup>3</sup>.

Given the the difficulties with bonding to root dentin, and the demand for procedures that optimize clinical time, self-adhesive resin cements have been developed and do not require prior preparation of the tooth surface<sup>4-6</sup>. The multifunctional organic matrix of these cements is composed of phosphoric acid/ methacrylate. The group of phosphoric acid molecules etches the surface and helps with bonding<sup>7</sup>.

The bond to dentin may nonetheless degrade over time<sup>8</sup> and the use of ethanol as a pre-treatment has been proposed to control dentin moisture and promote a long lasting bond<sup>9,10</sup>. Accordingly, monomers that have hydrophobic characteristics appear to penetrate into the ethanol-saturated dentin more deeply than dentin saturated with water<sup>2</sup>. Moreover, this would leave the tissue less hydrophilic, prevent phase separation of the hydrophobic monomer and result in a more stable bond over time<sup>11</sup>. Despite the encouraging results when used on coronal dentin<sup>12</sup>, there has been little investigation into this approach to cementation on root dentin<sup>9</sup>.

Furthermore, the short durability of root bond is linked to hydrolysis of the collagen network within the hybrid layer<sup>13</sup>. It is known that enzymes such as matrix metalloproteinases (MMP) are involved in connective tissue turnover and are capable of degrading almost all components of the extracellular matrix<sup>14</sup>. The exposed collagen fibrils, without the protection afforded by minerals, become susceptible to hydrolytic and enzymatic degradation mediated by matrix metalloproteinases (MMPs) that are exposed and activated during acid etching. This phenomenon allows continuous infiltration of a combination of fluids, bacterial metabolites and saliva MMPs that will result in degradation of the unprotected collagen fibrils in the lower portions of the hybrid layer. In this scenario, the use of chlorhexidine (CHX) has been proposed to optimize the hybrid layer by inhibiting MMPs<sup>15</sup>. MMP activity has also been confirmed in root dentin<sup>16</sup> and this information encouraged the use of CHX as a dentin pre-treatment prior to cementing fiber posts<sup>8</sup>.

Studies have evaluated the application of ethanol with the purpose of promoting the use of adhesive systems containing hydrophobic monomer units<sup>11,17</sup> and chlorhexidine as a MMP inhibitor<sup>18</sup>. It is still unclear, however, whether the combination of these two substances may act synergistically in the bonding of self-adhesive resin cements, in cases where the application of conventional adhesive systems is contraindicated.

Considering the aforementioned context, the aim of this study was to evaluate the influence of treating the root canal dentin with 100% ethanol and 2% chlorhexidine on the bond strength and longevity of a dual-cure self-etching resin cement at different root depths.

The null hypothesis tested was that the bonding durability of a dual-cure self-etching resin cement to root dentin in any root third would not be influenced by pretreatment with chlorhexidine or ethanol, after 48 hours and at 180 days.

# MATERIAL AND METHOD

#### Experimental Design

*Experimental units*: root slices obtained from roots restored with fiber posts.

*Dependent variable*: bond strength measured by the push-out test. Failure mode was verified qualitatively using a scoring system.

*Independent variable*: Root dentin treatment at 4 levels: no treatment; treatment with 2% chlorhexidine; treatment with 100% ethanol; treatment with both chlorhexidine and ethanol.

Storage time at two levels: 24 hours and 180 days

Root depth at three levels: Coronal, Middle and Apical.

### Tooth Selection

This study was approved by the local Animal Research Ethics Committee (#2014/0217). Bovine incisors were kept in 0.1% thymol solution until use. They were then debrided using periodontal curettes and subsequently sectioned horizontally at the cervical level, close to the cement-enamel junction with a double-faced diamond disk so that all roots measured 17 mm in length.

#### Preparation of the canals for post cementation

Canal preparation was performed with rotatory burs measuring 2, 3 and 4 mm in diameter. A working length of 9 mm was established by using a rubber stop placed on the bur shaft, and burs were replaced after every 5 preparations. The root canals were aspirated with cannulas connected to a suction tube and excess water was removed with # 80 paper cones (Dentsply, Maillefer, Petrópolis, RJ, Brazil). The roots were positioned in a  $21 \times 34$  mm acrylic mold filled with condensation silicone (Speedex Coltène, Whaledent, Vigodent, Rio de Janeiro, RJ, Brazil) to facilitate handling of the specimens and to allow the curing light to be emitted from the coronal aspect of the root at all times. Table 1 lists the brand names, manufacturers, composition and the steps for applying the materials used in this study.

In Group 1, the dual-cure self-etching resin cement (U200 3M/ESPE, St Paul, MN, USA) was applied in accordance with the manufacturer's recommendations. In Group 2.2% chlorhexidine (FGM Joinville, SC, Brazil) was applied prior to the dual-cure resin cement (U200 3M/ESPE, St Paul, MN, USA); and in Group 3, 100% ethanol was applied prior to the dual-cure resin cement (U200 3M/ESPE, St. Paul, MN, USA). In Group 4, ethanol was applied for 1 minute; the canal was dried with paper points and light jets of air; chlorhexidine was applied for 1 minute, the excess

Table 1. Brand name, manufacturer, composition and steps for applying the materials employed

Brand Name Manufacturer (batch number)	Composition (main components)	Application steps					
2% Chlorhexidine solution (FGM Joinville, SC, Brazil) BATCH:155	2% chlorhexidine digluconate	The 2% chlorhexidine solution was applied with the aid of a disposable syringe and $0.5 \times 25$ caliber needle in the root canal, remaining there for a period of 60 seconds. The excess was then removed using absorbent paper cones, while keeping the dentin moist.					
Ethanol (Chemco Ltda., Brazil) BATCH: 24631	100% Ethanol	The root canal was completely filled with ethanol with the aid of a disposable syringe and $0.5 \times 25$ caliber needle for 1 minute. The excess ethanol was then removed using absorbent paper cones.					
Rely X U200 3M/ESPE (St Paul, MN, USA) BATCH: 1329500658/528731	Silanized glass powder, silica treated with silicon, calcium hydroxide, substituted pyrimidine and sodium persulfate	Manipulate the pastes in identical quantities using the cement's own doser and insert it into the canal with the help of a Centrix insertion syringe.					
Reforpost no. 2 (Angelus, Londrina, PR, Brazil) BATCH: 29393/29846	Fiber glass, Epoxy resin, inorganic load, silane, polymerization catalysts	The post was positioned and pressure applied for 10 seconds, the excess cement being removed with a disposable brush.					
Silane (Angelus, Londrina, PR, Brazil) BATCH: 21845	Silane polysulfide, hydrocarbon nucleus, elastomeric composition and ethanol	Application on the post surface for 60 seconds.					

removed with an absorbent paper point, and finally the dual-cure resin cement was applied (U200 3M/ESPE, St Paul, MN, USA).

# Preparation of the fiber posts for luting and the luting procedure

The fiberglass posts (Reforpost, Angelus, Londrina, PR, Brazil) were cleaned and disinfected with 70% ethanol for 30 seconds and then air-dried. Subsequently, a layer of silane (Silano, Angelus, Londrina, PR, Brazil) was applied. The posts were then fitted into the root canals at 9 mm to match the working length.

The cement was inserted with the aid of a Centrix applicator and a metal tip. The post was then positioned and held in place using finger pressure for 10 seconds, so that the excess cement could be removed with a disposable brush (Microbrush, Vigodent, Rio de Janeiro, RJ, Brazil). The tip of the light-curing equipment was positioned at the cervical end of the root at a 45° angle to its long axis. The resin cement was light-cured for 40 seconds by using a halogen light set to 450 mW/cm<sup>2</sup>, verified with a radiometer (Newdent, Ribeirão Preto, SP, Brazil). The roots were then removed from their supporting base and stored in a moist environment in a bacteriological incubator at 37 °C for 7 days.

#### Preparation of the samples for the push-out test

The samples were fixed to acrylic plates with wax so that the long axis of the root remained parallel to the surface of the plate. The plates were then fixed to a metallographic precision cutter (Buehler, Lake Bluff, Illinois, USA) fitted with a high-precision diamond disc (Buehler, Lake Bluff, Illinois, USA), and seven parallel cuts were made in the buccal-lingual direction to obtain two slices approximately 0.7 mm thick from each third: coronal, middle and apical. One of the slices was kept in distilled water, which was replaced every other day, until evaluation of bond strength 180 days later.

#### Push-out test

At time intervals of 48 hours and 180 days, the specimens were positioned on a metal base made of stainless steel, and fixed to the testing machine (DL2000, EMIC, São José dos Pinhais, PR, Brazil) for the push-out test. The metal base had an orifice 3 mm in diameter in the central region and the samples were positioned so that the part corresponding to the post was in the same direction as the orifice. A metal rod with an active 1-mm diameter tip fixed to the load cell (50 KN) was positioned over the center of the post and the push-out test was carried out at 0.5 mm/min. To enable comparison of the thirds, the Kgf value obtained was converted to MPa (MPa = KgF \* 9.8/area). The area was calculated considering the diameter of the post and thickness of the section. Therefore, area =  $2\pi^*r^*h$ , where  $\pi = 3.1416$ ; r = radius of the post and h = height of the root section.

#### Fracture Pattern

At the end of the push-out test, the specimens from each group were observed under an optical microscope, at a  $40 \times$  magnification, in order to determine the type of failure, classified as: 1) adhesive failure between resin cement and dentin; 2) adhesive failure between post and resin cement; 3) mixed failure and 4) cohesive failure.

#### Statistical Analysis

The Shapiro-Wilks normality test and the Levene homogeneity of variance test were applied to the bond strength data. As heterogeneity of variance was observed, the data were transformed using a logarithmic function, which also improved normality. The transformed data were evaluated using three-way analysis of variance for randomized blocks. In order to break down the interactions, the Tukey test was used. The level of significance adopted was 5%, using SPSS for the statistical calculations.

# RESULT

The three-way Analysis of Variance for randomized blocks did not reveal any significant triple-interaction effect (p = 0.922), nor did the pairings treatment/time (p = 0.506) or time/root third (p = 0.165). Table 2 shows the means and standard deviations of the groups in relation to root third and storage time. There was no difference between the bond strength values at 48 hours or 180 days, irrespective of treatment or root third. The treatment/third interaction was significant (p = 0.041) showing that when chlorhexidine was used, the bond strength in the coronal third was significantly lower than the values obtained for the other treatments. In the middle third, no difference in bond strength values was detected when different treatments were used. In the apical third, bond strength with ethanol exceeded the values obtained with chlorhexidine or without any treatment (Table 3). With the chlorhexidine + ethanol combination, the result in the apical third was borderline. Where no treatment was used, bond strength in the apical third was significantly lower than the values found in the other thirds, which were identical. With chlorhexidine, bond strength was significantly lower in the coronal third, whereas, when ethanol was used the lowest bond strength value was noted in the middle third. When chlorhexidine + ethanol were used, no significant difference in bond strength values was observed (Table 3).

Figure 1 illustrates the failure modes for the specimens after the push-out bond strength test and shows that for all experimental conditions, adhesive failures between the cement and dentin prevailed, equaling a failure rate of between 55 and 90%.

#### DISCUSSION

Dentin is a complex tissue as regards composition, which translates into a substantial challenge when bonding it to hydrophobic materials<sup>19</sup>. In order to check the long-term bonding performance of a dual-cure cement to root dentin in the presence of chlorhexidine and ethanol, bovine incisors were selected. This choice was based

Table 2. Means and Standard Deviations of the push-out bond strength considering treatment and root third

Treat	Mean	Std. Deviation		
		Coronal	0.85	0.78
	48h	Middle	0.96	0.71
Chlasheri Jine - Ethanal		Apical	0.75	0.83
Chiornexidine + Ethanol		Coronal	0.65	0.58
	180 d	Middle	0.61	0.78
		Apical	0.71	0.40
		Coronal	0.56	0.75
	48h	Middle	0.83	0.66
Chlankaridina		Apical	0.69	0.73
Chiornexidine		Coronal	0.29	0.65
	180 d	Middle	0.63	0.56
		Apical	0.58	0.48
		Coronal	0.84	0.72
	48h	Middle	0.67	0.77
Ethanol		Apical	0.93	0.65
Ethanoi		Coronal	0.80	0.60
	180 d	Middle	0.63	0.69
		Apical	0.82	0.67
		Coronal	0.81	0.54
	48h	Middle	0.88	0.73
No tractment		Apical	0.47	0.76
no treatment		Coronal	0.76	0.44
	180 d	Middle	0.52	0.84
		Apical	0.57	0.97

Tabl	e 3.	General	means a	nd stand	ard o	deviations	of tl	he	breakdow	n of	th	e Thir	'd and	Treatment	interac	tion
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	Core	onal	Mide	dle	Apical		
	Mean	Mean SD		SD	Mean	SD	
Chlorhexidine + Ethanol	0.75 Aa	0.68	0.79 Aa	0.76	0.73 ABa	0.64	
Chlorhexidine	0.43 Bb	0.70	0.73 Aa	0.62	0.64 BCa	0.61	
Ethanol	0.82 Aa	0.65	0.65 Ab	0.72	0.88 Aa	0.66	
No treatment	0.78 Aa	0.49	0.70 Aa	0.80	0.52 CBb	0.86	

Means followed by identical uppercase letters within each column are no different from each other. Means followed by identical lowercase letters within each line are no different from each other.



**Figure 1.** Columnar diagram of frequency as a percentage observed in the fracture modes after the push-out bond strength test according to the type of root treatment, the time elapsed between the bond strength test and test of the root thirds, namely 1) adhesive failure between cement and dentin; 2) adhesive failure between post and cement; 3) mixed failure; and 4) cohesive failure.

on the study by Kato et al.<sup>16</sup> who concluded that bovine dentin was a reliable substrate for studies involving metalloproteinase activity.

The findings of the present study demonstrated that bond stability was found throughout the studied time intervals of 48 hours to 180 days, not only in groups treated with ethanol and chlorhexidine solutions, but also in the control group, confirming the null hypothesis in relation to length of time. Storing teeth for 6 months may not be sufficient time for the hybrid layer to undergo degradation, and consequently reduce bond strength. Instead, storage for 12 months might be more effective for ascertaining the effect of time on root dentin bonding8. Furthermore, this outcome can be explained by the performance of the resin cement used. Self-adhesive resin cements contain multifunctional monomers with phosphoric acid groups, which simultaneously demineralize and infiltrate into enamel and dentin<sup>20</sup>. The setting reaction may be initiated by exposure to light or chemicals<sup>5</sup>, resulting in cross-links that form high-molecular-weight polymers. A dash of glass ionomer was added to the composition of the self-adhesive cements to guarantee neutralization of this initially acidic system, resulting in an increase in pH from 1 to 6 by means of reactions between the phosphoric acid and alkaline groups. The phosphoric acid groups also react with the apatite from the tooth structure, and

the water yielded from the neutralization processes contributes to the initial hydrophilicity of the cement, leading to a better fit to the tooth structure and tolerance to moisture<sup>20</sup>. Moreover, water is then reused via a reaction with functional acid groups, and also during the reaction of the cement, resulting in a hydrophobic matrix. The bond obtained relies upon the micro-mechanical retention and chemical interaction between the acid monomer and hydroxyapatite groups<sup>4,21</sup>.

Chlorhexidine and ethanol, the solutions used for pre-treatment of the root dentin, can affect the adhesion of luting agents, by altering the structure of the dentin or interfering in polymerization of the resin<sup>6</sup>. In the present study, the self-adhesive resin cement, in combination with ethanol, demonstrated better bond strength values in the apical third, while chlorhexidine reduced the bond strength values in the coronal third.

Ethanol-assisted bonding has been demonstrated to reduce hydrophilicity in the dentin<sup>17</sup>. The composition of resin materials should preferably be based on hydrophobic monomers, as these are more chemically and mechanically stable, resulting in greater durability of the bond to dentin<sup>17</sup>. Hydrophobicity is, however, incompatible with dentin moisture. Therefore, the use of hydrophobic resin systems demands a reduction in the hydrophilicity of the naturally moist dentin. In this regard, ethanol has been proposed as a substitute for water in dentin bonding<sup>12,17</sup>. The concept of replacing water with ethanol infers the possibility that hydrophobic monomers are capable of encapsulating the collagen fibrils in an effective and enduring manner<sup>22</sup>. Given that the bond strength of the resin cement is highest in the cervical region and lowest in the apical region, probably due to reduced light penetration into the latter scenario, this leads to a strict polymerization of the material<sup>6</sup>. In the present study, ethanol may have favored bond strength in the apical third by making the substrate less moist, thereby enabling the formation of a more hydrophobic hybrid layer. This outcome corroborated the results of Bitter et al.<sup>23</sup> who also found better bond strength in the middle and apical thirds when bonding with dual-cure resin cement in the presence of ethanol.

The use of chlorhexidine as dentin pre-treatment has been suggested, based on inhibiting the degradation of the bond interface over time<sup>18,24</sup>. Dentin contains MMPs that regulate both the physiological and pathological homeostasis of collagen-based tissues<sup>13</sup>. In a study conducted by Pashley et al.<sup>19</sup>, the action of the MMPs was inhibited by using proteinase inhibitors, thus preserving the structural integrity of the collagen fibrils, which could slow down the hybrid layer degradation. It has been shown that chlorhexidine has the desirable property of inhibiting matrix metalloproteinases -2, -8 and -9, even in low concentrations<sup>15</sup>. When chlorhexidine is applied to exposed collagen fibers and then sealed in by means of a resin bonding agent, it is capable of protecting collagen from collagenolytic attack, thereby retarding one of the degradation pathways<sup>24</sup>. In addition, chlorhexidine has been proposed as a substitute for sodium hypochlorite as a root canal irrigant during endodontic treatment, based on its bactericidal effects on the root canal<sup>8,23</sup>. The use of chlorhexidine was, however, not beneficial to the bond in the coronal third. This is not commonplace in the pertinent literature<sup>23,25</sup>, in which better bonding performance has been found in the coronal (cervical) third when compared with the middle and apical thirds. Di Hipolito et al.<sup>26</sup>, however, reported that when a resin cement was used, there was incompatibility between self-adhesive systems and chlorhexidine solutions<sup>26</sup> due to the formation of precipitates in the form of crystals containing chlorine related to the calcium-chelating properties of chlorhexidine<sup>26</sup>. According to the authors, Chlorhexidine may react with the remaining apatite crystals during the self-adhesive cement setting, by interfering in polymerization. Therefore, it should be stressed that because the

cervical region is close to the external root surface, it was more difficult to dry the coronal third when compared with the middle and apical thirds. As a result, a larger quantity of excess of chlorhexidine may have remained in the coronal third, which could have reacted with the self-adhesive resin cement, thus explaining the negative influence of chlorhexidine only in the coronal third and not in the middle and apical thirds. Furthermore, Baldea et al.<sup>27</sup> also found higher bond strength values in the middle and apical thirds when bonding fiberglass posts to root dentin. The authors reinforced that the push out bond strength in root sections has a friction component that depended on the anatomy of the root canal in the region tested. Thus, the morphology of the apical and middle thirds was close to the shape, diameter and taper of the posts, thereby improving the bond performance in these regions.

Furthermore, chlorhexidine was found to have no effect on the immediate or long-term bond strength of intraradicular posts<sup>23</sup>, even when self-adhesive cement was used<sup>8</sup>. Given these results, the null hypothesis was rejected with regard to the effect of the treatment in the different root thirds, as treatment with chlorhexidine and ethanol did influence the bond strength to the root dentin.

A failure mode analysis revealed that the majority of failures were adhesive between the cement and the dentin, corroborating the results of other studies<sup>4,26</sup>. The majority of type 2 fractures (adhesive between post and cement) occurred at 180 days, which may also be attributed to the characteristics of the self-adhesive resin cement that favor dentin bonding.

Considering the results obtained in this study, the authors suggest that the bond strength of dual-cure resin cements to root dentin might be favored by treatment with ethanol in the apical third of the root, and the use of chlorhexidine as a dentin pre-treatment still needs to be further investigated, since it reduced the bond strength values in the coronal third.

# CONCLUSION

Treatments with chlorhexidine and ethanol, either separately or combined, did not yield a significant difference between bond strength values over time. Treatment with chlorhexidine caused lower bond strength in the coronal third, and treatment with ethanol promoted higher bond strength values in the apical third of the root.

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### CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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