EFFECTS OF DENTINARY CONTAMINATION BY ENDODONTIC IRRIGANTS ON BONDING STRENGTH OF ADHESIVE SYSTEMS

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ABSTRACT

This study aimed to evaluate if 2.5% sodium hypochlorite (NaOCl) compromises the adhesion of bonding materials. The factors in the study were the irrigation solutions in two levels: 2.5% sodium hypochlorite and saline solution; and the adhesive systems used in three levels: three-step adhesive, universal adhesive, and two-step self-etch adhesive systems. The answer variable used was the microshear bond strength obtained through a universal testing machine and fracture mode. Six groups were obtained (n=10) : Saline/Etch-and-rinse (saline solution + 3-step adhesive system - Scotchbond Multipurpose, 3M ESPE); Saline/Universal (saline solution + universal adhesive system – Prime&Bond,); Saline/Self-etch (saline solution + 2-step self-etch adhesive systems - Clearfil SE Bond); NaOCl/Etch-and-rinse (sodium hypochlorite 2,5% + 3-step adhesive system - Scotchbond Multiuso); NaOCl/Universal (sodium hypochlorite 2,5% + universal adhesive system – Prime&Bond); NaOCl/Self-etch (sodium hypochlorite 2,5% + 2-step self-etch adhesive system - Clearfil Se Bond). The specimens were obtained from 60 healthy bovine incisors. The crowns were separated from the roots, and the buccal surface was regularized. The groups received saline solution and 2.5% sodium hypochlorite for 30 minutes. A matrix of 1mm and 3mm oh height was stabilized by Scotch tape to obtain the resin sticks. Afterward, the bond strength test was performed at 1mm/min speed in a universal testing machine. The data were analyzed with normality Shapiro-Wilk, two-way ANOVA, and Tukey's tests ($P < 0.001$). Etch-and-rinse and self-etch adhesives presented the highest bond strength values after irrigation with saline solution and 2.5% NaOCl, respectively ($P < 0.01$). The irrigation with 2.5% NaOCl decreased the bond strength values of etch-and-rinse and universal ($P < 0.01$). On the other hand, 2.5% sodium hypochlorite improved the bond strength values of self-etch adhesive ($P < 0.01$). The 2.5% NaOCl negatively impacted the bond strength of etch-and-rinse and universal but improved the adhesion of self-etch adhesives.

KEYWORDS: Irrigation solutions. Adhesive systems. Adhesion.
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INTRODUCTION

After the endodontic treatment, the leading cause for failure is the bacteria that survive the disinfection process or infiltration through restorative and obturation materials. Unfortunately, the gutta-percha cannot make a hermetic sealing of the root canals.[1,2] The success of the endodontic treatment does not depend only on the correct endodontic access, localization, and

preparation of the root canal system, but also on the use of chemical agents such as irrigation solutions, due to the complex anatomy of the root canals, being the mechanical action of the files limited to the main canals, thus the effective cleaning in guaranteed by irrigation solutions. [3,4]

During the instrumentation, the irrigation solutions favor the debris cleaning, the canal lubrification, and the dissolution of the organic and inorganic tissue at the same time. They guarantee a suitable antibacterial effect. Therefore, the irrigant solution is essential to endodontic treatment success.[5] The sodium hypochlorite (NaOCl) is a halogenated compound commonly used in endodontic treatments due to its great bacteriostatic and bactericide action promoted by the hypochlorous acid (HClO) formation and the subsequent chlorine liberation.[6] Also, the HClO exercises an immediate inhibitory effect on mitochondrial function and bacterial DNA synthesis. Additionally, the sodium hydroxide created by the NaOCl can dissolve the remaining pulp and the organic component of the dentin.[7]

Studies prove that the quality of the coronal restoration exercises significantly influences the global prognosis of endodontically treated teeth since 54% of failure occurs during the resettlement of the lost dental structure, i.e., the crown sealing after endodontic treatment.[8]

The possibility of restoration with composite resin and the development of bonding systems revolutionized contemporary dental practice. More preservation of the healthy dental tissue, with less invasive preparation, led to more effective survival rates of endodontically treated teeth. Besides, the restorative materials of composite resin amongst adhesive systems can transmit functional tensions through the interface bonded to the tooth, which contributes to the reinforcement of the remaining dental structure.[9]

Therefore, chemical substances used during the biomechanical preparation of root canals may alter the composition of the dentin surface and change the interaction of the adhesive bonding properties and the restorative material due to the oxidizing properties of NaOCl, [10-13] which may create an abundant oxygen layer.[14,15] This oxygen layer formed in the dentin can inhibit the resinous material's polymerization, increasing microleakage.

The structural alterations and the current state of the endodontic therapy and the restorative procedures were reviewed by Dietschi et al.[16], between 1990 and 2005, about the biomechanical considerations for restoration of endodontically treated teeth. It was observed that the vitality loss and the endodontic therapy cause only a slight alteration in the humidity of the tissue. The loss of dental tissue due to endodontic access affects only 5% of the tooth strength, while the principal strength reduction, 4% to 44% and 20% to 63%, is found after the occlusal preparation and the MOD preparation, respectively. The depth of the cavity, the isthmus width, and the cavity configuration are the most critical factors in determining dental strength reduction and fracture risk.

When the composed resin is used to restore the cavity of access of a tooth with endodontic treatment, it is fundamental to get a proper hybridization with the dentin of the pulp-chamber floor to increase the retention and the seal of the tooth crown.[15] There are, in the market, countless adhesive systems available, which are classified according to the adhesive strategy. The first adhesive strategy consists of the three-step etch-and-rinse (acid, primer and adhesive are applied in distinct steps) and two-step etch-and-rinse (the acid is applied first, and after rinsing and humidity removal, primer, and adhesive are applied in a single bottle). The second strategy is the self-etch, in which there is no previous step for etching with phosphoric acid. Thus, the two-step system (where the acid and the primer are a single solution followed by the adhesive) and the single-step system (where a single solution has the acid, primer, and adhesive function).[17,18]

Although there are studies that evaluated the influence of sodium hypochlorite in the dental surface, many are related to the fiber posts cementation,[19] (i.e., the influence of hypochlorite in the root dentin). There is a lack of information about the effect of sodium hypochlorite on the pulp chamber for direct restorative procedures. Besides, the studies that evaluate the hypochlorite effect in the dentin used microtensile tests that used preparations and cuts different from the microshear tests. There are doubts about the NaOCl irrigation's role in the adhesive strength of adhesive systems in endodontically treated teeth. Characterizing the mechanical and biological events that occurred in the context of adhesive systems will inevitably lead to the discovery of better therapeutic results that impact the quality of life of patients who need endodontic treatment. Therefore, this study aims to evaluate using microshearing tests the effect of 2.5% sodium hypochlorite on the bond strength of three adhesive systems (three-step etch-and-rinse, two-steps self-etch, and universal adhesive) to bovine dentin. The null hypothesis is that the irrigant would not interfere on the bond strength of the tested adhesive systems.

MATERIALS AND METHODS

Experimental design

The factors in the study were the irrigation solutions in two levels: 2.5% sodium hypochlorite and saline solution; and the adhesive systems used in three levels: three-step adhesive, universal adhesive, and two-step self-etch adhesive systems. The answer variable used was the microshear bond strength obtained through a universal testing machine and fracture mode.

Sample calculation and preparation

Based on previous studies, ten specimens per group were used.[19,20] Sixty healthy bovine incisors were used. The teeth were cleaned and stored in distilled water before use. The specimens were analyzed to discard those with preexisting pigmentation, fracture lines, cracks, or other surface defects that could influence the study results. After, the roots were separated from the crowns with a diamond disc under water-cooling, 2mm under the cementoenamel junction. Next, the crowns were regularized at the buccal surface with dental polishing strips of 100, 400, 600, and 800 grains until a plain and regular surface was obtained. Each tooth was fixed in a thermo-polymerizable acrylic resin inside a PVC pipe, where the dentin was facing up, while the lingual surface of the crowns was built-in the acrylic resin.

Experimental groups and list of materials

After preparing the specimens, they were randomly divided into six groups according to the irrigating solution and the adhesive system (**Table 1**). The list of materials and their composition is described in **Table 2**.

Adhesive procedures

Specimens from the groups Saline/Etch-and-rinse, Saline/Universal, and Saline/Self-etch were immersed in 0.9% solution saline solution, while NaOCl/Etch-and-rinse, NaOCl/Universal, and NaOCl/Self-etch were immersed in 2.5% sodium hypochlorite. All remained immersed for 30 minutes with the renewing of the solutions every 5 minutes. After the immersion of the specimens in their respective irrigation solutions, they were dried with air jets. Afterward, the adhesive system corresponding to each group was applied following the manufacturer's instructions.

Table 1. Experimental groups.

Group	Irrigation Solution	Adhesive System
Saline/Etch-and-rinse	Saline Solution	3-step adhesive system (Adper Scotchbond MultiPurpose)
Saline/Universal		Universal adhesive system (Prime&Bond)
Saline/Self-etch		2-step self-etch adhesive systems (Clearfil SE Bond)
NaOCl/Etch-and-rinse	2.5% Sodium Hypochlorite	3-step adhesive systems (Adper Scotchbond MultiPurpose)
NaOCl/Universal		Universal adhesive system (Prime&Bond)
NaOCl//Self-etch		2-step self-etch adhesive systems (Clearfil Se Bond)

The groups Saline/Etch-and-rinse and NaOCl/Etch-and-rinse received the application of the three-step adhesive system (Scotchbond Multiuso, 3M) following the protocol below:

1. Profilaxis (Pumice stone + distilled water);
2. Acid conditioning for 15 seconds in dentin;
3. Rinsing for 20 seconds;
4. Removal of the water excesses using absorbent paper;
5. Active primer application for 20 seconds;
6. Slight drying to evaporate the solvent;
7. Active application of the adhesive for 15 seconds;
8. Light curing for 10 seconds (VALO Cordless 1000mW/cm^2 Standard potency).

The Saline/Self-etch and NaOCl/Self-etch groups received the application of the two-step self-etch adhesive systems (Clearfil SE Bond, Kuray Dental), following the protocol below:

1. Profilaxis (Pumice stone + distilled water);
2. Primer application with microbrush for 20 seconds;
3. Smooth air jet for 5 seconds;
4. Application of two adhesive layers;
5. Light curing for 10 seconds (VALO Cordless 1000mW/cm^2 Standard potency).

The groups Saline/Universal and NaOCl/Universal received the application of the universal adhesive (Prime&Bond Universal), following the protocol below:

1. Profilaxis (Pumice stone + distilled water);
2. Active application of the adhesive for 20 seconds;
3. Slight drying to evaporate the solvent;
4. Light curing for 10 seconds (VALO Cordless 1000mW/cm^2 Standard potency).

Table 2. List of meterials, composition, and manufacturer.

Material used	Composition	Manufacturer
Flow Resin	35% in weight of multifuncional methacrylate ester; 65% in weight of inorganic particles. Methacrylic Ester; Camphorquinone; Butylated hydroxytoluene; Strontium Glass; 65,5% Silica; Sodium Fluoride; Pigment.	WAVE SDI
Adper ScotchBond MultiPurpose adhesive system	Primer: Composed by HEMA and polyalkenoic acid copolymer. Adhesive: Light Curing compound by Bis-GMA; HEMA and Camphorquinone. Activator: Compound by ethyl sulfinic acid salt solutionand Camphorquinone. Catalyst: Compound by Bis-GMA e HEMA.	3M ESPE
Clearfil Se Bond adhesive system	Primer- MDP (monômero ácido) and water. Bond- Adhesive based on Bis-GMA	KURARAY, OSACA, JAPAN
Prime&Bond Universal adhesive system	UDMA resin; Penta; R5-62-1 resin; Camphorquinone; EDAB (Ethyl Dimethyl Aminobenzoate); BHT (Butyl Hydroxytoluene); Bisphenol A Dimethacrylate Powder; Cetylamine Hydrochloride and Acetone PA.	DENTSPLY SIRONA
2,5% Sodium Hypochlorite	Caustic soda (NaOH) – 2.5 %; Sodium Chloride (NaCl); Water; Sodium Hypochlorite.	ASFER
0.9% Saline Solution	0.9% NaCl in mass dissolved in 100ml of distilled water.	MATERVET

Sample preparation for microshearing

A matrix with 1mm dimensions of diameter for 3mm of height made from silicon was positioned over the tooth surface and stabilized by Scotch tape (Durex, 3M), allowing the delimitation of the bonding area between the adhesive and the dental surface. After positioning the matrix in the dental surface with Scotch tape, a perforation in the Scotch tape was made through an explorer probe to release the matrix hole, thus allowing the filling with Flow resin (Wave resin, SDI) after the light-curing was made. After the polymerization, the matrix was removed gently, avoiding possible fractures in the resin sticks. In each specimen, three resin Flow sticks were made (incisal, middle, and cervical third) with the same diameter and height.

Bond strength test

The plastic cylinders with the built-in samples were positioned using a template to standardize the specimen positioning in the universal testing machine. A stainless-steel handle with a 0.2 mm section was put around the resin sticks parallel to the bonding interface. The microshear test was performed at a 1mm/minute velocity until a failure happened. The data were obtained in kg/cm^2 and transformed into MPa.

Fracture pattern analysis

The fracture mode was analyzed in an optical microscope with a magnification of 40x. Each specimen was classified according to the fracture pattern.

- Adhesive failure – in the adhesive layer
- Cohesive failure in the resin - in the resin
- Cohesive failure in the dentin - in the dentin
- Mixed failure - two or more interfaces involved

Statistical analysis

The results were submitted to the test of normality of Shapiro-Wilk. Next, the data were analyzed by ANOVA two-way and Tukey's tests ($P < 0.001$).

RESULTS

Table 3 presents the values of bond strength test. After immersion of the specimens in 0.9% saline solution, the highest bond strength values were obtained with Socthbond Multipurpuse ($P < 0.001$). Cearfil SE and Prime&Bond showed similar bond strength values ($P = 0.905$). When the specimens were immersed in 2.5% sodium hypochlorite, Clearfil SE showed the highest bond strength mean values ($P < 0.001$), and no differences were observed between Scotchbond Multipurpuse and Prime&Bond ($P = 1.000$). The immersion in 2.5% NaOCl descreased

Table 3. The means and standar deviations (in MPa) after the bond strength test.

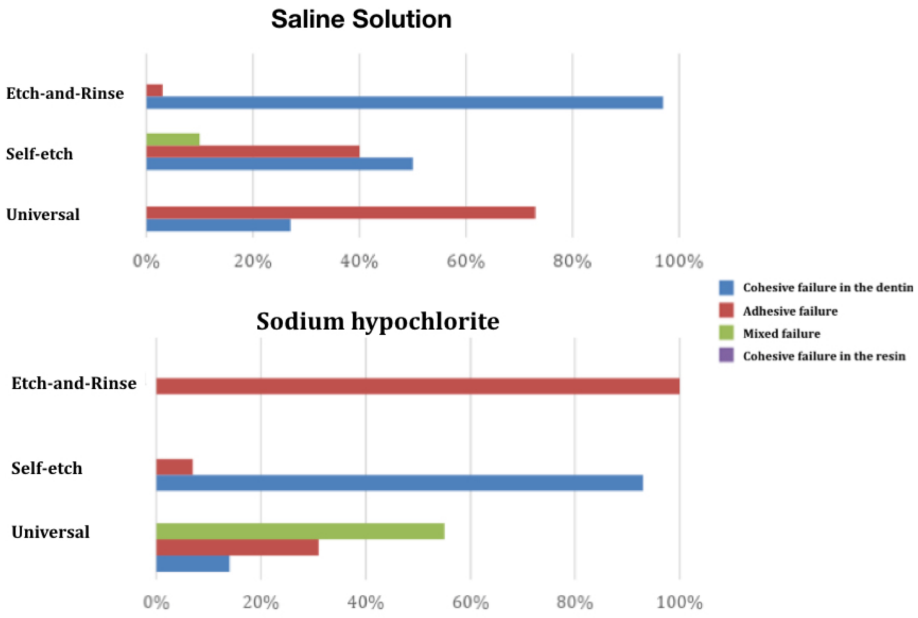
Groups	Etch-and-Rinse (Adper Scotchbond)	Self-etch (ClearFil SE)	Universal (Prime&Bond)
0.9% saline solution	23.21 ± 6.01 ^{Aa}	13.93 ± 4,01 ^{Ab}	13.06 ± 2,86 ^{Ab}
2.5% sodium hypochlorite	6.95 ± 0.69 ^{Bb}	21.67 ± 3.29 ^{Ba}	6.92 ± 0.82 ^{Bb}

Different lowercase letters on the same line indicate statistical difference (p<0.001). Different uppercase letters in the same column indicate statistical difference (p<0.001).

the bond strength values of Scotchbond Multipurpose and Prime&Bond but increased the Clearfil SE values ($P < 0.001$).

A predominance of dentin cohesive failures was observed in the Scotchbond Multipurpose group (97%) and Clearfil SE (50%) after immersion in 0.9% saline solution. Prime&Bond/saline presented a predominance of adhesive failures (65%) (**Figure 1**). After immersion in 2.5% NaOCl, all failures of the Scotchbond Multipurpose group were adhesive (100%), while the Clearfil SE group showed 93% of dentin cohesive failures. The Pime&Bond/NaOCl group presented mixed (55%), dentin cohesive (14%), and adhesive (30%) failures (**Figure 1**).

Figure 1. Fracture pattern in the groups that used 2.5% NaOCl and saline solution as irrigant.



DISCUSSION

The results of this laboratory study rejected the null hypothesis that there would not be an influence of the irrigant on the bond strength of the tested adhesives systems (three-step, two-step self-adhesive, and universal) to the bovine dentin. The microshear bond strength test was used to allow the test in little adhesive areas, enabling multiple specimens in the same tooth, as the microtensile test, however, without the sectioning procedures that perhaps would have allowed inducing a premature weakening of the adhesive interface before the mechanical tests.[21]

In this study, the two-step self-etch adhesive system (ClearFil SE) showed greater bond strength than the three-step and the single bottle universal adhesive system after the irrigation of the dentin with 2.5% NaOCl. This result agrees with previous studies.[15,22,23] The findings could be justified because ClearFil SE Bond is considered a self-etch system, which dismisses the use of phosphoric acid in the dentin, having as a trait a mild pH of around 2.7. Besides, its monomer MDP (10-Methacryloyloxydecyl dihydrogen phosphate) decalcifies, penetrates, and creates a chemical bond with the calcium ions and the hydroxyapatite, allowing double bonding simultaneously (i.e., a micromechanical and chemical bonding).[24] The calcium salt created by the phosphate monomer is highly insoluble. Thus, according to the adhesion-decalcification concept, the less soluble the calcium salt of an acid molecule is, the more intense and stable the molecular adhesion is, showing more excellent stability in an aqueous medium.[25]

The acidic functional monomer 10-MDP was present in this study only for the adhesive system ClearFil SE, absent in the other adhesive used. Consequently, the adhesive superiority observed after the dentin was bleached with 2.5% NaOCl may be attributed to the functional monomer, suggesting the MDP-Ca salt formation. These salts show excellent stability, strength to hydrolysis, and high longevity, granting the bonding interface more excellent stability and longevity.[11,26,27]

Regarding the influence of the irrigating solution used in this study, sodium hypochlorite is the most studied irrigant used during root canal preparation. NaOCl has the unique capacity of dissolving pulp remains and the organic component of the smear layer. In an aqueous solution, a dynamic chemical balance is found, where it may be presented as a non-dissociated salt, originating the other substances, or being presented as totally dissociated.[28] The chemical reactions between the tooth tissue components and the sodium hypochlorite may be responsible for the tissue dissolution. The sodium hydroxide formation is a powerful organic and fat solvent creating soaps (saponification); the hypochlorous acid is an antimicrobial agent due to its release of nascent chlorine that combines with the amino group of the proteins, forming the chloramines and releasing the free chlorine and nascent oxygen. These combined reactions can neutralize the medium acidity, making it improper for bacterial development.

Perhaps the pH alterations justify the ClearFil SE superiority, suggesting that this is due to its light conditioning effects, which would result in fewer alterations in the dentinal wall than the strong acids

used in the three-step adhesive system (Scotchbond Multipurpose) that may have been neutralized.[23] However, when the results were observed after being immersed in 0.9% saline solution, the ScotchBond Multipurpose showed better behavior than the other adhesives used ($P < 0.001$). This result may be attributed to the more significant demineralization effect because of the use of phosphoric acid at 37%, which increases the porosity and the opening of the dentin tubules, being able to take a significant number of resinous tags, with the possibility of side branches in the peritubular dentin. The complete removal of the smear layer and smear plug may contribute to greater adhesive effectiveness than the self-etch adhesive systems that do not eliminate this smear layer, where only an interaction happens.

On the other hand, the significant reduction of the adhesive strength attributed to the three-steps (Scotchbond Multipurpose) and the universal (Prime&Bond) adhesives after immersion with hypochlorite is according to previous studies that attribute the differences in the sensibility of the adhesive system to the oxidizing effect of NaOCl.[29,30] In this context, omitting the conditioning stage of the self-etch adhesive system was a suggestion of Machinck et al.[31] and Tay et al.[32], who hypothesized that endodontic irrigation with a demineralizing and chelating effect could produce a bond surface like the acid conditioning. These hypotheses diverged from the findings of Fawzi et al.[33] The discrepancy in the Machinck et al.[31] results may be attributed to the differences in the irrigation protocols and the nature of the examined substrate.

The results of this study agree with previous findings.[2,26,34,35,36,37] The bond strength is compromised because sodium hypochlorite is an oxidizing substance that takes to potent inhibition of the polymerization in the adhesive-dentin interface.[28,35,36] Besides, the treatment with sodium hypochlorite reduces the mechanical properties of the dentin, such as the flexion, traction, and microhardness resistance, producing a weakened composed dentin-resin interface.[11,38] One study showed loss of the demineralized peritubular dentin and a decrease of the intertubular dentin area after using sodium hypochlorite.[27]

Although bovine teeth were used for the bond strength tests in the dentin to microshear, the possibility of the reason being related to the specimens is discarded, once many authors[39,40,41] applied physical and chemical tests comparing the bovine and human substrate. It was concluded that the bovine substrate suits the researcher's needs for the adhesion test. Furthermore, the preparation of the specimens for the microshear test allowed a more significant similarity of the morphological traits of the pulp chamber since cuts in the occlusal surface is made for the microtensile test, simulating the characteristics of a class I preparation.

There are already studies that found methods of reversing the collagen fibers compromising that occurred during an endodontic treatment due to the hypochlorite irrigation. These denominated natural antioxidants restore the redox potential of the oxidized dentin substrate leading to a typical polymerization of the composite.[42] Currently, the literature shows that the treatment with natural antioxidants as grape seed, green tea, and tannic acid extract may bring satisfactory results in the bond strength recovery to the dentin treated with sodium hypochlorite, becoming an alternative to the immediate use after the endodontic treatment before the adhesive system application and restoration.[11,26]

Therefore, because of the immediate restorative need after endodontic treatments that used irrigation solutions based on sodium hypochlorite, self-etch adhesive with functional monomers based on MDP is suggested.

Because of the inherent limitations of this study, it is possible to highlight that other irrigation solutions, such as EDTA (ethylenediaminetetraacetic acid) and chlorhexidine, could be tested with the same methodology, being also possible to observe the antioxidant or surface treatment solutions to evaluate the possibility of reversion of the effects of the endodontic irrigation solutions action.

CONCLUSION

After dentin immersion, the three-step adhesive system presented the highest bond strength values in 0.9 % saline solution. However, when 2.5% sodium hypochlorite was used, the two-step self-etch adhesive system with functional monome 10-MDP presented better results. NaOCl negatively impacted the three-step (Scotchbond Multipurpose) bond strength and the universal (Prime&Bond) adhesives. On the other hand, it improved the two-step self-etch adhesive system (Clearfil SE).

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