Effect of Calcium Hypochlorite and Sodium Hypochlorite as Root Canal Irrigants on Push-out Bond Strength of Fiber Post Cemented with Etch-and-Rinse Resin Cement: An in Vitro Study

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ABSTRACT

Objectives: Calcium hypochlorite (CH) has been recently used as a root canal irrigant. The aim of the present study was to compare the effect of CH and sodium hypochlorite (SH), as root canal irrigants, on the push-out bond strength of fiber posts cemented with an etch-and-rinse resin cement.

Materials and Methods: In this experimental in-vitro study, 40 human anterior teeth with similar root lengths were randomly divided into five groups (N=8) according to the protocol of root canal irrigation as follows: group 1: saline (control); group 2: 2.5% SH; group 3: 5.25% SH; group 4: 2.5% CH; group 5: 5% CH. Before post placement, the post space was irrigated using the same irrigation protocol, and after that, they were irrigated by distilled water. Fiber posts were cemented with All-Bond 3 bonding and Dou-Link Universal cement. After thermocycling (1000 cycles, 5-55°C), a push-out test was performed, and data were analyzed using one-way analysis of variance and Tukey's post-hoc test with SPSS version 23 (α=0.05).

Results: The highest and lowest mean bond strengths were detected in groups 2 and 5, respectively. There was no significant difference between group 1 and the SH groups (P>0.05), but the difference between group 1 and the CH groups was significant (P<0.001). There was a significant difference between SH groups and CH groups (P<0.001).

Conclusion: Compared to SH, as a root canal irrigant, CH decreased the push-out bond strength of fiber posts cemented with an etch-and-rinse resin cement.

Keywords: Calcium Hypochlorite; Resin Cements; Dental Bonding; Root Canal; Irrigation; Sodium Hypochlorite

INTRODUCTION

Disinfecting and cleaning the root canal system from microbial flora and pulpal tissue are prerequisites for a successful root canal treatment [1]. Although root canal shaping can be efficiently attained with advanced instrumentation technology, effective cleaning of the entire root canal system remains a challenge [2]. The most favorable properties of irrigants are their flushing action, tissue dissolving, antimicrobial activity, and low toxicity [3]. Sodium hypochlorite (SH) is the most commonly used root canal irrigant during the
Chemo-mechanical debridement of root canals because of its broad antimicrobial effect [4] and its ability to promote organic tissue dissolution [5]. However, SH is highly irritating in contact with periausal tissues [6], reduces the resistance of teeth to fracture [7], and may adversely affect bond strengths [8]. Posts and cores are frequently used in endodontically treated teeth that have suffered the excessive loss of coronal tooth structure [9]. Fiber-reinforced composite posts are probably the most interesting choice for clinical use because their modulus of elasticity is similar to that of dentin, which allows equal distribution of forces on root canal walls [10]. Common complications of luted endodontic posts include the debonding of the post and endodontic lesions [10]. Therefore, luting of posts inside the root canal is still a challenge because of the high cavity configuration factor (C-factor) inside the root canal [11], limited access and visibility, moisture, and deposition of cementum and secondary dentin [12]. After post space preparation, the dentin walls are covered with a heavy smear layer containing rough debris and remnants of sealer and gutta-percha that may hamper bonding to the root canal dentin [13]. Consequently, irrigation after post space preparation and its effects on the bond strength of different adhesive strategies are still of interest, in particular, because the manufacturers’ recommendations vary from using SH to no recommendations at all.

Because of the adverse effects of this irrigant, researchers have developed alternative endodontic irrigants. The search for new irrigation protocols and chemical solutions had led to experimental studies using calcium hypochlorite (CH). SH and CH, which belong to the same family of chemicals, are primarily used as bleaching agents or disinfectants. In industry, CH has been traditionally used for disinfection and purification of water and milk [14]. Unlike SH, CH can be easily stored in powder form without losing its stability [15].

In a preliminary study, no differences were found among the tissue dissolving properties of 5% or 10% CH and 4.65% SH (Chlorax solution) or 1.36% SH solutions after 60 minutes [5]. Furthermore, it was revealed that similar to 10% SH, 10% and 15% CH could not cause any difference in microleakage when used as pretreatment agents before an acetone-based adhesive system [16]. In another study, Gürduysus et al [17] showed that neither SH nor CH was effective in removing the smear layer and dentinal debris. There are limited investigations on the antibacterial efficacy of CH in the endodontic field [18, 19]. Recently, the antibacterial efficacy of 2.5% CH and 2.5% SH in the elimination of Enterococcus faecalis (E. faecalis) from contaminated root canals was investigated. It was found that CH had better antibacterial activity than SH when agitated with ultrasonic energy [18]. In another study, Schmidt et al [19] indicated that 2.5% CH can be effectively used for the decontamination of gutta-percha points. According to Sedigh-Shams et al [20], CH at a minimum inhibitory concentration of 5% was effective in eliminating E. faecalis in the planktonic state and exhibited comparable cytocompatibility to that of 0.5% SH, and the cytotoxicity of 5% CH and 0.5% SH was similar.

In another study, Leonardo et al [21] showed that CH solutions are extremely alkaline and tend to have more available chlorine content than SH, but they have a higher surface tension than SH. Regarding the available chlorine content, these solutions tend to be stable up to 30 days of storage when kept at 4°C or 25°C temperatures. Because of limited investigations about CH in dentistry, the aim of the present study was to compare the CH and SH, as root canal irrigants, on the push-out bond strength of fiber posts cemented with an etch-and-rinse resin cement. Thus, the null hypothesis of the study was that CH and SH, as root canal irrigants, compared to NS (control), do not decrease the bond strength of fiber posts cemented with an etch-and-rinse resin cement.

**MATERIALS AND METHODS**

**Specimen preparation:** In this experimental in-vitro study, 40 extracted anterior maxillary human teeth with fully formed apices, a single canal, and without caries, cracks, or previous endodontic treatments were selected. The teeth were stored in a 0.2% thymol solution at room temperature for 3 months, and external debris was removed using a hand scaler. The coronal portion of each tooth was sectioned at 1 mm below the cementoenamel junction with diamond disks (Ref. 070, D&Z, Berlin, Germany) to achieve a...
uniform length of 13mm. The teeth were randomly divided into five groups (N=8) according to the protocol of root canal irrigation as follows: group 1: saline (control); group 2: 2.5% SH; group 3: 5.25% SH; group 4: 2.5% CH; group 5: 5% CH. The root canals were prepared using #15-35 files (Mani Inc., Tochigi, Japan) and #2-3 Gates Glidden drills (Mani Inc., Tochigi, Japan).

Irrigation was performed using 1ml of specific irrigation solution for each group after every change of file size. Finally, canals were irrigated using distilled water. Then, they were dried by absorbent paper points (AriaDent, Tehran, Iran) and filled using the cold lateral condensation technique with gutta-percha points (AriaDent) and a silver-free resin-based endodontic sealer (AH26, Dentsply DeTrey GmbH, Konstanz, Germany). Then, the endodontically treated teeth were coronally sealed with composite restorative material (Denfil Flow, Vericom, Anyang, Korea) and apically with wax, and were stored in water at 37°C for one week to allow the endodontic sealer to set.

**Cementation procedure:**
Gutta-percha was removed from the coronal portion of each root with a #3 Gates Glidden drill (Mani Inc., Tochigi, Japan) leaving 3mm of gutta-percha in the apices for adequate apical seal. Then, 10mm-long post spaces were prepared to receive prefabricated tapered fiber-reinforced posts (911 series; Micro.Medic, Robbio PV, Italy; Table 1). The root canals of 40 teeth were enlarged using a slow speed drill provided by the manufacturer of the fiber post system. Before post placement, the post space was irrigated using the same irrigation with 5ml for one minute followed by 5ml of distilled water. Then, the canals were dried using absorbent paper points. The posts were immersed in alcohol for one minute to remove residues and oil. After drying, one layer of bonding agent (All-Bond3, Bisco Inc., Schaumburg, IL, USA) was applied to the canal surfaces using micro-brushes, dried with paper points to remove the excess bonding agent, and then, gently air-dried and cured for 10s. The cement (Universal Duo-Link, Bisco Inc., Schaumburg, IL, USA) was inserted with a Centrix syringe and needle head (Centrix, DFL Ind. e Com. S.A., Rio de Janeiro, RJ, Brazil).

After post insertion, finger pressure was applied for 30s to assure the complete seating of the posts. The posts were light-cured for 40s after the excess cement was removed. A 2mm layer of composite resin (DenFil™, Vericom Co., Korea) was placed over the root access and light-polymerized for 40s. All roots were stored at 37°C and 100% humidity for 24h. Then, all specimens underwent 1,000 thermo-cycles at 5-55°C with a dwell time of 30s (Thermo-cycling Machine, Delta Tpro2, Mashhad, Iran).

**Push-out test:**
All the prepared roots were mounted in acrylic resin (Marlic Medical Industries Co., Tehran, Iran). Three sections were obtained from the middle part of the root, and a push-out test (Universal Testing Machine, LFM-L, PCS1000, Walter + Bai AG, Switzerland) was performed for 24 test specimens (8 root specimens x 3 sections) in each subgroup at a crosshead speed of 0.5 mm/minute.

Each slice was positioned on the loading machine with the apical aspect facing the plunger to assure the application of the force in the apical-coronal direction to move the post towards the larger part of the slice. The plunger was positioned so that it only contacted the post on loading, introducing shear stresses to the bonded interfaces. Bond failure was manifested by the extrusion of the post segment from the root slice. To express the bond strength in megapascal (MPa), the failure load, recorded in Newton (N), was divided by the area of the bonded interface in square millimeters. The area of the bonded interface was calculated as the lateral surface area of a truncated cone using the following formula:

\[ A = (R + r) \sqrt{h^2 + (R-r)^2} \]
Table 1. Materials used in this study and their chemical compositions and instructions of application

| Product                           | Company                                      | Composition                                                                 | Instructions                                                                 |
|-----------------------------------|----------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Glass fiber post (90V;911 series)-Conical type 2 | Micro. Medica, Robbio PV, Italy             | Glass fiber + epoxy resin matrix                                           | Clean with ethanol before cementation                                       |
| Duo-Link universal resin cement (Paste-paste dual syringe, automix) | Bisco Inc., Schaumburg, IL, USA             | Base: Bis-GMA, TEGDMA, urethane dimethacrylate, glass filler Catalyst: Bis-GMA, TEGDMA, glass filler | Etch and bond intracanal dentin and inject into canal                      |
| All-Bond 3 (universal adhesive system; Part A and B) | Bisco Inc., Schaumburg, IL, USA             | Part A: Ethanol, NTG-GMA salt                                             | Etch the canal using Uni-Etch for 15s, rinse thoroughly and remove excess water with a brief burst of air and paper point. Dispense an equal number of drops of All-Bond 3 Parts A and B (1:1), mix well for 5s. Remove excess pooling, thoroughly air dry. Apply one layer of All-Bond 3 to the post and air-dry, light cure for 10s |
| AH 26 silver-free powder AH resin  | Dentsply DeTrey; Konstanz, Germany           | Bismuth oxide, Methenamine Epoxy resin                                   | Powder and liquid are mixed on a glass slab using a metal spatula. 2 to 3 volume units of powder are mixed with 1 volume unit of resin. |
| Calcium hypochlorite              | Merck, Darmstadt, Germany                    |                                                                            | Powder of Calcium hypochlorite is mixed with distilled water to prepare of 5 and 2.5% solutions |
| Sodium hypochlorite               | Raga, Iran                                   |                                                                            |                                                                            |

Where A represents the area of the bonded interface, R denotes the coronal post radius, r refers to the apical post radius, and h shows the thickness of the slice (millimeters). The diameter of the post and the thickness of the slice were individually measured using a digital caliper (Mitutoyo digital caliper 500-714-10 Mitutoyo Co., Tokyo, Japan) with 0.01mm accuracy. All debonded specimens were analyzed using a stereomicroscope (x40, Vanox, Olympus, Tokyo, Japan), and the fracture mode was classified as an adhesive between post/cement (AP) or between cement/dentin (AD) and mixed (M). The mean bond strengths were analyzed with one-way analysis of variance and post-hoc Tukey's test. Fisher’s exact and chi-square tests were used to compare the frequency of failures (α=0.05) utilizing SPSS software (SPSS v.23, SPSS Corp., Chicago, IL, USA). Moreover, one prepared root in each group was cross-sectioned for scanning electron microscopic (SEM) analysis. The samples were dehydrated in ascending concentrations of ethanol (50, 70, 95, and 100%) for 1.5h and embedded in acrylic resin. The surfaces were polished with 600-, 800-, 1,000-, and 1,200-grit silicon carbide papers under running water. Between each polishing step, the specimens were put in an ultrasonic device for 15 minutes. The exposed interfaces were treated with 6N hydrochloric acid for 35s followed by a 10-minute immersion in 2.5% sodium hypochlorite (NaOCl). After 15 minutes of ultrasound treatment, the specimens were dehydrated for 36h and affixed to an aluminum mounting stub, and finally sputter-coated with platinum-gold for SEM analysis.

RESULTS

The mean bond strength values in the control and experimental groups are presented in Table 2. Groups 2 (SH 2.5%) and 5 (CH 5%) showed the highest (9.01±1.38MPa) and lowest bond strengths (5.01±1.00MPa), respectively. There was no significant difference between the controls (group 1) and the SH groups (groups 2 and 3). However, the difference between the controls (group 1) and
Table 2. Descriptive statistics of the push-out strengths (MPa) in each experimental group

| Group     | Mean (SD) | SE       | 95% CI Lower Bound | 95% CI Upper Bound | Min   | Max   |
|-----------|-----------|----------|--------------------|--------------------|-------|-------|
| 1 Saline (control) | 8.29±1.07 | 0.22     | 7.83               | 8.74               | 6.46  | 10.08 |
| 2 2.5% SH   | 9.01±1.38 | 0.28     | 8.43               | 9.59               | 6.54  | 10.97 |
| 3 5.25% SH  | 8.07±1.34 | 0.27     | 7.50               | 8.63               | 6.33  | 10.94 |
| 4 2.5% CH   | 5.14±1.21 | 0.25     | 4.63               | 5.66               | 3.46  | 6.84  |
| 5 5% CH     | 5.01±1.00 | 0.20     | 4.58               | 5.43               | 3.24  | 6.63  |
| Total      | 7.10±2.07 | 0.19     | 6.73               | 7.48               | 3.24  | 10.97 |

SH: Sodium hypochlorite; CH: Calcium hypochlorite; CI: Confidence Interval for the Mean; SD: Standard Deviation, SE: Standard Error. Data with different lowercase letters indicate significant differences (P<0.05, Tukey's test).

Table 3. P-values of bond strength between groups

| Groups     | Saline | 2.5% SH | 5.25% SH | 2.5% CH | 5.25% CH |
|------------|--------|---------|----------|---------|----------|
| 2.5% CH    | 0.0237 | 0.0969  | <0.001   | <0.001  |          |
| 5% CH      |        |         | <0.001   | <0.001  |          |

S: Saline; SH: Sodium hypochlorite; CH: Calcium hypochlorite

Table 4. Percentage of failures recorded in each group

| Groups     | AP  | AD  | M   | Total |
|------------|-----|-----|-----|-------|
| Saline     | 16.66 | 8.34 | 75  | 100   |
| 2.5% SH    | 41.68 | 4.16 | 54.16 | 100   |
| 5.25% SH   | 50   | 12.5 | 37.5 | 100   |
| 2.5% CH    | 4.16 | 70.84 | 25  | 100   |
| 5% CH      | 12.5 | 45.84 | 41.66 | 100   |

SH: Sodium hypochlorite; CH: Calcium hypochlorite; M: Mixed, AP: Adhesive between post/cement; AD: Adhesive between cement/dentin.

Table 5. P-values of failure modes between groups

| Groups     | Saline | 2.5% SH | 5.25% SH | 2.5% CH | 5.25% CH |
|------------|--------|---------|----------|---------|----------|
| 2.5% CH    | 0.204* | 0.017*  | P<0.001  | 0.015*  |          |
| 5% CH      |        |         | <0.001   | 0.002** |          |

S: Saline; SH: Sodium hypochlorite; CH: Calcium hypochlorite *Fisher's exact test; **Chi-square test.

The results of the failure modes are summarized in Tables 4 and 5. The SEM photomicrographs are shown in Figures 1 and 2. In group 5, the resin tags are apparently larger.

DISCUSSION

In this study, the effects of CH and SH, as root canal irrigants, on the push-out bond strength of fiber posts cemented with an etch-and-rinse resin cement were compared. The hypothesis of the present study was partly rejected because there were significant differences between the bond strengths of the control
group and CH groups and between CH and SH groups. Irrigating solutions are used in endodontics to complete the mechanical action of endodontic instruments in the cleaning and disinfection of root canals [22]. They act by causing physical and chemical changes in the ultra-structure of the root dentin, thus decreasing the micro-hardness and increasing dentin permeability [23,24]. Concerning the intensity of the effects of these solutions, there is a direct correlation between the concentrations of the irrigants and the changes in the dentin substrate [25].

Both decreases [26,27] and increases [28] in the bond strength have been reported following the application of SH to acid-etched dentin. Some dentin-bonding systems showed no compromised bond strength after pretreatment with SH [27,29,30], as we presented in this study. The decrease in the bond strength was explained by the change of the redox potential of the bonding substrate due to the residual SH [27] while the increase was explained by the removal of the ‘collagen smear layer’, which resulted in the rough surface of the dentin [28].

In the present study, the lack of adverse effects on the bonding was probably because of less SH remaining at the bonding interface due to using an etch-and-rinse cement. Using 5.25% SH as an irritant has extreme cytotoxicity [31]. Sedigh-Shams et al [20] indicated that the cytotoxicity of 5% CH and 0.5% SH was similar. SH 5.25% showed higher pH compared to 5.25% CH. Oliveira et al [32] showed that there were no significant differences in dentin roughness between CH and SH at the same concentrations. CH and SH, both at 5%, significantly changed dentin roughness.

CH concentration ranging from 1% to 2.5% could represent suitable solutions for root canal irrigation protocols. CH showed lower concentrations of available chlorine than SH, but according to the study conducted by Leonardo et al [21], CH solutions are extremely alkaline and tend to have more available chlorine content than SH. The high concentration of active chlorine in the solutions, which likely causes increased degradation of the organic portion of the root dentin, results in a higher dentin permeability and roughness [33]. The tested hypothesis is that high concentrations of CH and NaOCl significantly increase dentin permeability and may lead to greater sequestration of calcium ions and surface demineralization. These results support the hypothesis that 5% CH could act as a chelating solution causing erosion of the canal walls. On that account, the use of CH and SH at concentrations greater than 5% should be re-evaluated as an irrigating solution because it significantly changes dentin roughness and could prevent the adhesion of endodontic sealers to dentin.

The changes in the permeability and roughness of root canal dentin could provide an extended area of dentin surface for the adhesion of endodontic sealers [34]. However, these large areas may contain voids, which interfere with the spreading of the sealer on the dentin surface, compromising the interface [35]. These results support the reason that CH, as a root canal irrigant, decreased the bond strength of fiber posts cemented with an etch-and-rinse resin cement. If the collagen exposed by acid-etching cannot be adequately filled by adhesives, cathepsins and metalloproteinases can be activated by phosphoric acid, causing degradation [36]; they can also cause leakage and decrease the bond strength. Calcium hypochlorite deposits can be among these barriers. Perdigao et al [26] reported that the increase in the SH application time resulted in a progressive decrease in the shear bond strength. After the long application of SH, the residual SH in the porous dentin could result in incomplete polymerization of resin monomers at the adhesive-dentin, which contributes to the decrease in the bond strength. This can also be true for CH, which is a family of hypochlorites.

In the present study, according to the failure modes, the lowest rate of adhesive failures between dentin and the luting agent have been reported in groups 1 and 3, which confirm the results of the higher bond strength in the two groups while most of the adhesive failures between dentin and the luting agent have been reported in groups 4 and 5, which confirm the results of the lower bond strength in these two.
groups. Moreover, according to the SEM evaluation (Fig. 1 and 2), the highest penetration of the resin in the dentinal tubules was observed in the 5% CH and 5.25% SH groups, and these observations were consistent with the results obtained by Estrela et al [35], showing that superficial dentin roughness due to 5% CH and SH is higher than that due to 2.5% CH and SH. In this study, although the length of resin tags is long in the 5% CH group, the bond strength has decreased. Hybrid layer thickness and resin tag morphology contribute only slightly to the bond strength; the reduced bond strength to the intraradicular dentin is possibly because of factors other than substrate morphology, including the challenges posed by limited endodontic space and high C-factor [36].

CONCLUSION
Considering the limitations of this study, compared to SH, as a root canal irrigant, CH decreased the push-out bond strength of fiber posts cemented with an etch-and-rinse resin cement.

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CONFLICT OF INTEREST STATEMENT
None declared.

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