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Effect of sodium and calcium hypochlorite with or without surfactant on the adhesion of epoxy resin-based endodontic sealer

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Aim: To evaluate the effect of sodium hypochlorite (NaOCl) and calcium hypochlorite [$Ca(OCl)_2$] in the presence or absence of surfactant benzalkonium chloride (BAK) on the bond strength of an epoxy resin-based sealer to root dentin. **Methods:** Fifty decoronated permanent human maxillary lateral incisors, with a single main canal and complete root development, were divided into 5 groups (n=10) according to the irrigant: 0.9% sodium chloride (NaCl), (control); 2.5% NaOCl; 2.5% NaOCl + 0.008% BAK; 2.5%$Ca(OCl)_2$; and 2.5% $Ca(OCl)_2$ + 0.008% BAK. Irrigation was performed using the syringe and needle. The root canal was prepared with 40.06 nickel-titanium instruments, under irrigation with 20 mL of the solution corresponding. Roots were filled using the single cone technique with gutta-percha and epoxy resin-based sealer. A slice from each third was obtained and subjected to the push-out test by applying an apical-coronal force until failure. Data were analyzed by post-hoc pairwise comparisons were performed using the Kruskal-Wallis test adjusted using the Bonferroni method (α = 0.05). **Results:** The groups treated with 2.5% NaOCl and 2.5% $Ca(OCl)_2$ showed bond strength similar to the control group ($p > 0.05$). The use of an additional 0.008% of BAK was able to increase the bond strength after the use of 2.5% $Ca(OCl)_2$ ($p < 0.05$) and did not change the bond strength after the use of 2.5% NaOCl ($p > 0.05$). **Conclusions:** The NaOCl and $Ca(OCl)_2$ exhibited comparable bond strength values. Nevertheless, when the surfactant BAK was addition into both solutions, only $Ca(OCl)_2$ demonstrated an increase in adhesion.

Keywords: Benzalkonium compounds. Calcium oxychloride. Endodontics. Epoxy resins. Sodium hypochlorite.

Introduction

The root canal system presents a challenging internal configuration, featuring lateral canals, isthmuses, branching, and deltas, areas that are not readily accessible to instrumentation[1]. In this sense, root canal irrigation is indispensable for the success of endodontic treatment, due to the use of antimicrobial chemical substances to enhance disinfection and reach inaccessible areas[2]. An effective irrigating solution for endodontic purposes should exhibit a wide antimicrobial spectrum, act on endotoxins, have the capacity to dissolve organic matter [2,3], and maintain a low surface tension[4].

Sodium hypochlorite (NaOCl) is the main irrigant used in endodontic clinical practice, as it has excellent antimicrobial action and the ability to dissolve organic tissue[3]. However, its great chemical instability can negatively impact the desired characteristics of the irrigant[5]. Due to this inconvenience, new solutions have been investigated as an alternative, with an emphasis on calcium hypochlorite [$Ca(OCl)_2$][6-7]. $Ca(OCl)_2$ is a granulated powder with a greater amount of available chlorine and greater stability[7]. In addition, it has antimicrobial action and tissue dissolution capacity similar to NaOCl[8]. As advantages, $Ca(OCl)_2$ seems to cause less structural changes in dentin[9], does not have adverse effects on adhesive procedures[10], and does not produce toxic by-products when it interacts with other solutions used in Endodontics[11]. Nevertheless, both solutions (NaOCl and $Ca(OCl)_2$) have a relatively high surface tension, limiting their reach in anatomical complexities[7]. Additionally, it's worth noting that neither of these solutions excels in removing debris and the smear layer from the canal walls, making it necessary to use a chelating solution afterward[12]. Consequently, the irrigation protocol also plays a significant role and generates reflections on the filling, influencing the interaction of the endodontic sealer with the dentin substrate, which can directly affect the bond strength of the material to dentin[13].

An alternative to improve the efficiency of irrigants is the addition of a surfactant agent in its composition[14], giving the solution a lower surface tension, and consequently, deeper penetration into the root canal system[15]. Benzalkonium chloride (BAK) is a cationic surfactant capable of reducing the surface tension values of NaOCl and $Ca(OCl)_2$[16]. In addition, the use of BAK can increase the antimicrobial potential of the solutions, through changes in the resistance of the bacterial cell membrane[17] and delay in the initial bacterial adhesion to dentin[18]. In this sense, the potential for improvement conferred on irrigating solutions makes BAK a promising compound for clinical applicability, and for this reason the body of evidence available on its use is constantly growing.

Despite the aforementioned presupposes, the influence of NaOCl containing a surfactant on the bond strength of endodontic sealers to root dentin has been the subject of only one previous study[19]. Furthermore, to our knowledge, there are no previous studies on the influence of $Ca(OCl)_2$ alone or containing a surfactant for the same outcome. Therefore, this study aimed to evaluate the effect of NaOCl and $Ca(OCl)_2$ in the presence or absence of surfactant BAK on the bond strength of an epoxy resin-based sealer to root dentin. The study adopted the null hypotheses that there would be no difference in bond strength from the use of different irrigants [I]; nor after the use of a surfactant agent [II].

Materials and methods

Ethical approval and sample selection

This study was approved by the ethics committee of the Federal University of Santa Maria (no. 50355021.0.0000.5346). The sample size was selected based on previous similar studies[20,21]. Fifty permanent human maxillary lateral incisors were used. For the selection of teeth, periapical radiographs were previously performed, to include single-rooted teeth, with a single main canal and complete root development, free of root caries, previous endodontic treatment, calcifications, resorption, and cracks/fractures. The radiographs were taken in the buccolingual and mesiodistal directions to confirm eligibility criteria. The external root surfaces were cleaned using periodontal curettes, and the teeth were then stored in distilled water at 4ºC until the subsequent methodological procedures were carried out.

Sample preparation

The crowns of the teeth were sectioned 14 mm from the apex with a diamond disc under constant irrigation in a cutting machine set at 300 rpm (Isomet 1000; Buehler Ltd, Lake Bluff, USA), to standardize the length of the roots. A #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the root canal until it was possible to observe its crossing over the apical foramen, and this length was measured with a millimeter ruler. The working length was determined by subtracting 1 mm from this measurement, thus being set at 13 mm. The samples were randomly allocated (http://www.randomized.org), according to the irrigant used, in five groups (n=10): 0.9% sodium chloride (NaCl/Control); 2.5% NaOCl; 2.5% NaOCl + 0.008% BAK; 2.5%$Ca(OCl)_2$; and 2.5% $Ca(OCl)_2$ + 0.008% BAK. Irrigation was performed using the conventional method (syringe and needle). The preparation of the $Ca(OCl)_2$ solution and the addition of BAK to the solutions were carried out according to Iglesias et al.[16]. Table 1 shows the description of the tested solutions. The root canal was prepared with the X1 Blue 40.06 file (MK Life Medical and Dental Products, Porto Alegre, RS, Brazil), under irrigation with 20 mL of the solution corresponding to the experimental group. After instrumentation, we used 5 mL of 17% EDTA for 5 minutes, and a final irrigation with 10 mL of distilled water was performed. The root canals were dried with compatible absorbent paper points with the preparation. Next, root canal obturation was performed using the single cone technique (40.06; MK Life Medical and Dental Products) and AH Plus endodontic sealer (Dentsply Maillefer). The sealer was inserted into the canal with a size B finger spreader (Dentsply Maillefer), and the gutta-percha cone coated with sealer was inserted to the working length. The excess gutta-percha was removed by employing a flame-heated plugger positioned 1 mm below the canal orifice, followed by the application of cold vertical compaction. Periapical radiographs were taken in both the buccolingual and mesiodistal directions to verify the quality of the filling. The roots were sealed using interim restorative material and stored in distilled water at 37ºC and 100% relative humidity for one week.

Table 1. General description of the investigated irrigation solutions

Irrigation solution	Manufacturer
0.9% Sodium chloride	Eurofarma, São Paulo, SP, Brazil
2.5% Sodium hypochlorite	Dermapelle Farmácia de Manipulação, Santa Maria, RS, Brazil
65% Calcium hypochlorite	Grupo Rodoquimica, Taquari, RS, Brazil
50% Benzalkonium chloride	Êxodo Cientifica, Sumaré, SP, Brazil

Push-out assessment

After the storage period, the samples were sectioned using a precision cutting machine (Isomet 1000; Buehler Ltd) set at 300 rpm and equipped with a diamond disc, obtaining three slices per sample, with a thickness of 1mm ± 0.1mm. The cuts to obtain the slices were made at distances of 1.0 mm, 5.0 mm and 9.0 mm from the cervical third of the root.

Each slice was placed on a metal base with a central opening larger than the canal diameter within a universal testing machine (Emic DL-2000; Emic, Pinhais, PR, Brazil). The most coronal portion of the specimen was identified and oriented face down. For the push-out test, a force in the apical-coronal direction using a stainless-steel plunger (Ø = 0.5 mm) was exerted on the filling material at a speed of 0.5 mm/min until failure. A standard stainless-steel plunger was used to provide the most extensive coverage of the obturator material without touching the canal walls. The data obtained from the push-out test, in newtons (N), were converted to megapascals (MPa) by the following formula: $\sigma = F / A$. F represented the force (N) recorded by the testing machine at the time of specimen failure and A represented the bond area (mm^2). The following formula was used to determine the bonded interface area:

$$A = \pi(R + r)\sqrt{(h^2 + (R - r)^2)}$$

In the formula, π = is the constant 3.14, R = coronal radius, r = apical radius, and h = slice thickness[22]. A digital caliper was used to obtain measurements (CD-15C; Mitutoyo Co., Kawasaki, Japan).

Failure modes analysis

After failure, the samples were evaluated by a blinded examiner, through a stereomicroscope (Discovery V20; Carl-Zeiss, Gottingen, Germany) at ×30 magnification. The method proposed by Seballos et al.[23] to classify failure patterns at the resin cement/root dentin interface was adapted to evaluate the sealer/root dentin interface. Thus, failure patterns were classified into: As/d = Predominant adhesive at sealer/root dentin interface; As/g = Predominant adhesive at sealer/gutta-percha interface; C = Cohesive of dentin (Figure 1). The calibration consisted of repeating the analysis of the fracture pattern of 30 slices, with an interval of two weeks. Examiner reproducibility, which was calculated using the Kappa test, was 0.926.

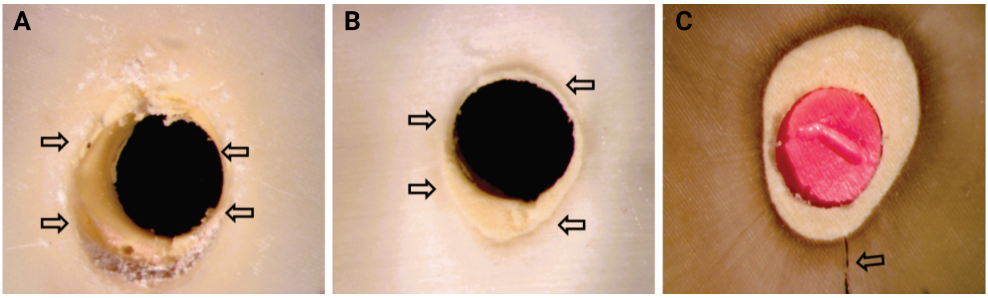

Figure 1. Representative images of the failure modes obtained under stereomicroscope at ×30 magnification. (A) = predominant adhesive at sealer/root dentin interface (As/d); (B) = predominant adhesive at sealer/gutta-percha interface (As/g); and (C) = Cohesive of dentin (C).

Statistical analysis

After the Shapiro-Wilk and Levene tests, the bond strength values did not show a normal homoscedastic distribution. Therefore, post-hoc pairwise comparisons were performed using the Kruskal-Wallis test adjusted using the Bonferroni method. Statistical significance was established when $p < 0.05$. All analyses were performed using the SPSS Statistics V.26 program (SPPS Inc., Chicago, USA).

Results

Push-out bond strength

The median and percentile of bond strength are shown in Table 2. The type of irrigant had a significant effect on the bond strength ($p < 0.05$). The groups treated with 2.5% NaOCl and 2.5% $Ca(OCl)_2$ showed bond strength similar to the control group ($p > 0.05$). The use of an additional 0.008% of BAK was able to increase the bond strength after the use of 2.5% $Ca(OCl)_2$ ($p < 0.05$) and did not change the bond strength after the use of 2.5% NaOCl ($p > 0.05$).

Table 2. Push-out bond strength values (Median [P25–P75]) in MPa of filling material to root dentin after treatment with different solutions

Groups	Bond Strength
0.9% NaCl (control)	1.62 (1.27-3.57)^{ab}
2.5% NaOCl	1.63 (1.27-3.90)^{ab}
2.5% NaOCl + 0.008% BAK	1.83 (1.30-3.48)^{ab}
2.5% $Ca(OCl)_2$	1.44 (1.17-1.88)^{a}
2.5% $Ca(OCl)_2$ + 0.008% BAK	1.92 (1.53-4.74)^{b}

NaCl, sodium chloride; NaOCl, sodium hypochlorite; BAK, benzalkonium chloride; $Ca(OCl)_2$, calcium hypochlorite. Different lowercase letters represent significant differences between groups ($p < 0.05$).

Failure modes

Failure modes can be seen in Figure 2. In all groups, the most common failure was predominant adhesive at sealer/gutta-percha interface, followed by predominant adhesive at sealer/root dentin interface, and finally cohesive failures.

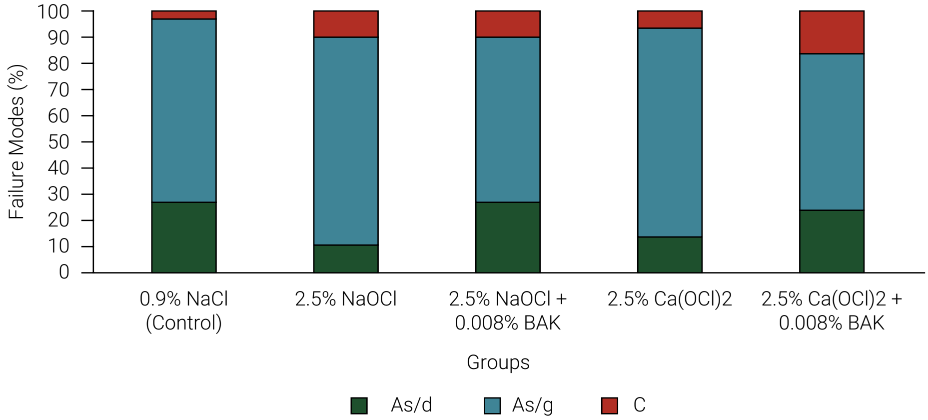

Figure 2. Failure modes (%) in each group after push-out. As/d = predominant adhesive at sealer/root dentin interface; As/g = predominant adhesive at sealer/gutta-percha interface; C = cohesive failures of the dentine.

Discussion

This study seems to be the first one designed with the aim of analyzing whether $Ca(OCl)_2$ with the presence or absence of a surfactant affects the bond strength of an epoxy resin-based sealer to root canal dentin. In our results, irrigating the root canal with a NaOCl solution or with a $Ca(OCl)_2$ solution resulted in similar bond strength of the filling material, with data comparable to the control. Thus, the first previously listed null hypothesis was accepted. In turn, when the surfactant BAK was added to the $Ca(OCl)_2$ solution, higher bond strength values were found compared to the group treated only with $Ca(OCl)_2$. Thus, the second formulated null hypothesis was rejected.

AH Plus is an epoxy resin-based sealer used worldwide in Endodontics, which carries with it the gold standard status, much in consideration of its resistance to resorption and dimensional stability. Chemically, the sealer AH Plus bonds to dentinal collagen[24]. Thus, the collagen fibers and their interconnections need to be exposed, using a decalcifying agent, to ensure the effectiveness of the bonding process[25]. A previous study demonstrated that final irrigation with NaOCl removes organic material from exposed dentin tissue and the bond strength of the sealer can be reduced[26]. In addition, due to NaOCl being a potent oxidizing agent, it is capable of leaving a layer rich in oxygen on the dentin surface, which may impair the infiltration of the sealer into the dentinal tubules[27]. Thus, it is recommended to use an inert solution in the final irrigation to act as an oxygen reducing agent[28]. In this sense, the experimental protocol of this study used a decalcifying agent (EDTA) and final irrigation of the root canal with distilled water, establishing such parameters to make the investigation compatible with an effective endodontic treatment protocol.

Statistical analysis indicated that the groups treated only with NaOCl or $Ca(OCl)_2$ showed bond strength similar to the control group. Although $Ca(OCl)_2$ causes fewer structural changes to dentin than NaOCl[6,9], this point was not crucial for the findings of this study. A possible justification is based on the fact that the adhesion of an epoxy resin-based sealer to the root dentin occurs largely through a mechanical penetration in the dentinal tubules[29], and is not dependent on the formation of a hybrid layer equal originated with the use of adhesive systems[30]. Therefore, it is assumed that, although the quality of the dentin tissue is important for the adhesion of the filling material, it seems to be more essential for adhesive restorative procedures. In addition, to improve the penetration of the sealer into the dentinal tubules, the removal of debris and the smear layer from the canal walls is essential[31]. In this context, prior studies have shown that both hypochlorites exhibit similar effectiveness in this regard, underscoring the necessity of using a chelating solution after their application[12]. Therefore, the comparable performance of both hypochlorites in this aspect may also explain the bond strength results we observed.

The addition of BAK surfactant to NaOCl maintained bond strength values similar to the group treated with NaOCl alone. This finding is in line with a recent investigation[19]. According to a previous study, the use of BAK reduced the surface tension of NaOCl by about 33% (NaOCl: 46.30 mN/m; NaOCl + BAK: 30.92 mN/m)[16]. Therefore, it is possible to imagine that this reduction was not enough to significantly increase the diffusion of the irrigant in the dentinal tubules, and consequently improve cleaning, and increase dentin permeability and the penetration of the filling material. In turn, in our study, the addition of BAK to $Ca(OCl)_2$ increased the bond strength values when compared to the group treated only with $Ca(OCl)_2$. Based on the same study cited above, BAK is able to reduce the surface tension of $Ca(OCl)_2$ by about 55% ($Ca(OCl)_2$: 72.13 mN/m; $Ca(OCl)_2$ + BAK: 31.86 mN /m)[16]. Thus, it is possible to assume that, in this scenario, the reduction of the surface tension of the $Ca(OCl)_2$ solution with the addition of BAK was enough to increase the penetrability of the solution in the dentinal tubules and thus, created better conditions for the penetration filling material, justifying the increase in bond strength. Moreover, future research should place emphasis on assessing the impact of incorporating BAK into both hypochlorite solutions in terms of their antibacterial effectiveness against the main microorganisms involved in endodontic pathologies and tissue dissolution capacity. A comparative analysis between the solutions should be conducted, as these properties are the primary characteristics that influence the choice of an endodontic irrigant.

The push-out test is a method capable of accurately estimating the bond strength, as the failure occurs parallel to the bonding interface, thus obtaining the true shear load, similar to the clinical condition. Ideally, adhesion test results should have a predominance of adhesive failures[32], a fact observed in the present study. In all groups, the most frequent failure mode was the predominant adhesive at sealer/gutta-percha interface. Lower bond strength at the sealer/gutta-percha interface was also reported in a previous study[33]. A possible explanation is that sealer can bond to dentin through mechanical penetration in dentinal tubules or by chemical adhesion, whereas gutta-percha lacks adhesion to dentin or sealer[34]. In addition, the AH Plus sealer can form, through chemical bonds, bonds between its epoxide ring and exposed amino groups in the collagen network[24]. Accordingly, its high flowability and long setting time are beneficial for forming tags[34], supposedly increasing penetration with root dentin. Therefore, it is logical to imagine that adhesion to dentin is superior to that of gutta-percha, reflecting lower percentages of failures at the interface with dentin.

In our study, we discovered that the bond strength values were similar between the groups treated with only NaOCl or $Ca(OCl)_2$. Additionally, the addition of the surfactant BAK to NaOCl maintained values similar to the use of NaOCl without the surfactant. In contrast, when BAK was added to the $Ca(OCl)_2$ solution, it increased the bond strength of the sealer to root dentin in comparison to using $Ca(OCl)_2$ without the surfactant. Consequently, the combination of both solutions appears to be a promising alternative. It's reasonable to assume that filling materials with low bond strength may exhibit more defects on the dentin surface, which could promote microbial reinfection and contribute to endodontic failure. A limitation of our study was the use of only one diameter of a stainless-steel plunger for all root thirds during the push-out test. We used a standard diameter of a plunger that provided filling material coverage without touching the canal wall, for all slices and groups. However, due to the complex internal configuration of the root canal, using a plunger of different diameters for each root third may be a more suitable strategy to better represent bond strength values[35]. In addition, our study lacks complementary analyzes to investigate the penetration of the sealer and the investigated irrigants, and this point can also be cited as a limitation. However, although simple, our experimental design was adequate for the purpose of the study, which was to evaluate bond strength[33]. For more comprehensive insights into the behavior of irrigating solutions and sealers in intratubular penetration, future studies employing techniques such as confocal laser scanning microscopy or scanning electron microscopy are recommended. Furthermore, to solidify these findings, additional research is necessary to assess the effectiveness of various surfactants. It is also important to investigate the impact of surfactants on other steps related to endodontic treatment. Moreover, well-designed clinical studies are needed to evaluate the long-term outcomes of endodontic treatments.

Conclusion

Considering the limitations of this study, it is concluded that the bond strength of the AH Plus sealer can be improved by using the $Ca(OCl)_2$ solution with the addition of BAK, with better results than using the same irrigant without any surfactant. In an antagonistic line, the addition of BAK was not able to increase the bond strength of the sealer after irrigation with NaOCl.

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Data availability

Datasets related to this article will be available upon request to the corresponding author.

Declaration of interest

The authors declare no conflict of interests.

Author contribution

Guilherme Pauletto - Conceptualization; Data curation; Investigation; Formal analysis; Methodology; Validation; Visualization; Writing - original draft; Writing - review & editing. **Natália Franco Brum** - Data curation; Investigation; Methodology; Validation; Writing - review & editing. **Israel Bangel Carlotto** - Conceptualization; Data curation; Methodology; Software; Validation; Visualization; Writing - review & editing. **Lucas Saldanha da Rosa** - Data curation; Formal analysis; Methodology; Software; Supervision; Validation; Writing - review & editing. **Carlos Alexandre Souza Bier** - Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing - review & editing. All authors revised and approved the final version of the manuscript.

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