






# Effect of sodium hypochlorite and calcium hypochlorite on the apical sealing ability of endodontic sealers

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**Aim:** This study aimed to compare the influence of sodium hypochlorite (NaOCl) and calcium hypochlorite [Ca(OCl)<sub>2</sub>] on the apical sealing capacity of AH Plus (Dentsply Maillefer) and Bio-C Sealer (Angelus) endodontic sealers. **Methods:** Sixty permanent human lower incisors were randomly allocated (<http://www.randomized.org>), according to the irrigant used, into three groups (n=20): 0.9% sodium chloride (NaCl/Control); 2.5% NaOCl; and 2.5% Ca(OCl)<sub>2</sub>. The root canal was prepared with rotary files under 10 mL of the solution corresponding to the experimental group. Each group was subdivided into two (n=10) according to the sealer used for filling: AH Plus (Dentsply Maillefer) or Bio-C Sealer (Angelus). Then, all samples were immersed in black India ink for one week. After the storage period, the roots were then grooved longitudinally and split, and the ink penetration was measured from the apical part to the coronal part of the root canal into which the ink penetrated using a stereomicroscope. Data were analyzed by one-way ANOVA and Tukey's post-hoc tests. **Results:** There was no statistical difference in ink penetration between the different endodontic sealers tested for the same irrigating solution (p > 0.05). However, when the Bio-C Sealer (Angelus) was used, the group treated with 2.5% Ca(OCl)<sub>2</sub> was associated with lower values of apical leakage, compared to 2.5% NaOCl (p < 0.05). For the AH Plus sealer (Dentsply Maillefer), there was no difference between the irrigants (p > 0.05). **Conclusions:** Associating Ca(OCl)<sub>2</sub> irrigant with Bio-C Sealer (Angelus) seems to be a good option to reduce apical leakage.

**Keywords:** Dental leakage. Calcium compounds. Sodium hypochlorite. Root canal obturation.

## Introduction

During chemical-mechanical preparation, many areas of the root canal system may remain untouched by endodontic instruments<sup>1</sup>. Therefore, irrigating solutions need to be associated with the use of instruments during treatment to reach inaccessible areas<sup>2</sup>. Among the irrigation solutions used in Endodontics, sodium hypochlorite (NaOCl) is the most widely used. As its main features, it has an excellent antibacterial action and tissue dissolution capacity<sup>3</sup>. However, it presents considerable chemical instability<sup>4</sup>. In addition, it causes irreversible damage to dentin morphology<sup>5</sup>. Due to the inconveniences of NaOCl, new irrigation solutions are constantly investigated, with recent emphasis on calcium hypochlorite  $[\text{Ca}(\text{OCl})_2]$ <sup>6-8</sup>. It is a granular powder, with a higher amount of available chlorine and greater chemical stability when compared to NaOCl<sup>6,9</sup>. It has an antibacterial effect and tissue dissolution capacity similar to NaOCl<sup>8</sup> and does not affect the properties of dentin<sup>10,11</sup>.

AH Plus (Dentsply Maillefer, Ballaigues, Switzerland) is an epoxy resin-based sealer considered the gold standard in Endodontics, due to its resorption resistance and dimensional stability<sup>12</sup>, presenting good sealing capacity<sup>13</sup>. However, the main limitation of AH Plus (Dentsply Maillefer) is its absence of bioactive properties<sup>14</sup>. Calcium silicate-based endodontic sealers were recently developed, with excellent bioactive potential<sup>15</sup>. These sealers can be used in conjunction with thermomechanical compaction techniques, the single-cone technique<sup>16</sup>, or the lateral condensation technique<sup>17</sup>. Bio-C Sealer (Angelus, Londrina, PR, Brazil) is a root canal sealer containing calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and dispersing agent in its composition. This sealer has biocompatibility; bioactivity; and high pH, radiopacity, and flow values<sup>18</sup>. However, the apical sealing ability of the Bio-C Sealer (Angelus) still requires investigation.

One of the major reasons attributed to the clinical failure of endodontic therapy is apical leakage<sup>19</sup>. In this sense, irrigating solutions, especially NaOCl, can cause structural damage to the dentin that can affect the interaction of endodontic sealers with the root dentin, which may compromise adequate apical sealing<sup>20</sup>. Thus, in the search for superior results, investigating the influence of  $\text{Ca}(\text{OCl})_2$  on the apical sealing of different endodontic sealers is necessary, and as far as we know, there are no studies that have evaluated this association.

Therefore, the purpose of this *in vitro* study was to compare the influence of NaOCl with  $\text{Ca}(\text{OCl})_2$  on the apical sealing capacity of AH Plus (Dentsply Maillefer) and Bio-C Sealer (Angelus) endodontic sealers. The study adopted null hypotheses are that there would be no difference in apical leakage with the use of the same irrigating solution for different endodontic sealers (I); and that the different solutions would not have a significant effect on apical leakage for the same endodontic sealer (II).

## Materials and methods

### Ethical approval and sample selection

Ethical approval for the study was obtained from the local ethics committee (no. 60114022.5.0000.5346). The sample size was estimated based on data from a previous study<sup>21</sup>. The ANOVA: fixed effects, omnibus, one-way test was selected from the F-tests family in G\*Power 3.1 software for Windows (Heinrich-Heine-Universität, Düsseldorf, Germany). Accordingly, for the analysis with  $\alpha = 0.01$  and 95% testing power, and considering an effect size = 0.81, a total of 48 specimens (eight teeth per test group) were indicated as the minimum size required for observing significant differences. From this, the sample was defined as 60 permanent human lower incisors. Digital periapical radiographs were performed to select 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 teeth were kept in distilled water at 4°C until the following methodological steps.

### Sample preparation

The crowns of the teeth were sectioned 13 mm from the apex with a diamond disc under constant irrigation in a cutting machine (Isomet 1000; Buehler Ltd, Lake Bluff, USA), to standardize the length of the roots. A #10 K-file (Dentsply Maillefer) 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. The samples were randomly allocated (<http://www.randomized.org>), according to the irrigant used, in three groups (n=20): 0.9% sodium chloride (NaCl/Control); 2.5% NaOCl; and 2.5% Ca(OCl)<sub>2</sub>. The root canal was prepared with the Bassi Logic rotary files (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil), sizes 40.01 and 40.05, under 10 mL of the solution corresponding to the experimental group. After instrumentation, we used 2 mL of 17% EDTA for 1 minute, repeated 3 times, and a final irrigation with 5 mL of distilled water was performed. Then, the root canals were dried with compatible absorbent paper points. Afterward, each group was subdivided into two (n=10) according to the endodontic sealer used: AH Plus (Dentsply Maillefer) or Bio-C Sealer (Angelus) (Table 1). All roots were filled using the lateral condensation technique with gutta-percha and the respective sealer. Digital periapical radiographs were performed to confirm the quality of the filling. The roots were sealed using interim restorative material (Coltosol; Coltene, Alstätten, Switzerland) and stored in distilled water at 37°C during one week.

**Table 1.** Experimental samples grouping.

Groups (n)	Subgroups (n)
0.9% NaCl (n=20)	AH Plus (n=10)
	Bio-C Sealer (n=10)

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2.5% NaOCl (n=20)	AH Plus (n=10)
	Bio-C Sealer (n=10)
2.5% Ca(OCl) <sub>2</sub> (n=20)	AH Plus (n=10)
	Bio-C Sealer (n=10)

NaCl, sodium chloride; NaOCl, sodium hypochlorite; Ca(OCl)<sub>2</sub>, calcium hypochlorite.

## Apical leakage assessment

The root surfaces were coated with nail varnish (except the apex). All samples were immersed in black India ink for one week. After the storage period, the samples were thoroughly rinsed under running tap water. The roots were then longitudinally grooved with a diamond disc and split with a chisel, ensuring that the root canal filling was not penetrated, and then split into halves by levering with a plaster knife.

The ink penetration was measured from the apical to the coronal part of the root canal to which the ink had penetrated using a stereomicroscope (Discovery V20; Carl-Zeiss, Gottingen, Germany) at ×10 magnification. A blinded observer was responsible for this analysis. All computational work was performed using Image J software (National Institutes of Health, Bethesda, USA). The calibration consisted of repeating the analysis of the apical leakage in 10 samples, with an interval of two weeks. Examiner reproducibility, which was calculated using the intraclass correlation coefficient, was 0.949.

## Statistical analysis

After the Shapiro-Wilk and Levene tests, the apical leakage values presented a normal homoscedastic distribution. Based on that, data were analyzed by one-way ANOVA and Tukey's post-hoc tests. The level of statistical significance was set at  $p < 0.05$ . All analyses were performed using the SPSS Statistics V.26 program (SPSS Inc., Chicago, USA).

## Results

The mean and standard deviation of apical leakage are shown in Table 2. All groups demonstrated apical leakage. There was no statistical difference in ink penetration between the different endodontic sealers tested for the same irrigating solution ( $p > 0.05$ ). However, when comparing the same endodontic sealer to different irrigating solutions, there was a statistical difference ( $p < 0.05$ ). When the Bio-C Sealer (Angelus) was used, the group treated with 2.5% Ca(OCl)<sub>2</sub> was associated with lower values of apical leakage, compared to 2.5% NaOCl ( $p < 0.05$ ). For the AH Plus sealer (Dentsply Maillefer), there was no difference between the irrigants ( $p > 0.05$ ).

**Table 2.** Mean and standard deviation of apical leakage for the experimental groups (mm).

Irrigation Solutions	Endodontic Sealer	
	AH Plus	Bio-C Sealer
0.9% NaCl (control)	3.98 ± 2.42 <sup>abA</sup>	4.99 ± 2.37 <sup>abA</sup>
2.5% NaOCl	3.07 ± 2.44 <sup>abA</sup>	5.32 ± 2.82 <sup>abA</sup>
2.5% Ca(OCl) <sub>2</sub>	3.27 ± 1.95 <sup>abA</sup>	2.03 ± 1.25 <sup>abA</sup>

NaCl, sodium chloride; NaOCl, sodium hypochlorite; Ca(OCl)<sub>2</sub>, calcium hypochlorite.

Equal lowercase letters in the column mean no statistically significant difference between irrigation solutions ( $p < 0.05$ ). Equal uppercase letters in row mean no statistically significant difference between sealers ( $p < 0.05$ ).

## Discussion

This study seems to be the first one designed to analyze whether Ca(OCl)<sub>2</sub> affects the apical sealing capacity of endodontic sealers. In our results, there was no statistical difference in apical leakage between the different endodontic sealers tested for the same irrigating solution (NaCl, NaOCl or Ca(OCl)<sub>2</sub>). Thus, the first previously listed null hypothesis was accepted. In turn, when comparing the same endodontic sealer with different irrigating solutions, when Bio-C Sealer (Angelus) was used in the group treated with 2.5% Ca(OCl)<sub>2</sub>, lower values of apical leakage were found, compared to 2.5% NaOCl. Thus, the second formulated null hypothesis was rejected.

Statistical analysis indicated that there was no difference in apical leakage between AH Plus (Dentsply Maillefer) and Bio-C Sealer (Angelus) for the same irrigating solution tested (NaOCl or Ca(OCl)<sub>2</sub>), with values similar to the control. AH Plus (Dentsply Maillefer) is an epoxy resin-based sealer little soluble, with good fluidity and with satisfactory dimensional stability<sup>22</sup>. In addition, it contains an acidic pH, which provides self-etching of the dentin. This is explained as a result of its reaction with amino groups present in collagen, which form covalent bonds to the epoxy resin<sup>23</sup>, supposedly favoring a three-dimensional apical sealing. In turn, Bio-C Sealer (Angelus) is an endodontic sealer based on calcium silicate, alkaline, and viscous, also with good fluidity<sup>18</sup>, with great potential to generate an excellent biological apical sealing. In this sense, according to the manufacturer, when reacting with carbon dioxide (CO<sub>2</sub>), it forms calcium carbonate (CaCO<sub>3</sub>), which releases ions that interact with dentin, producing an area of mineral deposits that favor its connection to dentin tissue, possibly avoiding infiltration. In addition, although the irrigating solution may alter the dentin surface and compromise sealer adhesion<sup>24</sup>, in a similar scenario (same irrigant used) both sealers performed similarly. Therefore, it is assumed that, even in the face of sealers with different compositions, both presented similar apical leakage values, and the effectiveness of their mechanisms of action help to explain this finding. In addition, it is reinforced that previous studies also obtained similar results, without differences in the apical sealing ability between AH Plus (Dentsply Maillefer) and calcium silicate-based sealers for the same irrigant (NaOCl)<sup>25,26</sup>.

On the other hand, when comparing the different irrigating solutions tested for the same endodontic sealer, the use of 2.5% Ca(OCl)<sub>2</sub> together with Bio-C Sealer

(Angelus) resulted in a greater apical sealing capacity, to the 2.5% NaOCl. This finding can be partially explained by the following assumption: after the use of  $\text{Ca}(\text{OCl})_2$ , higher percentages of calcium ions are observed on the dentin surface<sup>27</sup>. In turn, the Bio-C Sealer (Angelus) also involves the release of this mineral<sup>28</sup>, and during its setting process, the probable formation of hydroxyapatite occurs<sup>15</sup>, generating the dentin-sealer bond. Thus, assuming that the Bio-C Sealer (Angelus) chemically bonds to the hydroxyapatite through calcium ions, due to  $\text{Ca}(\text{OCl})_2$  leaving greater amounts of this mineral in the dentin after its use, this fact can enhance the adhesion of the sealer to the dentine tissue and consequently increase the apical sealing, a fact observed in our study. In addition, NaOCl causes damage to the organic matrix of dentin, generating collagen degradation<sup>5</sup> and affecting its main properties<sup>29</sup>. In contrast,  $\text{Ca}(\text{OCl})_2$  causes less dentin erosion<sup>30</sup> and does not alter the structural architecture of dentin<sup>10,11</sup>. Therefore, it is also consistent to assume that the quality of the dentin substrate is of paramount importance to the Bio-C Sealer (Angelus), since it chemically binds to dentin, and the negative impact of NaOCl on dentin tissue justifies a poorer apical seal found after use. Accordingly, previous studies also demonstrated differences in apical sealing capacity between AH Plus (Dentsply Maillefer) and calcium silicate-based sealers after the use of different irrigants, consolidating our findings<sup>20,31</sup>.

In our study, for the AH Plus (Dentsply Maillefer) the apical leakage values were similar between the different irrigants. Although  $\text{Ca}(\text{OCl})_2$  does not cause major damage to dentin<sup>10,11</sup>, this point was not crucial for this finding. A plausible hypothesis consists of the fact that the adhesion of an epoxy resin-based sealer to the root dentin occurs largely by mechanical penetration in the dentinal tubules<sup>32</sup>. Thus, it is logical to imagine that, although the quality of the dentin tissue is important for the adhesion of an epoxy resin-based sealer, it seems to be more essential for sealers based on calcium silicate, which are joined through a chemical bond the hydroxyapatite. In this sense, the negative impact of irrigants on dentin properties is more evident in calcium silicate-based sealers, which corroborates the better results obtained in the present study for the joint use of  $\text{Ca}(\text{OCl})_2$  and Bio-C Sealer (Angelus).

Apical leakage continues to be a topic of great interest because, despite advances in Endodontics, clinical failures still occur. The methodology of immersion of the teeth in dyes or inks is the most used method worldwide to evaluate apical leakage, due to its easiness<sup>33</sup>. Although linear ink penetration does not provide data on the infiltrated area, it does provide sufficient data on the apical leakage that occurred. In addition, black India ink has particles of appropriate diameter for use in this method<sup>34</sup>. Finally, in a comparison between methods, the method of immersion in dyes or inks is considered the most faithful and fast to evaluate apical leakage, than the methods of fluid transport and bacterial leakage<sup>33</sup>, justifying their employment in our study.

In our findings, the use of 2.5%  $\text{Ca}(\text{OCl})_2$  together with Bio-C Sealer (Angelus) showed the lowest values of apical leakage when compared to 2.5% NaOCl. Thus, when the endodontic treatment plan includes the use of Bio-C Sealer (Angelus), using  $\text{Ca}(\text{OCl})_2$  is a good option to reduce the rates of apical leakage, and consequently, reduce the chances of microbial reinfection and failure endodontic. However, further studies are needed to assess the effect of  $\text{Ca}(\text{OCl})_2$  on other outcomes strictly associated with

endodontic treatment, as well as the performance of well-designed clinical studies to assess the long-term longevity of the endodontic treatment.

## Conclusion

Considering the limitations of this in vitro study, it is concluded that for the use of the same irrigant (NaCl, NaOCl or  $\text{Ca}(\text{OCl})_2$ ), there is no difference in the quality of apical sealing between AH Plus sealer (Dentsply Maillefer) and Bio-C Sealer (Angelus). On the other hand, lower values of apical leakage are found using the Bio-C Sealer when the root canal was previously irrigated with 2.5%  $\text{Ca}(\text{OCl})_2$  instead of 2.5% NaOCl. Finally, for the AH Plus sealer, similar results were obtained when comparing both irrigants.

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## Data availability

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

## Conflict of interest

The authors declare no conflict of interests.

## Author contributions

**Israel Bangel Carlotto** - Conceptualization; Data curation; ; Investigation; Methodology; Software; Validation; Visualization; Writing - original draft; Writing - review & editing.

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**Guilherme Pauletto** - Conceptualization; Data curation; Formal analysis; Methodology; Validation; Visualization; Writing - review & editing.

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**Carlos Alexandre Souza Bier** - Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing - review & editing.

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