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Original Article

Consequences of chemical residue formation during potentiation of final irrigation – *in vitro* study

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Seeking to increase the efficiency of endodontic irrigation, the association of different solutions as final irrigant has been investigated, such as sodium hypochlorite with chlorhexidine. The literature shows that the combination of these substances leads to the formation of a brownish precipitate, but does not reveal measurements of the intensity of this precipitate and its consequences. **Aim:** The present study aimed to evaluate the change in dentin color and the obliteration of the dentinal tubules after the association of sodium hypochlorite (NaOCl) with chlorhexidine (CHX) in the final irrigation. **Methods:** Fifty sterile human lower premolars were prepared with a ProDesign R 35.05 files and divided into 6 groups. Four different NaOCl concentrations (0.5%; 1%, 2.5% and 5.25%) associated with 2% CHX were tested, in addition to 2 control groups, using only 2.5% NaOCl and 2% CHX, respectively. After the final irrigation protocol, the dentin color change was evaluated by spectrophotometry immediately and after 24 hours, and the dentinal tubule obliteration was assessed by scanning electron microscopy. **Results:** It was possible to verify that regardless the NaOCl concentration used when associated with CHX, a chemical residue was formed, with consequent dentin pigmentation and tubular obstruction. There was a trend towards increased dentin pigmentation and tubular obstruction due to the deposition of the chemical residue formed by this association. **Conclusion:** It can be concluded that all concentrations of NaOCl associated with CHX caused color changes and tubular obstruction, being proportional to the concentration of NaOCl used.

Keywords: Sodium hypochlorite. Chlorhexidine. Root canal irrigants. Endodontics.

Introduction

The purpose of endodontic treatment is to clean and model the root canal system (RCS). However, due to the anatomical complexity presented by this three-dimensionally complex system, only mechanized instrumentation is not able to remove all pulp and bacteria content present in the isthmus and branches. Therefore, it is necessary to use chemical agents during mechanized instrumentation for greater success rates in endodontic therapy[1-3].

Sodium hypochlorite (NaOCl) is one of the irrigating solutions widely used in endodontics due to its excellent antimicrobial and tissue dissolution properties, being present in concentrations of 0.5% to 5.25%[3,4]. Although sodium hypochlorite has advantages in its use, it still can not be considered the ideal solution. NaOCl does not have the full capacity for debridement of the dentinal tubules; it is irritating when in contact with periapical tissues, in addition to not having substantivity[5,6]. Seeking to increase the efficiency of irrigation, the possibility of associating different irrigating solutions to NaOCl has been studied due to the positive synergistic effects on antimicrobial activity[7].

Thus, other irrigating solutions are recommended for the association with sodium hypochlorite, such as chlorhexidine digluconate[6,7]. Chlorhexidine (CHX) in a concentration of 2% has a broad-spectrum antimicrobial effect used as an alternative to sodium hypochlorite[7-10], and demonstrates similar antimicrobial activity.

The literature shows that CHX can be used as a final irrigant after NaOCl due to its residual antimicrobial action[11]. However, sodium hypochlorite must be removed from the RCS since the concomitant use of these two substances leads to the formation of a brown chemical residue, that is difficult to remove and can cause discoloration of dentinal structures[12]. It also promotes the obliteration of the dentinal tubules, compromising the filling of the canals.

The literature shows that the precipitate formed may contain para-chloroaniline (PCA), a compound formed through the hydrolysis of CHX in a reaction dependent on time, alkaline pH, and temperature[2,12,13]. This precipitate is toxic and carcinogenic and may lead to methaemoglobinemia in humans. The International Agency for Research on Cancer (International Agency for Research on Cancer, 2006) classified PCA as a carcinogen of the 2B group, with limited evidence about its carcinogenic potential in humans[12,14,15].

The objective of this study was to evaluate the consequences of the interaction of sodium hypochlorite and chlorhexidine on the obliteration of the dentinal tubules and the alteration of the dentin staining, after the final irrigation, immediately and after 24 hours. The null hypothesis tested is that there is a tendency for greater pigment deposition as sodium hypochlorite concentration is increased.

MATERIALS AND METHODS

Preparation of teeth

The present study was approved by the Local Ethics Committee (CAAE: 04269418500005421). Fifty healthy human lower premolars were selected and

radiographed to confirm the absence of internal calcifications, the presence of more than one canal, stones, or pulp nodules. All procedures were performed by a single operator.

The external root surfaces of the specimens were cleaned of tissue remnants and stored in 0.9% saline solution until the moment of use[16,17]. The length was standardized at 15 mm using an Isomet cutting machine (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) with a diamond disc at 250 revolutions per minute (rpm), under irrigation, reducing the height of the tooth crown and gaining access to the canal[18]. The patency was performed by inserting a # 15 K file (Dentsply, Maillefer, Ballaigues, Switzerland) until the tip of the instrument was seen juxtaposed to the apical foramen[19,20]. Soon after, 1mm was subtracted from this measurement, obtaining a working length of 14mm. Subsequently, the teeth were immersed in 1% sodium hypochlorite for 12 hours for initial disinfection and dissolution of organic tissues. To open the dentinal tubules, the specimens received three 10-minute baths each in an ultrasonic tub (Odontobras, Ribeirão Preto, SP, Brazil) with 1% sodium hypochlorite (NaOCl) (Formula e Ação, São Paulo, SP, Brazil), 17% ethylenediaminetetraacetic acid (EDTA) (Biodynamic Chemistry and Pharmaceuticals, Ibiporã, PR, Brazil) followed by distilled water to neutralize the previous substances. The specimens were dried for 24 hours before being autoclaved at 121 °C[18].

The root apex of each specimen was closed with pink wax # 7, avoiding the extrusion of irrigants[20,21]. The specimens were inserted into a sterile metallic device and adjusted until they were firm.

All specimens were prepared with ProDesign R 35.05 files (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil) following the speed and torque indications suggested by the manufacturer.

During instrumentation, the canal was irrigated with distilled water, using a 5 milliliter (mL) syringe (Ultradent Products, South Jordan, UT, USA) with a 30 gauge needle (Ultradent Products, South Jordan, UT, USA) at 1 mm from the working length 3 times with 3 mL, totaling 9mL. Distilled water was used because it is an inert solution, which did not influence the association of compounds in the final irrigation. With a # 15 K file (Dentsply, Maillefer, Ballaigues, Switzerland), the working length was recapitulated[17,22].

Final irrigation protocol

After instrumentation, the specimens were flooded with 3mL of 17% EDTA for three minutes to remove organic residues from the instrumentation, followed by irrigation with 5mL of distilled water to neutralize the substance previously used.

Next, they were randomly divided into 6 groups, 4 experimental groups with n = 10 and 2 control groups with n = 5, which received the following final irrigation protocol:

Group 1: (n = 10) 2mL of 0.5% NaOCl followed by 2mL of 2% CHX;

Group 2: (n = 10) 2mL of 1% NaOCl followed by 2mL of 2% CHX;

Group 3: (n = 10) 2mL of 2.5% NaOCl followed by 2mL of 2% CHX;

Group 4: (n = 10) 2mL of 5.25% NaOCl followed by 2mL of 2% CHX

Group 5: (n = 5) 2mL of 2.5% NaOCl

Group 6: (n = 5) 2mL of 2% CHX

Sodium hypochlorite solutions in concentrations of 0.5%; 1%; 2.5% and 2% chlorhexidine were obtained through the manufacturer (Formula e Ação, São Paulo, SP, Brazil) and 5.25% by manipulation in a pharmacy (Specifica Ltda, Bauru, SP, Brazil). The canals were immediately dried with Logic Tanari 35.05 sterile absorbent paper tips (Tanariman Industrial Ltda., Manacapuru, AM).

Analysis of color change in a spectrophotometer

Color measurements were performed using a spectrophotometer (VITA Easyshade® compact; VITA Zahnfabrik, Bad Saichen, Germany) under standard conditions, calibrating the equipment before measuring each group.

The following intervals were used for the measurement: T = 0 (before the specimens received the proposed final irrigation protocols); T = 1 (just after the proposed final irrigation protocols); T = 2 (24 hours after the proposed final irrigation protocols). To avoid optical changes caused by dehydration and to simulate the environment of the oral cavity, the specimens were stored in a humid environment and kept in an oven at 37ºC for the third reading and their water excess removed with a sterile paper filter (Melitta, Minden, Germany). The readings were performed on the root vestibular surface of each specimen, in the region of the cervical and apical thirds[23].

The specimens were measured three times, with 5 seconds for each reading, so that the average of the measurements was used, avoiding possible bias in the reading. The measurement was standardized in the cervical and apical thirds. The values of (CIE) (International Lighting Commission, 1913), L, a and b were noted, and the color change (ΔE) concerning the interval between before the final irrigation protocol (T0), immediately after the association of the substances in the final irrigation (T1) and 24 hours after the proposed final irrigation protocol (T2), was calculated using the following formula:

$$\Delta E = [(\Delta L)\ 2 + (\Delta a)\ 2 + (\Delta b)\ 2]\ ½$$

Where "L" represents the luminosity values of the color, "a" is the measurement along the red-green axis, "b" corresponds to the measurement along the yellow-blue axis and "ΔE" is the measure of the difference between the color in the initial reading and the final reading[23,24]. The values obtained from "ΔE" were statistically evaluated by the Friedman test.

Scanning electron microscopy (SEM)

For scanning electron microscopy analysis (JEOL, JSMTLLOA, Tokyo, Japan), longitudinal sections of the specimens from each group were performed with a diamond disc and sterile saline solution on an Isomet machine (Isomet, Buehler, IL, USA) to obtain two halves. The sections obtained were dipped in absolute alcohol for 1 minute and allowed to dry at room temperature for 24 hours, and subsequently mounted on metal bases to receive the gold bath. They were sprayed with 200A - 300A (angstroms)

of gold (Hammer VI Sputtering System; Anatech Ltd, Alexandria, VA) to become electrically conductive. Images of the cervical, middle, and apical thirds were obtained at 10-15Kv with a standard increase of 750x[25].

Scoring system

The images obtained in SEM were classified according to the presence of the precipitate observed in the thirds and punctuated by three calibrated examiners using a classification system described by Pirani[26] (2009) and Prati[27] (2004):

0 - More than 75% of the tubules visibly exposed and free from the *smear layer*.

1 - *Smear layer* present and <75% of tubules visibly exposed.

2 - *Smear layer* visibly limited and <50% of tubules visibly exposed.

3 - *Smear layer* homogeneous in dentine and without exposed dentinal tubules.

The term *smear layer* in the present work corresponds to the presence of the precipitate. Intra- and an inter-examiner agreement was assessed using the KAPPA test ($p < 0.05$).

Statistical analysis

In the analysis of the color change by spectrophotometry, the tabulated data showed non-normal distribution, being subjected to the Kruskal-Wallis test followed by the Dunn's test when all groups were compared. The evaluation of these data by time was performed using the Friedman test. In the scanning electron microscopy, the analyses by scores were also submitted to the Kruskal-Wallis test, followed by the Dunn's test using the Prism 6.0 software (GraphPad Software Inc., La Jolla, USA) as an analytical tool and the level of significance was established at $P < 0.05$.

RESULTS

Color change

In all tested groups, the precipitate was formed with consequent dentin color change (ΔE) of the specimens. In Figure 1, it is possible to observe the color and appearance of this precipitate right after the irrigators' interaction.

Figure 1. Precipitate formed after the association of the proposed irrigators.

Table 1 shows the median, maximum and minimum color change (ΔE) values for all groups before final irrigation (T0), after final irrigation (T1) and 24 hours after the proposed final irrigation protocols (T2). Although without statistically significant differences when comparing ΔE between times T0 (before final irrigation) and T1 (after final irrigation); G4 showed higher values than the other groups (Figure 2).

Table 1. Values for color change (ΔE) in the different groups evaluated before the irrigation protocol, immediately after and 24h after. Friedman test p <0.05.

Groups	Median (min-max)		
	ΔE		
	T0	T0	T0
G1 – NaOCl 0.5% + CLX 2%	15.22 (4.80-32.25)	15.22 (4.80-32.25)	15.22 (4.80-32.25)
G2 – NaOCl 1% + CLX 2%	16.13 (11.00-24.00)	16.13 (11.00-24.00)	16.13 (11.00-24.00)
G3 – NaOCl 2.5% + CLX 2%	14.72 (5.90-26.30)	14.72 (5.90-26.30)	14.72 (5.90-26.30)
G4 – NaOCl 5.25% + CLX 2%	15.98 (13.20-26.50)	15.98 (13.20-26.50)	15.98 (13.20-26.50)
G5 – NaOCl 2.5%	7.93 (4.20-11.90)	7.93 (4.20-11.90)	7.93 (4.20-11.90)
G6 – CLX 2%	7.26 (3.40-14.90)	7.26 (3.40-14.90)	7.26 (3.40-14.90)

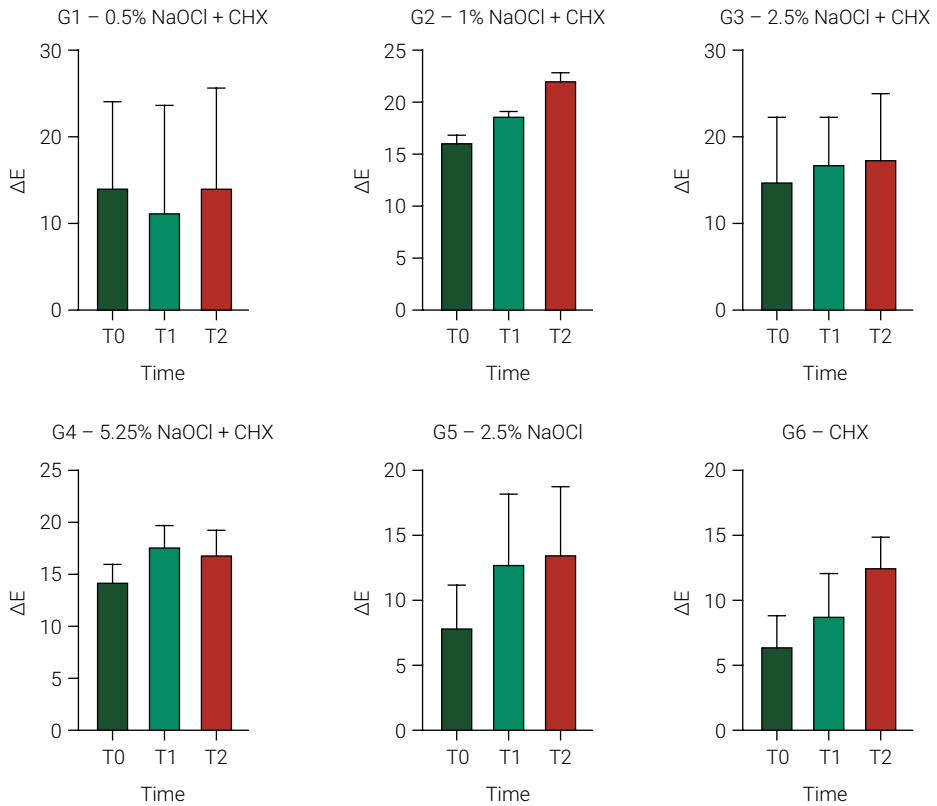

Figure 2. Mean and standard deviation of variation of the color (DE) of the experimental and control groups measured at the moment after biomechanical preparation (T0), immediately after the final irrigation (T1) and 24 hours after (T2).

When T1 (just after the final irrigation) and T2 (24 hours after the final irrigation) were compared, there was a reduction in ΔE in groups G2 and G4, but without statistically significant differences. As a result, it is suggested that pigmentation of the dental structure occurs immediately after the association of irrigants, but it is not maintained after 24 hours. Groups G1 and G3, on the other hand, showed an increasing trend in their ΔE between T1 and T2, without statistically significant differences. The positive control groups, G5 and G6, showed small variations in ΔE between the proposed times, with no statistical differences (P> 0.05) (Figure 3).

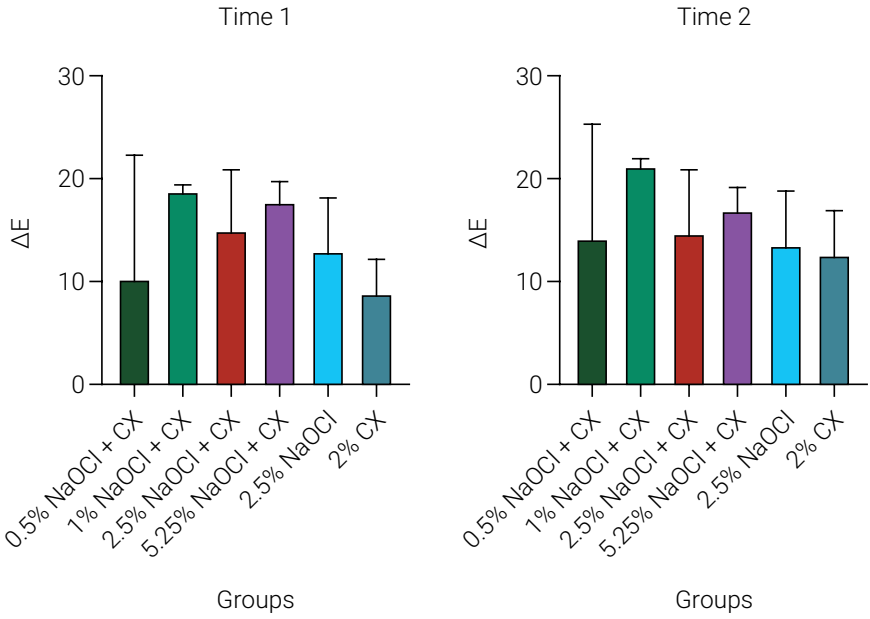

Figure 3. Mean and standard deviation of variation of the color (DE) between groups in the moments immediately after irrigation (T1) and 24 hours after the irrigation protocol (T2).

Scanning electron microscopy

In all experimental groups, there was residue formation, regardless of the concentration of NaOCl associated with CLX. In G2, this precipitate was more visible and obliterated most dentinal tubules, according to the classification proposed to blind examiners. The control groups showed fewer chemical residues deposited in the dental tubules (tubular obstruction) when compared to the experimental groups (Figure 4). However, the results showed that there was no statistical difference between the groups regarding the presence of residue in the dentin. Table 2 shows the median, maximum, and minimum values of the scoring system used to classify the images according to the presence of precipitate in the three thirds. Figures 5 and 6 show some SEM images related to the cervical, middle and apical thirds, respectively, of different groups, where the formation and deposition of residue were greater in the cervical and middle thirds when compared to the apical.

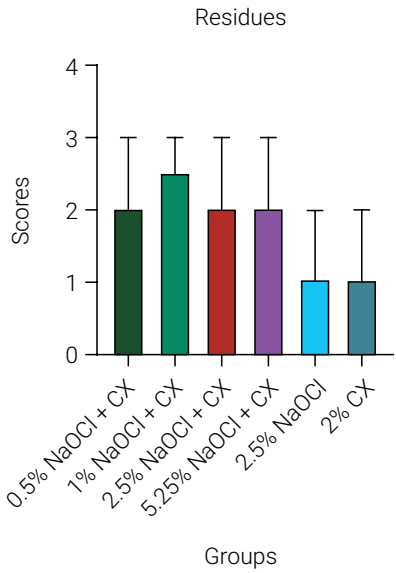

Figure 4. Mean and standard deviation of Residue formation based on the examiner's analysis and score classification.

Table 2. Score values for the presence of precipitate on the dentin surface in the three thirds of the different groups. Kruskal-Wallis test p <0.05.

Groups	Median (min-max)
	Score (SEM)
G1 – NaOCl 0.5% + CLX 2%	2.13 (0.00-3.00)
G2 – NaOCl 1% + CLX 2%	2.20 (0.00-3.00)
G3 – NaOCl 2.5% + CLX 2%	1.86 (0.00-3.00)
G4 – NaOCl 5.25% + CLX 2%	2.10 (0.00-3.00)
G5 – NaOCl 2.5%	1.46 (0.00-3.00)
G6 – CLX 2%	1.26 (0.00-3.00)

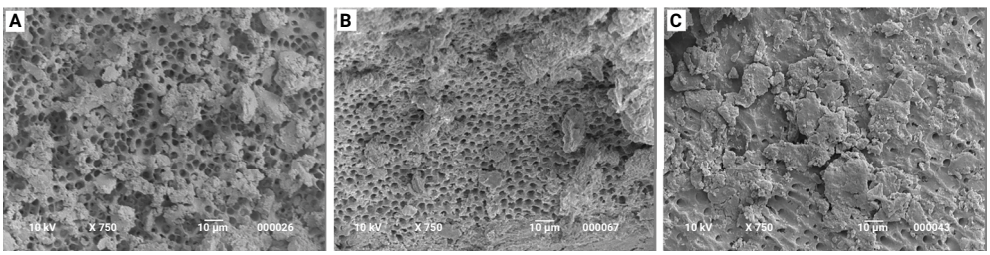

Figure 5. SEM images (magnification 750x) of dentin surface after irrigants association showing precipitate formation in the cervical (a) and middle (b) thirds.

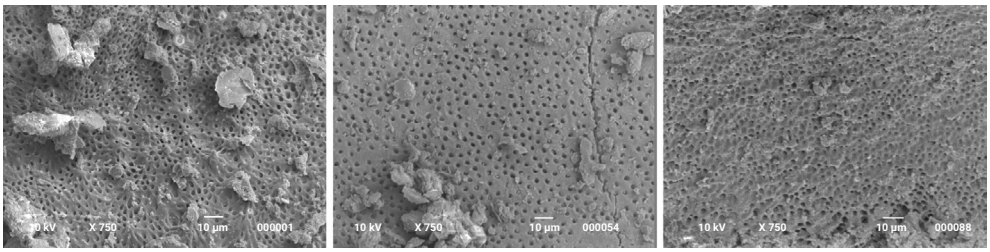

Figure 6. SEM images (magnification 750x) of precipitate formation in the apical third of several specimens, but in smaller amounts.

DISCUSSION

The present study evaluated the chemical residue formed by the association of irrigants commonly used in endodontic therapy[11]. This knowledge is important because this association results in a *smear layer* with the potential to obliterate the dentinal tubules, compromising the sealing of the root canal during obturation[14,16,17], in addition to aesthetically compromising some roots. The null hypothesis was partially accepted since there were no statistical differences between the groups in the analyses carried out, but there was a tendency for greater deposition of residues and pigmentation as the NaOCl concentration increased.

In the spectrophotometric analysis, the values of L, a and b established by the CIEE (International Lighting Commission, 1913) were observed to detect color changes (ΔE) using the formula ΔE = [(ΔL) 2 + (Δa) 2 + (Δb) 2] 1/2, concerning the established reading protocols. The findings of the present study are in agreement with other results presented in the literature[14,19]. When the association of NaOCl in concentrations of 0.5% to 5.25% with the 2% CLX solution was carried out, it was possible to observe the formation of dense chemical residue, whose intensity of the staining varied according to the concentration of NaOCl used. When T0 (before final irrigation) and T1 (immediately after final irrigation) were compared, the results in the spectrophotometry showed an increase in ΔE in most of the tested groups, although without statistically significant differences. G1 showed the highest standard deviation when compared to the other groups, which can be associated with chemical instability of 0.5% NaOCl. However, values of greater variations of ΔE were detected for G4 where the association of 5.25% NaOCl + 2%CHX was used. These results reinforce the findings of the literature[6,19].

In the results obtained in T1 (just after the final irrigation) and T2 (24 hours after the final irrigation), the G1, G2, G3, G5, and G6 groups showed an increase in ΔE, diverging from G4, which showed a reduction in ΔE between the times. The result obtained in G4 suggests that the color change did not last due to the low stability of sodium hypochlorite in high concentrations, which rapidly loses active chlorine, as stated in the study by Clarkson and Moule[28] (1998). The control groups, G5 and G6, had their concentrations selected based on the concentrations of the solutions with the greatest scientific relevance[29]. In the evaluation of ΔE between the times, they presented lower values concerning the formation of residues, without statistical differences between them.

In the analysis by scanning electron microscopy, residue formation was found in all groups of the present study to a greater or lesser degree, however, there was no statistical difference between the groups[25]. In the cervical and middle thirds of G1, G2, G3, and G4, there was a greater amount of chemical residue formed (Figure 5) when compared to the apical third of each group (Figure 6), corroborating the findings in the literature[6,19]. G2 was the group that showed the greatest evidence of the chemical residue formed, a fact that may be associated with the use of stabilizers in the formulation of 1% NaOCl[30] (Figure 3). Its presence is probably responsible for less loss of active chlorine and, therefore, there is a greater formation of final chemical residue.

Our findings are in line with other studies in the literature[19,31,32], showing that this chemical residue from the association of these two different irrigants obstructs the dentinal tubules. Although the association of sodium hypochlorite with chlorhexidine in the final irrigation may result in positive synergistic effects on antimicrobial activity[11], it triggers the formation of a chemical precipitate capable of altering dentin staining and obstructing the dentinal tubules.

According to the limitations of this *in vitro* study, it was concluded that there was the formation of a precipitate, in greater concentration in the cervical and middle thirds of the root canal, and pigmentation in all concentrations of NaOCl, when associated with 2% chlorhexidine, observing that the pigmentation in extracted teeth, remains for at least 24 hours. Clinically, the use of sodium hypochlorite followed by chlorhexidine is not recommended without the use of an intermediate solution, such as saline or distilled water.

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