Antagonistic Interactions between Sodium Hypochlorite, Chlorhexidine, EDTA, and Citric Acid

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Abstract

Introduction: Root canal irrigants play a significant role in the elimination of microorganisms, tissue dissolution, and the removal of debris and smear layer. No single solution is able to fulfill these actions completely; therefore, their association is required. The aim of this investigation was to review the antagonistic interactions occurring when sodium hypochlorite (NaOCl), chlorhexidine (CHX), EDTA, and citric acid (CA) are used together during endodontic treatment. **Methods:** A search was performed in the electronic database Medline (articles published through 2011; English language; and the following search terms or combinations: "interaction AND root canal irrigant or endodontic irrigant or sodium hypochlorite or chlorhexidine," "sodium hypochlorite AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent or chlorhexidine," and "chlorhexidine AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent") to identify publications that studied unwanted chemical interactions between NaOCl, CHX, and EDTA and CA. **Results:** The search identified 1,285 publications; 19 fulfilled the inclusion/exclusion criteria of the review. Their research methodology was classified as either *in vitro* or *ex vivo*. **Conclusions:** Antagonistic interactions included the loss of free available chlorine for NaOCl when in contact with chelators, which consequently reduced the tissue dissolution capability and to a lesser extent antimicrobial activities. When CHX and NaOCl are mixed, a precipitate forms that can present detrimental consequences for endodontic treatment, including a risk of discoloration and potential leaching of unidentified chemicals into the periradicular tissues. CHX and EDTA mixtures cause a precipitate, whereas CHX and CA do not exhibit interaction. *(J Endod 2012;38:426–431)*

Key Words

Chlorhexidine, citric acid, EDTA, endodontic irrigant, interaction, root canal irrigants, sodium hypochlorite

Root canal cleaning and disinfection during chemomechanical preparation relies heavily on irrigants because of the anatomic complexities of the pulp canal system. Irrigants should ideally have antimicrobial and tissue-dissolution actions as well as other advantageous properties, such as lubrication, demineralization, and the ability to remove debris and the smear layer (1).

Sodium hypochlorite (NaOCl) is recommended as the main endodontic irrigant because of its ability to dissolve organic matter together with its broad antimicrobial action (2). NaOCl is commercially available as aqueous solutions with concentrations ranging from 1% to 15% and having an alkaline pH with values around 11 (3). Among other salts, they also contain sodium hydroxide salts in order to increase their stability (3), and they might contain surfactants as well as other components that are not always disclosed by the manufacturer (4).

No irrigation solution has been found capable of demineralizing the smear layer and dissolving organic tissue simultaneously (5). Therefore, the adjunctive use of chelating agents such as EDTA or citric acid (CA) is suggested in order to remove and prevent the formation of the smear layer associated with root canal instrumentation (2).

EDTA is a polyprotic acid whose sodium salts are noncolloidal organic agents that can form nonionic chelates with metallic ions (2, 6). Its solutions are normally used at concentrations between 10% and 17%, and its pH is modified from its original value of 4 (7) to values between 7 and 8 to increase its chelating capacity (2, 6). Like many well-known chelating agents, EDTA exists in aqueous solutions as an equilibrium mixture of both protonated and unprotonated forms. CA is an organic acid normally used in endodontics at concentrations between 10% and 50% (2) with a pH value between 1 and 2 (8).

Although the role of smear layer removal has been widely debated, endodontic literature concerning the antimicrobial action of irrigants suggests that the combined use of EDTA and NaOCl is more efficient than NaOCl alone when measuring bacterial survival after multiple appointments (9); bacterial survival analysis is a surrogate measure of treatment outcome. A recently published outcome investigation indicated that 2.5% to 5% NaOCl followed by 17% EDTA had a profoundly beneficial effect on secondary nonsurgical root canal treatment success while having a marginal effect on the original treatment (10).

It has been suggested that variations of NaOCl pH will modify the antimicrobial and tissue-dissolution activities (11). A reduction of the pH to values around 6.0 to 7.5 has been found to improve the antimicrobial efficacy (11–13) but hinders tissue-dissolution action (11, 13–15). If the pH is lowered to values below 4, then the amount of chlorine gas in the solution will increase (16). Chlorine in gas form is volatile and therefore unstable (17). If NaOCl is mixed with other irrigants possessing low pH values, there is a possibility of altering its properties.

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Chlorhexidine (CHX), a bisguanide, is stable as a salt although it dissociates in water at a physiologic pH, releasing the CHX component (18). It is frequently used at concentrations between 0.2% and 2% (2) and exhibits an optimal antimicrobial activity at a pH of 5.5 to 7.0 depending on the buffering agent used and the organism studied (19). The most common preparation is CHX gluconate (20). It has been recommended that CHX be used as either an alternative or an adjunct root canal irrigant because of its antimicrobial qualities. Studies comparing its antimicrobial action versus NaOCl solutions present conflicting results (10, 21–29).

Some investigations suggest that NaOCl is more effective as an antimicrobial agent compared with CHX. One *in vivo* study showed 2.5% NaOCl was a more effective antimicrobial agent compared with 0.2% CHX (21). However, an *in vitro* study (22) using a bovine root model showed that CHX had a similar antimicrobial effect as NaOCl, whereas another investigation into bovine dentinal tubule disinfection comparing NaOCl and CHX 0.2% to 2% found no difference in antimicrobial efficacy between either solution at these concentrations (23). Similarly, an *ex vivo* investigation found no statistically significant difference when comparing 5.25% NaOCl and 2% CHX (24). Contemporary *in vivo* studies comparing 2.5% NaOCl and 0.12% CHX and their ability to reduce the numbers of cultivable bacteria (25) and the presence of bacteria, archaea, and fungi on teeth with apical periodontitis using molecular microbiology procedures (26) suggest no difference in effectiveness between the solutions. On the contrary, an *in vivo* investigation into the percentage of growing bacterial species after irrigation with 5.25% NaOCl or 2% CHX in teeth with pulpal necrosis, apical pathosis, or both found the latter to be significantly more effective at reducing growth (27). Some characteristics of the irrigants investigated are summarized in Table 1.

A seminal investigation comparing the use of 0.2% CHX and 2.5% NaOCl individually and in combination in human teeth presenting with periapical radiolucencies suggested that their combined use produced the greatest percentage reduction in cultures (28). Equally, the addition of 2% CHX to 1% NaOCl in teeth with infected necrotic pulps was found to enhance the disinfection of the root canal system because of the reduction of cultivable bacteria in those cases (29). Ng et al (10) in their outcome investigation suggested that the use of 0.2% CHX in addition to NaOCl significantly reduces the success rate in nonsurgical treatment (10). CHX lacks tissue dissolution capacity (30), an important quality desired from root canal irrigants.

It has been purported that the application of CHX before the application of adhesives prevents resin-dentin bond degradation because of its ability to inhibit collagenolytic enzymes (31). Concerns about the longevity of bonding to root canal dentin have been raised for bonded root filling techniques and resin cemented posts (32) so this should be taken into consideration when resin-based sealers are used even when gutta-percha is used as the core material.

Commercially available NaOCl solutions have an alkaline pH value, with the hypochlorite ion being the main chlorine species present (16). The chemical interactions of NaOCl with EDTA or CHX are redox reactions, with molecular groups being oxidized by NaOCl (20, 33); an acid-base reaction occurs when CHX and NaOCl are mixed because CHX has the ability to donate protons as a positive component, whereas NaOCl can accept them (20, 28, 34). In regard to EDTA associated with CHX, it may potentially degrade CHX, forming a salt (35). CA and CHX apparently pose no antagonistic reactions (36). Therefore, the purpose of this article was to review the undesired effects after interactions between NaOCl, CHX, and the commonly used chelating agents EDTA and CA.

Materials and Methods

A literature search using electronic database Medline was conducted on June 15, 2011, for articles published through to the date using the following search terms and combinations: "interaction AND root canal irrigant or endodontic irrigant or sodium hypochlorite or chlorhexidine," "sodium hypochlorite AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent or chlorhexidine," and "chlorhexidine AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent." Publications were included if they studied antagonistic interactions between NaOCl, CHX, EDTA, and CA by comparing 1 of the solutions against a mixture of them and were published in English. Titles and abstracts of the publications identified were initially screened by 2 independent reviewers (G.R.F. and E.J.D.). Publications were included for full-text evaluation by 1 reviewer (G.R.F.) if the content of the abstracts met the inclusion criteria. Full-text assessment and data extraction were performed by 1 reviewer (G.R.F.). Publications were excluded if they did not meet the inclusion criteria (ie, if they did not study antagonistic interactions between NaOCl, CHX and CA or EDTA by comparing 1 of these alone and when combined with a substance mentioned previously) or if they were not published in English. Of 1,285 publications identified, 19 were included in the review.

Interactions between NaOCl and Chelating Agents

The addition of chelators to NaOCl reduces its pH in a ratio and time-dependent manner (37–39). This affects the forms of free chlorine in the solution and causes an increase in hypochlorous acid and chlorine gas, which subsequently reduces the amount of the hypochlorite ion (3, 11). When 1% NaOCl was mixed with 17% EDTA (pH = 8) in ratios of 1:1, 1:5, and 5:1, the pH of the solutions ranged between 8.0 and 8.4 (37). The addition of 10% CA to 1% NaOCl in the same ratios resulted in pH values between 1.8 and 4.3 (37). Another study mixed 1% to 2% NaOCl with 17% EDTA in equal proportions, resulting in a final pH value of 8.0 from an initial value of 10 after an elapse of 48 hours. However, when mixed in a 1:3 ratio, although with a larger volume of EDTA, the pH value was stable during the 48-hour experimental time, probably because of an immediate interaction between the solutions (38).The reduction of pH values in the NaOCl solution causes the release of chlorine gas, which has potentially hazardous effects on humans (39). When EDTA is added to NaOCl, chlorine gas can be detected at relatively low levels. When CA is used, significantly more chlorine is detectable and present at a further distance. This is according to a laboratory-based investigation that studied the reactions between NaOCl (5.25%, pH = 12.12) and CA (50%,

TABLE 1. Characteristics of Some Root Canal Irrigants

Compound	Chemical structure	Type	Concentration of solution (%)	Typical pH of solutions	Commonly used oral preparation
Sodium hypochlorite	NaOCl	Chlorine-releasing agent	0.5 to 15	9 to 12	
EDTA	$C_{10}H_{16}N_2O_8$	Polyprotic acid	10 to 17	7 to 8	EDTA disodium salt
CHX	$C_{22}H_{30}Cl_2N_{10}$	Bisguanide	0.2 to 2	5.5 to 7	CHX (di)gluconate
CA	$C_6H_8O_7$	Organic acid	10 to 50	1 to 2	

pH = 1.28) or EDTA (15%, PH = 7.51). Portions of the chelator were added to the NaOCl at regular time intervals for a total time period of 2 hours; the release of chlorine gas was measured at 6 inches and 6 feet from the container (39).

The consequences of chemical interactions between chelating agents and NaOCl result in a loss in the free available chlorine (FAC) of the mixtures (6, 37, 40). The effects on FAC contents in a 1% NaOCl solution were assayed by mixing it with either 17% EDTA or water (1:1) and measured via an iodine/thiosulfate titration method. NaOCl's FAC was substantially modified by the presence of EDTA with a reduction to 0.06% when compared with 0.5% of the water dilution control (6). This research group subsequently looked into the impact on available chlorine in 1% NaOCl from the interaction with 17% EDTA (pH = 8) and 10% CA using the same methodology and taking into account the time factor. Their results indicated that when mixed with CA, FAC decreased to 0 in less than a minute, whereas EDTA required between 1 and 60 minutes to reduce the FAC to the same level (37). These results were confirmed by a different research group that looked into time-related effects (between 5 and 18 minutes) on active chlorine content because of dilution with a EDTA solution (17%, pH = 7.5, and containing a surfactant). Different NaOCl preparations (1%, 1.5%, 4%, and 4.5% with some containing surfactant) and NaOCl:EDTA ratios (9:1, 3:1, and 1:1) were tested via iodometric titration. Apart from modifications because of dilution, the available chlorine loss was extreme (ie, up to 80% even when adding small amounts of EDTA at the early stages of the process and then becoming more gradual, indicating that a chemical reaction occurs between the solutions). The presence of a surfactant made the reduction of the available chlorine less marked in time and was a far better predictor than the original concentration for chlorine loss (4). When gel-type preparations of chelators containing 15% EDTA and 10% Urea peroxide (RC-Prep; Premier Dental, Philadelphia, PA, and Glyde; DeTrey Dentsply, Konstanz, Germany) were tested for interaction with 1% NaOCl using spectroscopy, it was shown that both compounds depleted the solution from its chlorine content after 5 minutes (40).

The dramatic reduction of FAC in NaOCl mixtures caused by chemical interactions appears to explain the inability of NaOCl and EDTA mixtures to dissolve soft tissues. An investigation looking into bovine tissue dissolution of NaOCl (1%-2.5%) alone and combined with 17% EDTA in different ratios (2:2 and 1:3) showed that after 48 hours only unmixed NaOCl was able to completely dissolve the tissue (38). Similarly, the tissue dissolution effects of the interactions between NaOCl and EDTA were tested on porcine palatal mucosa by assessing the percentage of original tissue weight after different exposure periods up to 120 minutes; 8.5% EDTA, 0.5% NaOCl, and a 1:1 mixture of 17% EDTA together with 1% NaOCl were the test solutions. This investigation suggested that NaOCl alone was substantially more efficient than the other groups, with no statistically significant differences among them (6).

The degradation and consequent deactivation of EDTA after its interaction with NaOCl is extremely slow, and, therefore, it does not compromise its clinical performance with respect to its chelating, smear layer removal, and dentin softening effects (6, 7, 37). This phenomenon has been analyzed via nuclear magnetic resonance with no reactions detected in the first 7 minutes, and the process was not complete after 120 minutes (33).

NaOCl does not reduce the calcium chelating or smear layer ability of EDTA and CA (6, 7, 37). An investigation, using standardized dentin portions immersed into solutions of either 17% EDTA and distilled water or 17% EDTA and 0.5% NaOCl, found greater calcium chelation occurring in the solution containing NaOCl (7). Similarly, the chelating ability of 17% EDTA alone and in a mixture with 5% NaOCl (9:1 mixture) was compared using a calcium titration method in order to assess the amount of chelated calcium per mole of EDTA. The results indicated that NaOCl had little effect on EDTA's calcium chelating ability (6). Another investigation from the same research group studied calcium chelation and smear layer removal from root canals after irrigation *ex vivo* (37). Human single-rooted teeth were instrumented and subsequently irrigated with mixtures of the chelating agents and 1% NaOCl or water at a 1:1 ratio. After irrigation, the solutions were analyzed for their calcium content using atomic absorption spectrophotometry. No statistically significant differences for EDTA or CA were found between the combinations containing water or NaOCl. The teeth were subsequently split and observed using a scanning electron microscope for the presence or absence of smear layer in a semiquantitative manner; no differences were found among the irrigant combinations described earlier (37). The addition of NaOCl to EDTA does not alter EDTA's ability to decalcify human dentin, and this has been shown through studies assessing Vickers microhardness after adding either NaOCl or distilled water to EDTA in a 1:1 ratio and observing for 7 minutes (7).

Chelators can eliminate NaOCl's antimicrobial efficacy if the original FAC values are modest, whereas EDTA and CA performance does not seem to be jeopardized because of interactions with NaOCl (6, 37). The effects on antimicrobial ability, related to the interactions between EDTA and NaOCl, have been analyzed using an agar diffusion test against *Enterococcus faecalis* and *Candida albicans* using 0.5% NaOCl, 8.5% EDTA, and a mixture with 1% NaOCl and 17% EDTA (1:1 mixture) (6). Pure NaOCl produced smaller zones of inhibition when compared with pure EDTA or the mixture of EDTA/NaOCl, and there were no statistically significant differences among the EDTA-containing groups (6). An *in vitro* investigation testing the impact of CA and EDTA on NaOCl's antimicrobial action against was performed by the same group. *E. faecalis* was suspended in phosphate buffered saline and then added (1:1) to tubes containing chelating agent mixtures with 1% NaOCl and their 1:10 and 1:100 dilutions; after incubation, it was found that 10% CA and 17% EDTA eliminated NaOCl's antimicrobial action at the 1:100 dilutions because growth was present (37).

Interactions between NaOCl and CHX

From the review of the literature, it transpires that mixing NaOCl with liquid CHX results in the instant formation of a flocculate or precipitate (41–49). Basrani et al (41) looked into the minimum NaOCl concentration required to form a precipitate when mixed with 2% CHX (41). Concentrations ranging from 0.023% to 6% were tested, and an instant color change occurred in all samples from dark brown to light orange. A precipitate was induced with 0.19% NaOCl with varying amounts of material formed in the different mixtures (41).

Several investigations have been undertaken to elucidate the chemical composition of the flocculate produced by the association of NaOCl with CHX (41–45, 47–49). Different proportions and concentrations of NaOCl (0.5%, 2.5%, and 5%) and CHX (0.2%–2%) have been mixed, which results in the formation of a brownish flocculate evident when the solutions make contact with each other; atomic absorption spectrophotometry showed the presence of Ca, Fe, and Mg (42). Although most investigations report the presence of parachloroaniline (PCA) in the precipitate, 1 failed to detect its presence. The precipitate was analyzed using X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry, which detected that PCA was present at concentrations directly related to the NaOCl concentration (41). The same researchers used gas chromatography-mass spectrometry in order to further identify the precipitate composition after the mixture of 6% NaOCl with 2% CHX; PCA was detected again although no further aniline derivatives or chlorobenzene were found (43). Krishnamurthy and Sudhakaran (44) mixed 2.5% NaOCl with 2% CHX and were able to detect PCA in the precipitate by using Beilstein and HCl solubility tests followed by nuclear magnetic resonance. Despite Thomas and Sen (45) using nuclear magnetic resonance spectroscopy, they failed to detect PCA in the precipitate after combining 5.25% NaOCl with 2% CHX acetate. PCA has been suggested to be a toxic and carcinogenic substance, hence the significance of this subject (46).

Three studies have evaluated the cleaning efficacy after irrigation with CHX containing solutions (44, 47, 48). Bui et al (47) investigated the influence of irrigation on debris removal and patent dentinal tubules using 5.25% NaOCl and 2% CHX *ex vivo* and analyzed it with an environmental scanning electron microscope (47). The test group involved irrigation initially with NaOCl, which was either left inside the root canal or aspirated and dried with paper points; after which CHX irrigation was performed. The positive control group consisted of irrigation solely with NaOCl and then aspiration and drying with paper points. There was no difference in remaining debris and a reduction in number of patent dentinal tubules in the coronal and middle third between the 2 test groups. A scanning electron microscopic investigation into the percentage of open and closed tubules after root canal instrumentation on human teeth using 2.5% NaOCl and 2% CHX in liquid or gel forms, intercalated by physiologic saline, with half of the experimental groups receiving a final flush with 17% EDTA was performed by Valera et al (48). Their results indicated that 2% CHX gel produced the largest amount of open dentinal tubules, whereas 2% CHX liquid presented the worst result. The addition of EDTA and physiologic saline as a final flush improved cleaning and debris removal. The presence and thickness of the precipitate formed after irrigation with 17% EDTA followed by 2.5% NaOCl and a final flush with 2% CHX (test group) was compared against the same sequence, but they were intercalated between these other solutions to assess their ability to reduce formation of the precipitate (44). This was performed on *ex vivo* root canals and examined with a stereomicroscope (44). Isopropyl alcohol resulted in completely clean canals, whereas the use of saline or distilled water produced a sparse precipitate. The test group presented deposits all along the canal wall with a mean thickness 2 to 3 times greater than that of the saline and distilled water groups. The precipitate was present mainly in the coronal and middle thirds of the canals.

This precipitate has an effect on dentinal permeability (34) and dye penetration after root canal obturation (49). An *ex vivo* investigation compared the effects of combining 1% NaOCl and 2% CHX on dentinal permeability as measured by Rhodamine leakage in percentage (34). When compared against a "no irrigation" control, the mixture of NaOCl and CHX caused a reduction of permeability only in the apical third. This was explained by the formation of a brown mass suspended in the liquid that becomes a flocculate precipitate, which acts as a "chemical smear layer." Another *ex vivo* investigation assessed dye penetration in clear teeth after preparation using different irrigants and obturation (49). The results suggested that a precipitate formed when combining 1% NaOCl and 2% CHX gel, which stained the dentin and adhered to the canal walls. Therefore, this group presented the largest values of linear dye penetration. Statistically significant differences were found with the other groups, which included NaOCl alone, NaOCl and EDTA, CHX gel alone, and distilled water.

Interactions between CHX and Chelating Agents

CHX is easily mixed with CA, and no modification of its demineralizing ability or precipitation occurs (34, 36). An *in vitro* study on bovine dentin slices using atomic absorption spectrophotometry looked into the effect of adding 1% CHX and 10% to 20% CA on the

TABLE 2. Reported Interactions between Root Canal Irrigants

Mixture	Reaction product or byproduct	Undesirable result	Chemical reaction	First author	Journal	Year	Issue and page numbers
NaOCl + EDTA	Cl_2 (gas)	Loss on active chlorine content	Breakdown of NaOCl al low pH	Grawehr	J Endod	2003	36:411–5
				Zehnder	J Endod	2005	31:817–20
				Zehnder	J Endod	2006	32:389–98
				Baumgartner	J Endod	1987	13:47–51
				Clarkson	J Endod	2011	37:538–43
	Glioxilic acid; EDTA	Degradation of EDTA molecule	Oxidation of EDTA (after 7 minutes of reaction)	Grande	J Endod	2006	32:460–4
NaOCl + CHX	Orange-brown precipitate	Potentially Toxic compound; color change	Redox reaction	Krishnamurthy	J Endod	2010	36:1154–7
				Basrani	J Endod	2007	33:966–9
				Vivaqua-Gomes	Int End J	2002	35:791–5
				Zehnder	J Endod	2006	32:389–98
				Bui	J Endod	2008	34:181–5
				Marchesan	Oral Surg Oral Med Oral Pathol Oral Radiol Endod	2007	103:e103–5
NaOCl + CA	Cl_2 (gas)	Loss on active chlorine content	Breakdown of NaOCl al low pH	Baumgartner	J Endod	1987	13: 47–51
CHX + EDTA	Salt; white precipitate	Chemical degradation of CHX	Salt formed by neutralization between CHX and EDTA	Rasimick	J Endod	2008	34:1521–3
CHX + CA			No reaction known	González-Lopez	J Endod	2006	32:781–4

demineralizing capacity of the chelator (36). The results after 3, 10, and 15 minutes of immersion showed no alteration of the decalcifying effect (36). Another study looking into modification of dentinal permeability after the irrigation of human teeth found no statistically significant differences when compared against the "no irrigation" group although statistically significant differences were found when compared against the NaOCl and CHX group in the apical third of the root canal (34). It has been shown that 15% CA followed by 2% CHX causes the formation of a "milky" solution, which can be easily removed by using further CHX; no precipitation occurs (34).

When mixing CHX with EDTA, it is difficult to obtain a homogeneous solution; a precipitate composed chiefly of the original components forms (35, 36). It has been shown that it is not possible to obtain a homogenous solution by mixing 17% EDTA and 1% CHX because a highly insoluble pink powdery precipitate forms (36). An investigation using reverse-phase high-performance chromatography analyzed the precipitate that forms after the combination of 17% EDTA with 2% or 20% CHX in equal volumes and 3 different mixing conditions (35). Over 90% of the precipitate mass was either EDTA or CHX although PCA was not detected. It was suggested that the precipitate was most likely a salt formed by neutralization of the cationic CHX by anionic EDTA (36).

Discussion

This literature review highlights the importance of clinicians having a comprehensive understanding of possible antagonistic interactions among endodontic irrigants used in their routine clinical practice. Because these solutions are used in succession, they come into contact with each other inside the endodontic space. This might impact treatment due to the modifications to tissue dissolution, antimicrobial and cleaning efficacy, seal, the risk of discoloration, and most importantly the potential adverse effects to a patient's general health as a consequence of leaching chemicals in the periradicular tissues. Table 2 summarizes the deleterious effects of the associations described earlier.

Preventive Strategies

Apart from avoiding use of the aforementioned chemicals together to prevent or reduce the occurrence of the detrimental reactions described, the following strategies have been suggested:

1. NaOCl and EDTA: rinse out with copious amounts of NaOCl (37), making sure that fluid exchange occurs at all levels in the canal to prevent stratification of the solutions through the canal, which will lead to different mixtures of the irrigants at different levels (4). Alternatively, evacuation or drying before the placement of the next irrigant (4) can also help.
2. NaOCl and CHX: to prevent the formation of a precipitate associated with CHX and NaOCl interactions, a rinse with intermediate solutions after NaOCl has been suggested. They include saline (48); water (47, 48); alcohol (42, 47); isopropyl alcohol (44); or a demineralizing solution, which can be CA (34) or EDTA (42). Finally, if the flocculate is formed, then acetic acid can be used to dissolve the precipitate (41).
3. CHX and chelating agents: CA can be used in association with CHX because no interactions occur (34, 36). Alternatively, maleic acid can be used because it has been shown that this combination does not cause the formation of a precipitate, and only a marginal reduction of CHX availability occurs (50).

In summary, chelating agents have a dramatic effect on the free available chlorine contents of NaOCl and subsequently on its tissue dissolution capability, whereas its antimicrobial effect is reduced only when the initial NaOCl concentrations are modest. EDTA and CA do not suffer from a reduction of their chelating ability in mixtures containing NaOCl. CHX- and NaOCl-containing solutions develop a precipitate that might contain toxic substances that have an influence on root canal cleaning; however, further research is required to better understand its nature. When mixing CHX and EDTA, it is difficult to obtain a homogenous solution, and a precipitate composed mainly of those substances is formed. CA is not influenced by CHX, and no precipitate is formed when mixed with it.

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References

1. Gutmann JL, Dumsha TC, Lovdahl PE. *Problem Solving in Endodontics*. 4th ed. St Louis, MO: Elsevier; 2006.
2. Zehnder M. Root canal irrigants. J Endod 2006;32:389–98.
3. Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin Microbiol Rev 1997;10:597–610.
4. Clarkson RM, Podlich HM, Moule AJ. Influence of ethylenediaminetetraacetic acid on the active chlorine content of sodium hypochlorite solutions when mixed in various proportions. J Endod 2011;37:538–43.
5. Baumgartner JC, Brown CM, Mader CL, et al. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. J Endod 1984;10:525–30.
6. Grawehr M, Sener B, Waltimo T, et al. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J 2003;36:411–5.
7. Saquy PC, Maia Campos G, Sousa Neto MD, et al. Evaluation of chelating action of EDTA in association with Dakin's solution. Braz Dent J 1994;5:65–70.
8. Haznedaroğlu F. Efficacy of various concentrations of citric acid at different pH values for smear layer removal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:340–4.
9. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35–40.
10. Ng Y-L, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of non surgical root canal treatment: part 1: periapical health. Int Endod J 2011;44:583–609.
11. Rossi-Fedele G, Guastalli AR, Doğramacı EJ, et al. Influence of pH changes on chlorine-containing endodontic irrigating solutions. Int Endod J 2011;44:792–9.
12. Mercade M, Duran-Sindreu F, Kuttler S, et al. Antimicrobial efficacy of 4.2% sodium hypochlorite adjusted to pH 12, 7.5 and 6.5 in infected root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:295–8.
13. Camps J, Pommel L, Aubut V, et al. Shelf life, dissolving action, and antibacterial activity of a neutralized 2.5% sodium hypochlorite solution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:e66–73.
14. Christensen C, McNeal ST, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue *in vitro*. J Endod 2008;34:449–52.
15. Aubut V, Pommel L, Verhille B, et al. Biological properties of a neutralized 2.5% sodium hypochlorite solution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e120–5.
16. Fair GM, Morris JC, Chang SL, et al. The behaviour of chlorine as water disinfectant. J Am Water Works Assoc 1948;1:1051–61.
17. Lee SV, Hung YC, Chung D, et al. Effects of storage and pH on chlorine loss in Electrolyzed Oxidizing (EO) water. J Agric Food Chem 2000;50:209–12.
18. Greenstein G, Berman C, Jaffin R. Chlorhexidine: and adjunct to periodontal therapy. J Periodontol 1986;57:370–6.
19. Block S, Seymour B. Disinfectant and antiseptic. *Kirk-Othmer Encyclopedia of Chemical Technology*. New York: Wiley & Sons; 1998.
20. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J 2009;42:288–302.
21. Ringel AM, Patterson SS, Newton CW, et al. *In vivo* evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. J Endod 1982;8:200–4.
22. Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J 1998;31:8–14.
23. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules *in vitro*. Endod Dent Traumatol 1993;9:243–8.
24. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod 1994;20:276–8.
25. Siqueira JF, Roças IN, Paiva SSM, et al. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:122–30.

26. Roças IN, Siqueira JF. Comparison of the *in vivo* antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. J Endod 2011;37:143–50.
27. Ercan E, Özenkici T, Atakul F, et al. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: *in vivo* study. J Endod 2004;30:84–7.
28. Kuruvilla JR, Kamath P. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod 1998;24:472–6.
29. Zamany AZ, Safavi K, Spångberg LS. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol 2003;96:578–81.
30. Okino LA, Siqueira EL, Santos M, et al. Dissolution of pulp tissue by acqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. Int Endod J 2004;37:38–41.
31. Liu Y, Tjäderhane L, Breschi L, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res 2011;90:953–68.
32. Santos J, Carrilho M, Tervahartiala T, et al. Determination of matrix metalloproteinases in human radicular dentin. J Endod 2009;35:686–9.
33. Grande NM, Plotino G, Falanga A, et al. Interaction between EDTA and sodium hypochlorite: a nuclear magnetic resonance analysis. J Endod 2006;32:460–4.
34. Akisue E, Tomita V, Gavini G, et al. Effect of the combination of sodium hypochlorite and chlorhexidine on dentinal permeability and scanning electron microscopy precipitate observation. J Endod 2010;36:847–50.
35. Rasimick BJ, Nekich M, Hladek M, et al. Interaction between chlorhexidine digluconate and EDTA. J Endod 2008;34:1521–3.
36. Gonzalez-Lopez S, Cornejo-Aguilar D, Sanchez-Sanchez P, et al. Effect of CHX on the decalcifying effect of 10% citric acid, 20% citric acid, or 17% EDTA. J Endod 2006;32:781–4.
37. Zehnder M, Schmidlin P, Sener B, et al. Chelation in root canal reconsidered. J Endod 2005;31:817–20.
38. Irala LED, Grazziotin-Soares R, Azevedo Salles A, et al. Dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA. Braz Oral Res 2010;24:271–6.
39. Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridement. J Endod 1987;13:47–51.
40. Girard S, Paque F, Badertscher M, et al. Assessment of a gel-type chelating preparation containing 1-hydroxyethylidene-1, 1 biphosphonate. Int Endod J 2005;38:810–6.
41. Basrani BR, Manek S, Sodhi RN, et al. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod 2007;33:966–9.
42. Marchesan MA, Pasternak Junior B, Afonso MM, et al. Chemical analysis of the flocculate formed by the association of sodium hypochlorite and chlorhexidine. Oral Surg Oral Med Oral Pathol 2007;103:e103–5.
43. Basrani BR, Manek S, Mathers D, et al. Determination of 4-chloroaniline and its derivatives formed in the interaction of sodium hypochlorite and chlorhexidine by gas chromatography. J Endod 2010;36:312–4.
44. Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine. J Endod 2010;36:1154–7.
45. Thomas JE, Sem DS. An *in vitro* spectroscopic analysis to determine whether parachloroaniline is produced from mixing sodium hypochlorite and chlorhexidine. J Endod 2010;36:315–7.
46. Chhabra RS, Huff JE, Haseman JK, et al. Carcinogenicity of p-chloroaniline in rats and mice. Food Chem Toxicol 1991;29:119–24.
47. Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod 2008;34:181–5.
48. Valera MC, Chung A, Menezes MM, et al. Scanning electron microscope evaluation of chlorhexidine gel and liquid associated with sodium hypochlorite cleaning on the root canal walls. Oral Surg Oral Med Oral Pathol 2010;110:e82–7.
49. Vivacqua-Gomes N, Ferraz CCR, Gomes BPFA, et al. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root filling. Int Endod J 2002;35:791–5.
50. Ballal NV, Moorkhot S, Mala K, et al. Evaluation of chemical interactions of maleic acid with sodium hypochlorite and chlorhexidine gluconate. J Endod 2001;37:1402–5.