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Research Article

Ethylenediaminetetraacetic Acid as an Irrigant between 5% Sodium Hypochlorite and 2% Chlorhexidine in the Formation of Para-chloroaniline Related Precipitate

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Abstract

Objective: This *in vitro* study evaluated the effect of ethylenediaminetetraacetic acid as an intermediate irrigant to prevent the formation of para-chloroaniline related precipitate when reacting sodium hypochlorite and chlorhexidine.

Material and Methods: Different concentrations of sodium hypochlorite and ethylenediaminetetraacetic acid were mixed. 2% Chlorhexidine was added and the precipitate formation was visualized. Formed para-chloroaniline was quantified by spectrophotometry.

Results: Combinations of 0.05%, 0.5% and 5% sodium hypochlorite, 2% Chlorhexidine and most ethylenediaminetetraacetic acid dilutions formed precipitates of assorted colors. Mixtures of 0.05% sodium hypochlorite with 0.0010%, 0.0017%, 0.010% and 0.017% ethylenediaminetetraacetic acid plus 2% Chlorhexidine did not form any kind of precipitate. Extrapolation indicates that an ethylenediaminetetraacetic acid concentration of 0.03278% would prevent the formation of para-chloroaniline at a sodium hypochlorite concentration of 0.05%.

Conclusion: Ethylenediaminetetraacetic acid as an intermediate irrigant between sodium hypochlorite and chlorhexidine partially inhibits the formation of para-chloroaniline in a direct concentration ratio: the higher the concentration, the greater the formation inhibition of para-chloroaniline related precipitate.

Keywords: *Chlorhexidine; Ethylenediaminetetraacetic Acid; Para-Chloroaniline; Sodium Hypochlorite; Spectrophotometry*

Abbreviations

EDTA: Ethylenediaminetetraacetic Acid; PCA: Para-Chloroaniline; NaOCl: Sodium Hypochlorite; CHX: Chlorhexidine; RCS: Root Canals System

Introduction

Root canal cleaning and disinfection during chemomechanical preparation relies heavily on different irrigants used under specific protocol because one alone does not meet the requirements 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. When used as an irrigant, NaOCl concentrations ranging between 0.5 and 6% and an alkaline pH with values around 11 are required [2]. However, NaOCl is unable to remove the smear layer as a whole. It can eliminate the organic part of it, and should be associated with a subsequent irrigation with EDTA or citric acid (CA), prior to medication or sealing of the root canals system (RCS) to achieve complete removal of smear layer in the root canal [3].

EDTA is a polyprotic acid whose sodium salts are non-colloidal organic agents that can form non-ionic chelates with metallic ions. Its solutions are normally used at concentrations between 10% and 17%, and its pH is modified from its original value of 4 to values between 7 and 8 to increase its chelating capacity [4,5].

Intracanal medication is an adjuvant in endodontic therapy and consists in the placing of a drug into root canals at each appointment. This adjuvant becomes more important every day in Endodontics since it is known that even with a complete biomechanical preparation bacteria remain in the RCS [6]. 2% chlorhexidine (CHX) has proved to be the substance with better results in persistent infections because *Candida albicans* and *Enterococcus faecalis* can be eliminated at this concentration, something that calcium hydroxide cannot do by itself [7,8]. CHX, a bisguanide, is stable as a salt although it dissociates in water at a physiologic pH, releasing the CHX component [9,10]. Because its inability to dissolve organic tissues, 2% CHX should be used in combination with NaOCl for cleaning and disinfection of RCS [3,6,10].

When a combination of irrigants is used for endodontic therapy, the first irrigant used into the RCS could be partially removed before using the next. Consequently, they could come into contact with each other and chemical interactions can occur. The interactions between NaOCl and EDTA include the loss of free available chlorine for NaOCl which consequently reduces the tissue dissolution capability and to a lesser extent, the antimicrobial activity [3,10].

When CHX and NaOCl are mixed a precipitate forms that can present detrimental consequences for endodontic treatment, including risk of discoloration and potential leaching of unidentified chemicals into the periradicular tissues. This chemical reaction produces an orange substance, which for some authors is an aromatic amine called para-chloroaniline (PCA) proved to be toxic [11,12]. In 2007 Basrani., *et al*. [13] carried out a study about CHX and NaOCl interaction, focusing on the compound formed by them. In this study, the immediate reaction between these substances was shown, even when using low concentrations of hypochlorite (0.19%). As the NaOCl concentrations rose, the precipitate increased in coloration and thickness, since it depends directly on sodium hypochlorite concentration [13].

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 [12,14]. Chromatographic analysis of the precipitates formed from interacting EDTA with CHX showed that 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. The molar ratio of CHX to EDTA in the precipitate was found to be approximately 1.6 to 1. Para-chloroaniline was not detected in the precipitate (the limit of detection was 1%). The high recovery indicates that CHX is not chemically degraded by EDTA under normal conditions [14].

Small amounts of EDTA can influence the effectiveness of the solutions of NaOCl. Clarkson., *et al*. in 2011 [15] showed that the content of active chlorine of the sodium hypochlorite is reduced by mixing it with EDTA. A substantial decrease in the content of active chlorine occurs even with small concentrations of EDTA. Therefore, it is evident that EDTA may inactivate sodium hypochlorite prior to irrigation or medication with chlorhexidine and avoid the formation of PCA [3,4,15].

Hence, the purpose of this study was to evaluate the preventing effect of EDTA in the formation of PCA related precipitate when reacting sodium hypochlorite and chlorhexidine, analyzed by UV-Visible spectrometry.

Materials and Methods

All the following reactants were used: Sodium Hypochlorite solution (Sigma-Aldrich, St. Louis, MO., USA), 4-Chloroaniline (Sigma-Aldrich, St. Louis, MO., USA), Chlorhexidine digluconate solution 20% in H_2O (Sigma-Aldrich, St. Louis, MO., USA), EDTA (Titriplex III, Merck KGaA, Darmstadt, Germany). All chemical solutions were prepared by the chemistry laboratory at the school of dentistry based on the concentration requested.

PCA Determination: To determine the concentration of PCA in the samples, a modified protocol described in the European Pharmacopoeia (2005) [16] was applied to detect presence of para-chloroaniline in chlorhexidine gluconate solutions. We added 250 µL diluted (73g/L) hydrochloric acid (J.T. Baker, Mallinckrodt Baker, S.A. de C.V. Xalostoc, 55320, Mex State, Mexico, made in USA printed in Mexico) to the 1 mL sample and diluted to 2 mL with distilled water. The following solutions were added rapidly, stirring after each addition: 36 µL of Sodium Nitrite (100 g/L) (Merck Millipore, Frankfurter Str. 250, 64293 Darmstadt, Germany); 200 µL Ammonium Sulfamate (50 g/L) (Sigma-Aldrich Chemie Gmbh Munich, Germany); 500 µL (N-1-Naphthyl) ethylenediamine dihydrochloride (1 g/L) (Sigma-Aldrich 3050 Spruce St. St. Louis, MO 63103 USA); 100 µL Ethanol 96% (Merck Millipore, Frankfurter Str. 250, 64293 Darmstadt, Germany). The solution was diluted to 5 ml with distilled water and allowed to stand for 30 minutes and a change in color (red-blue) was observed. The protocol was applied to 5 standard solutions of PCA between 0.005% and 0.01%, absorbance was read in the visible range (350 - 800 nm) to determine λ_{max} and the calibration curve was constructed.

Preparation of Samples: 500 microliters of the following solutions: 5%, 0.5% and 0.05% sodium hypochlorite were mixed with equal volume of 10%, 1%, 0.1%, 0.01% and 0.001% EDTA, and 17%, 1.7%, 0.17%, 0.017% and 0.0017% EDTA. 1000 microliters of 2% chlorhexidine were added to all mixtures and PCA, produced by the reaction between NaOCl-EDTA-CHX, was determined spectrophotometrically using the calibration curve. PCA concentration ([PCA]) of samples was measured by reading the absorbance at the maximum wavelength (A) after applying the methodology described by the European Pharmacopoeia.

Results

With the spectral curve, it was determined that λ_{max} of PCA was at 552 nm. The obtained calibration curve was linear for PCA, with an equation of A = 68.747x[PCA]–0.0046, with R^2 = 0.9997.

Once mixed, most resulting solutions showed the formation of either orange-brown or white precipitates that did not allow the spectrophotometric measurement of PCA (Figure 1).

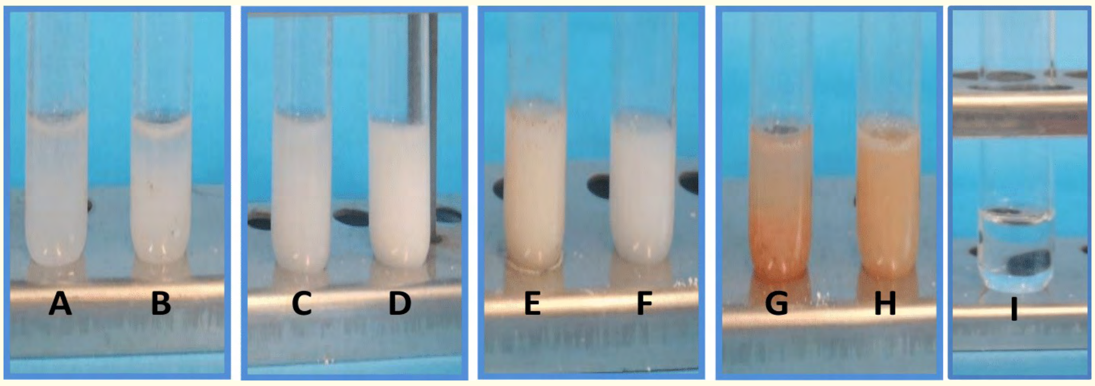

Figure 1: *Precipitates formed in reactions between NaOCl, EDTA and CHX. A: 5% NaOCl+10% EDTA+2% CHX. B: 5% NaOCl+17% EDTA+2% CHX. C: 0.5% NaOCl+10% EDTA+2% CHX. D: 0.5% NaOCl+17% EDTA+2% CHX. E: 0.5% NaOCl+1.0% EDTA+2% CHX. F: 0.5% NaOCl+1.7% EDTA+2% CHX. G: 0.05% NaOCl+0.1% EDTA+2% CHX. H: 0.05% NaOCl+0.17% EDTA+2% CHX. I: 0.05% NaOCl+0.01% EDTA+2% CHX.*

Only four mixtures with 0.05% sodium hypochlorite, EDTA dilutions at concentrations of 0.001%, 0.0017%, 0.01% and 0.017% did not form precipitates when adding 2% chlorhexidine. These solutions where analyzed by spectrophotometry (soluble samples). The absorbance values for samples as a function of the concentration of EDTA were 0.4589, 0.4063, 0.3061 and 0.2214. The Absorbance values and PCA concentrations of soluble samples decreased to $6.74x10^{-3}$%, $5.98x10^{-3}$%, $4.52x10^{-3}$%, $3.29x10^{-3}$% as the EDTA concentration increased. This relationship can be seen in Figure 2.

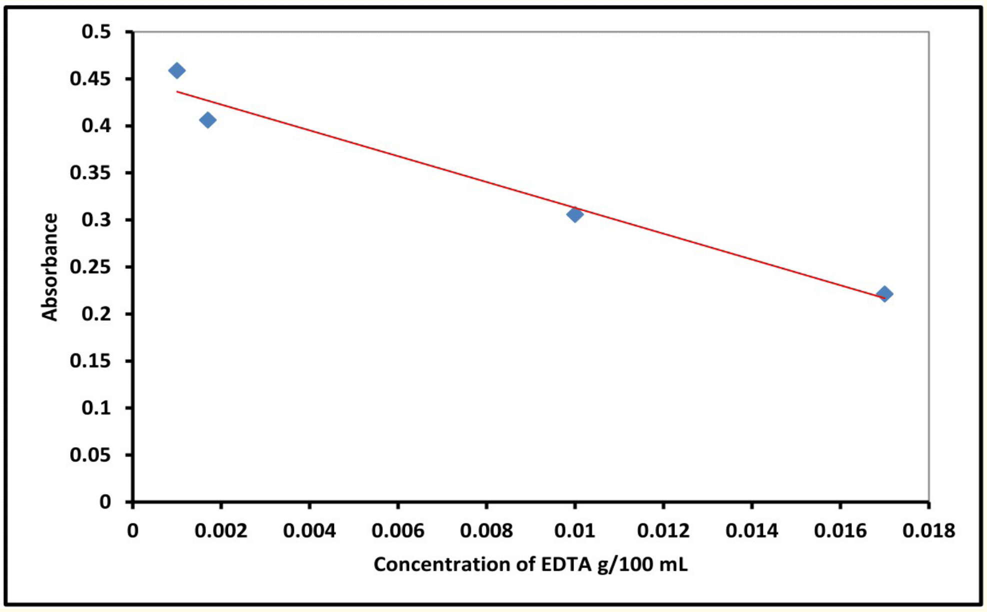

Figure 2: *Relationship for soluble samples between EDTA concentration and absorbance values.*

For soluble samples, the equation of the curve for the relationship absorbance vs. concentration of EDTA was: A = -13.729 [EDTA]+0.4501, a correlation coefficient, r = 0.9850. Using the above equation and seeking the concentration of EDTA that produces a zero absorbance value and therefore a zero concentration of PCA, a concentration of EDTA of 0.03278% was determined. This extrapolation of the absorbance values of the EDTA for the soluble samples interactions NaOCl, EDTA and CHX, leading to the formation of PCA, can be seen in Figure 3.

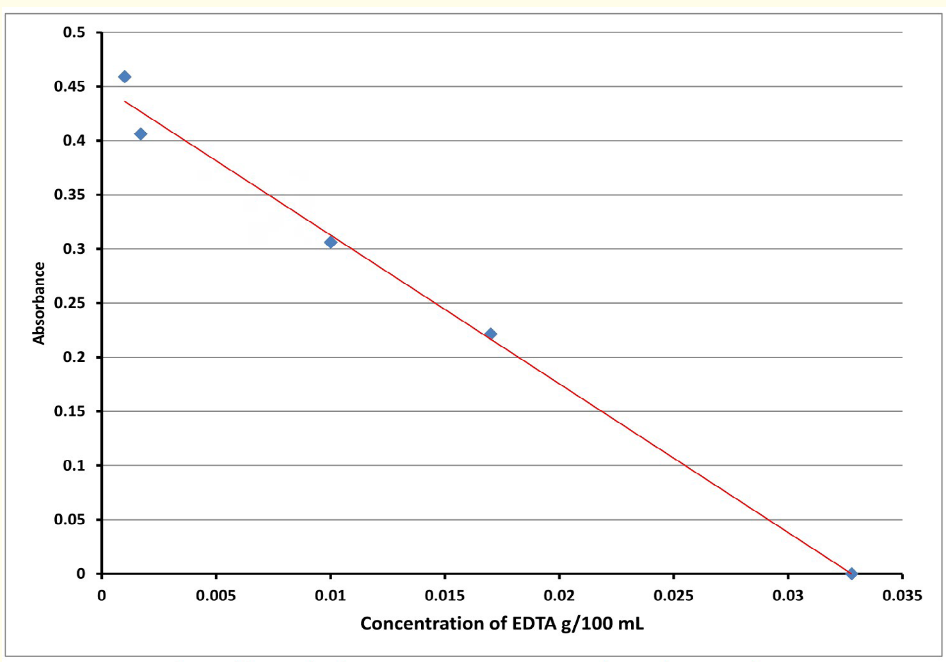

Figure 3: *Extrapolation of the PCA absorbance curve vs EDTA concentration for zero formation of PCA.*

Discussion

Although endodontic irrigants have been well characterized individually, their interactions are less understood. When mixed, NaOCl is inactivated by EDTA, and the EDTA remains functional for a few minutes [16-18]. On the other hand, the interaction between NaOCl and CHX produces a reddish-brown precipitate containing the suspected carcinogen para-chloroaniline [11-13], and is found even in the presence of relatively low levels of NaOCl [13].

The combination of CHX and EDTA produces a white precipitate. The analysis of the white precipitates formed when 2% and 20% CHX reacted with 17% EDTA indicated that over 90% of the precipitate was composed of EDTA or CHX, which means that the CHX forms a salt with EDTA rather than undergoing a chemical reaction [14].

In our study, the combination of NaOCl, EDTA and CHX produced a series of reactions shown in Figure 1. For higher concentrations of NaOCl (5%, 0.5%) and EDTA (17%, 10%) plus 2% CHX, white precipitates prevailed. The presence of reddish-brown precipitate in more diluted solutions of NaOCl shows that the chemical interactions between NaOCl, CHX and EDTA originate new products. The literature indicated that the reaction between NaOCl and CHX produces a precipitate of red-orange color, which for some authors is para-chloroaniline [12,13,19]. This precipitate is visible at concentrations of 0.05% NaOCl, but PCA presence has been demonstrated at very low NaOCl concentrations, no longer as precipitate but in its soluble form. Basrani., *et al*. [13] evaluated the chemical nature of this precipitate and reported that even at low concentrations (0.023% NaOCl), the product formed is para-chloroaniline (PCA). Others like Thomas and Sem [20] used 1H nuclear magnetic resonance (NMR) spectroscopy to determine the reaction between NaOCl and CHX, and concluded that PCA was not produced. Other authors have identified the precipitate as para chlorophenyl urea (PCU) and parachlorophenylguanidyl-1.6-diguanidyl-hexane (PCGH) [20,21].

Contact between 5% NaOCl and 2% CHX forms PCA with a strong orange coloration. The coloration slightly decreases due to sodium hypochlorite dilution in water and its concentration influences PCA formation directly. As the concentration of NaOCl decreased by dilution in water the orange coloration decreased, a sign that PCA formation decreases [22].

The results in Figure 1 showed a high chemical reactivity of irrigants used in endodontic processes, with only four prepared samples forming no precipitate. These soluble PCA containing samples had in common to have used sodium hypochlorite 0.05%, EDTA at concentrations between 0.001% and 0.017%, and 2% chlorhexidine.

In the soluble samples, absorbance was measured at the maximum wavelength, interpolating this value in the calibration curve of the PCA. Therefore, absorbance values reflected the concentration of PCA formed in reactions between CHX, NaOCl and EDTA. By relating the absorbance of these soluble samples with EDTA concentration, a straight line is obtained (equation $A = -13.729 [EDTA]+0.4501$, a correlation coefficient, $r = 0.9850$) with a negative slope. The absorbance of PCA formed and therefore their concentration in the soluble sample decreases as the concentration of EDTA increased (Figure 2).

This decrease in PCA formation could be explained by the reaction between EDTA and sodium hypochlorite. The addition of chelators to NaOCl reduces its pH in a ratio and time-dependent manner. 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 [23,24]. The consequences of chemical interactions between chelating agents and NaOCl result in a loss of the free available chlorine (FAC) of the mixtures [5,17,25,26]. The effects on FAC contents in a 1% NaOCl solution were assayed by mixing them 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 [5]. Thus, the evidence tells us that the reaction between NaOCl and EDTA undergoes a reduced concentration of hypochlorite and therefore less formation of PCA when reacting with 2% CHX (Figure 2).

The absorbance of the solutions will diminish as the concentration of EDTA is increased and there is a linear relationship between the two variables ($r = 0.9850$). It would be possible to calculate which EDTA concentration produces 0.000 absorbance by extrapolating the curve until it meets the "X" axis. This value of EDTA concentration is 0.03278%. This means that for these conditions (0.05% NaOCl and

2% CHX), a concentration of 0.033% EDTA would be required to avoid the formation of PCA (See Figure 3). A common NaOCl concentration used in endodontics is 5%. In theory, residues of 5% sodium hypochlorite remaining in root canals would need a concentration of EDTA of 3.3% to neutralize it.

The clinical significance of the EDTA/CHX precipitate is largely unknown, being difficult to measure how much precipitate adheres to the root canal dentin. Furthermore, it is unknown if any adhering precipitate interferes with the apical seal. Rasimick., et al. [14] evaluated the white precipitate formed after the interaction between EDTA and CHX, founding that this reaction does not produce significant quantities of para-chloroaniline. So, to prevent the formation of para-chloroaniline, EDTA can be used to flush NaOCl from the canal before the application of CHX [14].

Our findings indicate that in reactions involving 0.05% NaOCl, 0.001% to 0.017% EDTA and 2% CHX, the EDTA reduces para-chloroaniline formation *in vitro*. In theory, to prevent the formation of para-chloroaniline, 3.3% EDTA can be used to flush 5% NaOCl before the application of CHX. Other approach could be to dilute with distilled water the residual NaOCl to a near 0,05% concentration, and then rinse it with EDTA.

Conclusion

The use of EDTA between sodium hypochlorite and chlorhexidine results in no visible precipitates and partially inhibits the formation of soluble PCA at the low concentration of irrigants used. The action of EDTA is concentration related: the higher the concentration, the greater the inhibition in the formation of PCA.

Practical Implications: This intermediate irrigation with EDTA is fairly effective inhibiting PCA related precipitate formation *in vitro.* Further *in vivo* studies need to be conducted to assess the ideal scenario in which such inhibition happens in order to tune up the endodontic irrigation protocols and thus improve the cleanliness of the dentine walls prior the RCS sealing.

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Conflict of Interest

The authors deny any conflicts of interest related to this study.

Bibliography

1. Basrani B and Haapasalo M. “Update on endodontic irrigating solutions”. *Endodontic Topics* 27.1 (2012): 74-102.

2. Siqueira Jr JF., *et al*. “Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite”. *Journal of Endodontics* 26.6 (2000): 331-334.

3. Haapasalo M., *et al*. “Irrigation in endodontics”. *Dental Clinics of North America* 54.2 (2010): 291-312.

4. Zehnder M. “Root canal irrigants”. *Journal of Endodontics* 32.5 (2006): 389-398.

5. Yamashita JC., *et al*. “Scanning electron microscopy study of the cleaning ability of chlorhexidine as a root-canal irrigant”. *International Endodontic Journal* 36.6 (2003): 391-394.

6. Almyroudi A., *et al*. “The effectiveness of various disinfectants used as endodontic intracanal medications: an in vitro study”. *Journal of Endodontics* 28.3 (2002): 163-167.

7. Basrani B., *et al*. "Physical and chemical properties of chlorhexidine and calcium hydroxide-containing medications". *Journal of Endodontics* 30.6 (2004): 413-417.

8. Krithikadatta J., *et al*. "Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments". *Journal of Endodontics* 33.12 (2007): 1473-1476.

9. Greenstein G., *et al*. "Chlorhexidine: and adjunct to periodontal therapy". *Journal of Periodontology* 57.6 (1986): 370-376.

10. Gomes BP., *et al*. "Chlorhexidine in endodontics". *Brazilian Dental Journal* 24.2 (2013): 89-102.

11. Bernardi A and Teixeira CS. "The properties of chlorhexidine and undesired effects of its use in endodontics". *Quintessence International* 46.7 (2015): 575-582.

12. Mohammadi Z., *et al*. "Agonistic and antagonistic interactions between chlorhexidine and other endodontics agents: a critical review". *Iranian Endodontic Journal* 10.1 (2015): 1-5.

13. Basrani B., *et al*. "Interaction between sodium hypochlorite and chlorhexidine gluconate". *Journal of Endodontics* 33.8 (2007): 966-969.

14. Rasimick BJ., *et al*. "Interaction between Chlorhexidine Digluconate and EDTA". *Journal of Endodontics* 34.12 (2008): 1521-1523.

15. Clarkson RM., *et al*. "Influence of Ethylenediaminetetraacetic Acid on the Active Chlorine Content of Sodium Hypochlorite Solutions When Mixed in Various Proportions". *Journal of Endodontics* 37.4 (2011): 538-543.

16. C EPM. "Solution chlorhexidine digluconate. 5th ed" (2005).

17. Grawehr M., *et al*. "Interactions of ethylenediaminetetraacetic acid with sodium hypochlorite in aqueous solutions". *International Endodontic Journal* 36.6 (2003): 411-415.

18. Zehnder M., *et al*. "Chelation in root canal therapy reconsidered". *Journal of Endodontics* 31.11 (2005): 817-820.

19. Grande NM., *et al*. "Interaction between EDTA and sodium hypochlorite: a nuclear magnetic resonance analysis". *Journal of Endodontics* 32.5 (2006): 460-464.

20. Thomas JE and Sem DS. "An in vitro spectroscopic analysis to determine whether para-chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine". *Journal of Endodontics* 36.2 (2010): 315-317.

21. Vivacqua-Gomes N., *et al*. "Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root filling". *International Endodontic Journal* 35.9 (2002): 791-795.

22. Nowicki JB and Sem DS. "An in vitro spectroscopic analysis to determine the chemical composition of the precipitate formed by mixing Sodium Hypochlorite and Chlorhexidine". *Journal of Endodontics* 37.7 (2011): 983-988.

23. Riquelme MJ., *et al*. "Water and physiological saline to prevent the formation of p-chloroaniline". *International Journal of Odontostomatology* 9.3 (2015): 399-404.

24. Irala LE., *et al*. "Dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA". *Brazilian Oral Research* 24.3 (2010): 271-276.

25. Rossi-Fedele G., *et al*. "Influence of pH changes on chlorine-containing endodontic irrigating solutions". *International Endodontic Journal* 44.9 (2011): 792-799.

26. Girard S., *et al*. "Assessment of a gel-type chelating preparation containing 1-hydroxyethylidene-1, 1 biphosphonate". *International Endodontic Journal* 38.11 (2005): 810-816.

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