ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 3, Issue – 1, January - 2020, Page No. : 116 - 124

Does free active chlorine in sodium hypochlorite is effected by other root canal irrigants ?

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Citation of this Article: R.Rajasekhar Naik, S.Anitha Rao, S.Srinivas, T.Muralidhar, " Does free active chlorine in sodium hypochlorite is effected by other root canal irrigants ", IJDSIR- January - 2020, Vol. – 3, Issue -1, P. No. 116 – 124.

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Introduction: NaOCl is widely accepted as an irrigant in endodontics because of its tissue dissolution potential and antibacterial effect. The therapeutic effects of sodium hypochlorite solutions are dependent on the levels of of free available chlorine (FAC). The use of additional irrigation with 2 % Chlorhexidine, 17% EDTA and Biopure MTAD is one of the proposed strategies to enhance bacterial eradication. Mixing these solutions with NaOCl can result in significant reduction in free available chlorine so the aim of the present study is to evaluate the effect of different root canal irrigants on free active chlorine in sodium hypochlorite. Methodology: 5.2% sodium hypochlorite solution admixed with 2% chlorhexidine , 17% EDTA , Biopure MTAD and saline in the following proportions: 90:10, 80:20, and 50:50.Mixing of ssodium hypochlorite with these irrigants reduces free active chlorine. Changes in free active chlorine is measured by iodometric titration. Statistical differences between means were determined using a post hoc tukey analysis test after an analysis of variance. Results: 80:20 and 50:50 proportions of 2% CHX and 17% EDTA having the significant influence on FAC. CHX and EDTA both reduces the FAC of NaOCl. MTAD has no significant effect on FAC at all three proportions.

Conclusions: CHX and EDTA combined use with NaOCl solutions should be avoided. MTAD has no demonstrable effect on FAC.

Keywords: NaOCl - Sodium Hypochlorite, CHX - Chlorhexidine, EDTA- Ethylene diamine tetra acetic acid, FAC – Free active chlorine.

Introduction

The primary endodontic treatment goal is to optimize root canal disinfection and to prevent re-infection.[1] The purpose of endodontic treatment is to achieve thorough

debridement of root canals. Microorganisms considered to be responsible for all pulpal and periapical pathology are present not only in the root canals but also invade the dentinal tubules up to varying depths.[2,3] Irregularities in canal systems, narrow isthmi, and apical deltas prevent complete debridement by mechanical instrumentation alone. Thus, chemical disinfection through irrigation becomes a critical adjunct.[4] For clinical usage, an ideal irrigant should have certain properties, such as ability to flush out loose debris, to lubricate the dentinal walls, to dissolve organic matter in the canal, and to have antimicrobial effects.[5]

A wide range of irrigating solutions is available for endodontic use, such as NaOCl, EDTA and chlorhexidine (CHX).[6] Sodium hypochlorite (NaOCl) have been used as endodontic irrigants since 1920 for their antibacterial and tissue-dissolving properties, most likely used endodontic irrigant worldwide. These solutions are very strong oxidizing agents, and in solution they contain hypochlorite ion (OCl) and hypochlorous acid (HOCl) in varying proportions, which together constitute the active chlorine content. It is this active chlorine that provides the protein-dissolving ability and antibacterial properties of NaOCl.[7]

Despite the fact that in vitro tests utilizing higher concentrations of NaOCl have generally exhibited its antibacterial impact, the presence of dentin collagen, exudate, and microbial biomass inside root canals rapidly reduce its efficacy , bringing about bacterial persistence.[8] The collateral tissue damage seen with high concentrations of NaOCl (eg, a proportional increase in cytotoxicity and a lessening in the mechanical properties of dentin) is an extra concern.[7] The utilization of additional irrigation with secondary antibacterial irrigants is one of the proposed methodologies to enhance bacterial destruction.[8]

Several studies have shown that the use of a combination of sodium hypochlorite (2.5-5%) and EDTA (10-17 %) is particularly effective in the removal of organic and inorganic debris.[9–11] Sodium hypochlorite (NaOCl) is known for its antibacterial properties 12 and its ability to dissolve organic components[13], EDTA is a Ca2+ chelating agent and therefore capable of removing the smear layer.[14-16] The smear layer adheres to the canal walls and occludes the dentinal tubules (smear plugs). This negates the ability of medications to penetrate into deeper tissues[17], and prevents the filling material from optimally adhering to canal walls.[18-20] Most authors consider the removal of the smear layer important because it may be infected or it can prevent access to the dentinal tubules, which may contain bacteria and their by-products.[21] EDTA can be used as a final flush to open up the dentinal tubules thus allowing an increasing number of lateral canals to be filled. [22]

Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that has been advocated as an effective medication in endodontic treatment[23, 24]. When used as a root canal irrigant and intracanal medication, it has an antibacterial efficacy comparable to that of NaOCl [25,26], while being effective against certain NaOCl resistant bacterial strains[27]. Prolonged exposure of the root dentin to CHX may result in residual antimicrobial activity of the dentin surface[28]. CHX has a low grade of toxicity[29]; however, the inability of CHX to dissolve organic matter is a perceived drawback [30].

The disadvantages of NaOCl are its unpleasant taste, high toxicity,[31] and inability to remove smear layer when used alone.[32,33] EDTA chelates with calcium and removes the mineralized portion of the smear layer,[34] but has to be used with a proteolytic agent (NaOCl) to remove the organic component.[35] EDTA itself does not possess disinfecting ability[36] and also has been shown to inactivate

chlorine, the active agent in NaOCl.[37] CHX is reported to have good antibacterial properties but does not possess tissue-dissolving capabilities.[38]

Considering these limitations, a new irrigant was introduced that could fulfill all the ideal requirements. Since its introduction, MTAD (a mixture of doxycycline, citric acid, and a detergent [Tween 80]; Dentsply, Tulsa, OK) has been the focus of attention as an alternative root canal irrigant. MTAD, introduced by Torabinejad and Johnson[39] at the Loma Linda University in 2003, is an aqueous solution of 3% doxycycline, a broad-spectrum antibiotic; 4.25% citric acid, a demineralizing agent; and 0.5% polysorbate 80 detergent (Tween 80). In this product, doxycycline hyclate is used instead of its free-base doxycycline monohydrate, to increase the water solubility.[40] It is commercially available as a 2-part mixture (Biopure MTAD; Dentsply). It is considered to be clinically effective[41] and a biocompatible endodontic irrigant.[42]

Utilizing some of the present accessible irrigants in mix with NaOCl has its drawbacks, for example the formation of toxic reaction products and antagonistic interactions[7]. FAC is significantly diminished when NaOCl and EDTA are combined, even in small proportions[7]. CHX forms a precipitate when mixed with NaOCl, the composition and toxicity of which are still controversial[7].The effect of CHX on the FAC when it is mixed with NaOCl is unknown. Till now no study evaluated the effect of Biopure MTAD on the FAC when it mixed with NaOCl.

However, there are no data that support the use of any particular secondary irrigant solution. Nevertheless, there is a need to identify secondary irrigants with antibacterial and antibiofilm properties that can be used safely and effectively in root canal systems in combination with NaOCl without reducing the FAC.

The aim of this investigation was to measure the effect of CHX, EDTA, Biopure MTAD on the FAC content of NaOCl solutions when mixed at varying proportions

Materials and methods

The NaOCl solution was used as primary solution (Vishal dental care pvt. Gujarat,India), which had a nominal free chlorine content of 5.2% (w/v).

The 3 secondary solutions tested were the following:

1. 0.5% Normal saline (Infutec Healthcare Limited)
2. 2% CHX solution (Vishal dental care pvt. Gujarat,India)
3. EDTA (Prevest denpro, EDTA solution)
4. Bio pure MTAD (Dentsply)

Preparation of Mixture:

The test solutions were made by diluting the primary solution with the secondary solutions to make up 25-mL aliquots of different proportions. The proportions and their composition were as follows:

- 90:10, 22.5 mL 5.2% NaOCl was mixed with 2.5 mL secondary Solution
- 80:20, 20 mL 5.2% NaOCl was mixed with 5 Ml secondary solution
- 50:50, 12.5 mL 5.S2% NaOCl was mixed with 12.5 mL secondary solution

The active chlorine concentrations of all test solutions were established by iodometric titration. The baseline FAC of the primary solution was established by averaging three iodometric titrations immediately before mixing with the secondary solution. The FAC content of the mixtures was also established immediately after mixing. Three independent assessments were made for each 25-mL aliquot of each mixture in each of the 3 proportions for each test solution and averaged.

Data Analysis

Statistical analysis was performed using SPSS 22.0 (SPSS Inc, Armonk,NY). A post hoc Tukey test analysis was

performed after analysis of variance to compare the mean FAC between mixtures of NaOCl with different secondary solutions at different proportions. The level of significance was. *P<0.05; Significant; **P<0.001; Highly significant.

4. Results:

Table.1 shows free active chlorine available at different dilution in moles. Mixing NaOCl with other test solutions resulted in a decrease of FAC in all mixtures. When NaOCl mixed with saline at 90:10,80:20 and 50:50 dilution the available free active chlorine respectively 0.00513,0.00407,0.00387 moles.

Chlorhexidine

When NaOCl mixed with CHX at 90:10,80:20 and 50:50 dilution the available free active chlorine respectively 0.00467,0.00360 ,0.00100 moles. Figure: 1 shows highly significant decrease in FAC with chlorhexidine when compared to all other irrigating solutions.

Table :1 Shows Free Active Chlorine Available At Different Dilution

Irrigant Combination Use	Free Active Chlorine Available At Different Dilution					
	90 : 10		80 : 20		50 : 50	
	Mean	± SD	Mean	± SD	Mean	± SD
1. NaOCl + Saline	0.005133	0.0001155	0.004067	0.0001155	0.003867	0.0001155
2. NaOCl + CHX	0.004667	0.0005774	0.003600	0.0005292	0.001000	0.0000000
3. NaOCl + EDTA	0.005000	0.0000000	0.004000	0.0002000	0.002400	0.0000000
4. NaOCl + MTAD	0.005067	0.0001155	0.005133	0.0001155	0.005200	0.0000000
p value	0.304		0.001*		<0.001**	

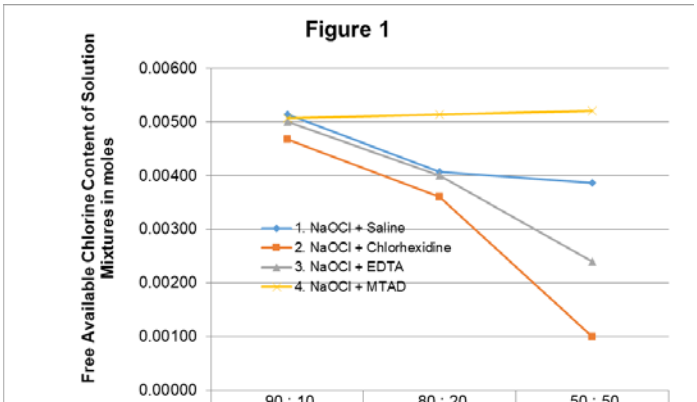
EDTA

When NaOCl mixed with EDTA at 90:10,80:20 and 50:50 dilution the available free active chlorine respectively 0.00500,0.00400 ,0.00240 moles. Figure: 1 shows decrease in FAC with EDTA at 80:20 and 50:50 dilution when compared to Biopure MTAD and saline.

Biopure MTAD

When NaOCl mixed with Bio pure MTAD at 90:10, 80:20 and 50:50 dilution the available free active chlorine respectively 0.00507,0.00513 ,0.00520 moles. Figure: 1 shows no significant change in decrease of FAC when compared to CHX and EDTA.

Figure: 1 Shows Free Available Chlorine Content of Solution Mixtures in moles at 90:10,80:20 and 50:50 propotion.



	90 : 10	80 : 20	50 : 50
1. NaOCl + Saline	0.00513	0.00407	0.00387
2. NaOCl + Chlorhexidine	0.00467	0.00360	0.00100
3. NaOCl + EDTA	0.00500	0.00400	0.00240
4. NaOCl + MTAD	0.00507	0.00513	0.00520

At 90:10 dilution there is no significant decrease in FAC P<0.304, At 80:20 and 50:50 dilution there is significant decrease in FAC respectively P<0. 0.001* significant, highly significant P<0.001**.

With saline, the loss of FAC was almost identical to what would be expected because of dilution, indicating that little if any reaction occurred between solutions. With all other mixtures, the reduction in FAC was much greater than would have been expected from dilution, indicating that a chemical reaction occurred between the solutions.

Discussion

Sodium hypochlorite solutions (NaOCl) have been used as endodontic irrigants since 1920 for their antibacterial and tissue-dissolving properties[43], Nevertheless, they are almost certainly the most common endodontic irrigants used worldwide[49–53]. These solutions are very strong oxidizing agents, and in solution they contain hypochlorite

ion (OCl -) and hypochlorous acid (HOCl) in varying proportions, which together constitute the active chlorine content. It is this active chlorine that provides the protein-dissolving ability and antibacterial properties of NaOCl [54].

A suggested clinical protocol for treating the dentin before root filling consists of irrigation with NaOCl to dissolve the organic components, irrigation with EDTA to eliminate the smear layer and irrigation with CHX to impart substantive antibacterial activity 1. Although such combination of irrigants may enhance their antimicrobial properties[55], possible chemical interactions among the irrigants have to be considered.

In considering FAC in mixtures of irrigants, it should be remembered that any reduction may be combined effect of dilution and a chemical reaction [7]. The impact of dilution alone on the FAC of NaOCl has been recently affirmed by Clarkson et al [7]. The expected reduction in values caused by dilution alone is indicated in Figure -1 when NaOCl and Saline combination used. Any reduction in FAC beyond that expected from dilution can be assumed to be a result of a chemical reaction.

However, a volume-based measurement of the mixture as previously reported by Clarkson et al [7] was used because it is a clinically relevant way of testing irrigant interaction. Clinically we have dentin and organic matter which evoke neutralizing effect on irrigants so iodometric titration is used in the present study to measure FAC.

Our results showed that the reduction of FAC with all combinations of CHX was more than the effect of dilution, confirming that NaOCl and CHX reacted with each other. The chemical interactions of NaOCl with EDTA or CHX are redox reactions, with molecular groups being oxidized by NaOCl[56]; 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[56].CHX produced a significant reduction in FAC, the poor clinical outcome reported when CHX was used concurrently with NaOCl [57] is likely because of the combined effect of the reduced FAC nullifying any antimicrobial effect of the mixture and in part because of the precipitate[47], which could affect the obturation seal[58]. The present study results coincide with the previous study done by Unni Krishnan et al (2017).[59] It is clear from this investigation that NaOCl and CHX should not be mixed in the canal.

Previous studies have shown that EDTA can reduce up to 80%–88% of available chlorine from NaOCl solutions, which was mirrored in the results of this study [7, 60]. What was surprising was the substantial reduction in FAC observed with EDTA. The loss of active chlorine content over time does give some insight into the nature of the reaction taking place. It seems likely that NaOCl reacts with the amide groups on the EDTA molecule to produce chloramines, reducing the available active chlorine in the process. The reaction between EDTA and NaOCl is exothermic.[7] The results of this investigation do confirm the findings of Grawehr et al[60], who reported an 88% loss of active chlorine concentration with a 50:50 mix of EDTA and NaOCl, It is clear from this investigation that NaOCl and EDTA should not be mixed in the canal.

With Biopure MTAD, the loss of FAC was almost identical to what would be expected because of dilution, indicating that little if any reaction occurred between solutions. Bench-top reproductions of the phenomenon revealed that the redox reaction between NaOCl and MTAD.61 In the present study MTAD and NaOCl combination shows highest amount of FAC when compared to CHX and EDTA. In the previous study done by Clarkson et al (2011) irrigants containing surfactants shows substantially high amount FAC when compared to irrigants which don't have sufactants[7] , So in the present study this could be possible reason for MTAD and NaOCl

combination presenting a substantially higher amount of FAC. Further studies are needed on Biopure MTAD and NaOCl irrigant interaction.

This article is not without limitations. The chlorine levels determined using the iodometric titration methods are generally higher than those that are clinically achievable because of the inherent instability of NaOCl and the neutralizing effect of dentin and organic matter. In addition, the inclusion of chlorate, chlorite, perchlorate, and bromate ions in FAC measurements in the iodometric titration makes it less accurate compared with FAC measurement with liquid chromatography and tandem mass spectrometry.[59]

Conclusion

In conclusion, when mixed with NaOCl solutions, CHX appears to cause a marked reduction in FAC in a similar manner to that of EDTA, and, thus, its combined use with NaOCl cannot be recommended. On the other hand, Biopure MTAD has no demonstrable effect on the available chlorine levels in all mixed proportions other than the effect of dilution. Its use as an irrigant in combination with NaOCl is a promising development. Further studies are required to evaluate the extent of synergism achievable using Biopure MTAD as a root canal irrigant in combination with NaOCl and also with EDTA at various pH levels because irrigant synergism decreases irrigation time and also provide synergistic effect . The ideal proportion and minimal concent of NaOCl with Biopure MTAD to achieve optimal antimicrobial and tissue dissolution actions require further investigation.

References

1. Zehnder M. Root canal irrigants. J Endod. 2006;32:389–398.
2. Baumgartner JC, Bakland LK, Sugita EI. Microbiology of endodontics and asepsis in endodontic practice. In: Ingle JI, Bakland LK, editors. Endodontics. 5th ed. Ontario, Canada: BC Decker; 2002; p. 63.
3. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endodon Dent Traumatol 1990;6:142.
4. Ingle JI, Himel VT, Hawrish CE, Glickman GN, Serene T, Rosenberg PA, et al. Endodontic cavity preparation. In: Ingle JI, Bakland LK, editors. Endodontics. 5th ed. Ontario, Canada: BC Decker; 2002; p. 498.
5. Siqueira JF, Rôças IN, Santos SRLD, Lima KC, Magalhães FAC, de Uzeda M. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. J Endod 2002;28:181-4.
6. Singla MG, Garg A, Gupta S. MTAD in endodontics: an update review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112(3):70–76.
7. 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.
8. Haapasalo M, Shen Y, Qian W, et al. Irrigation in endodontics. Dent Clin North Am 2010;54:291–312.
9. Goldman M, Goldman LB, Cavaleri R, Bogis J, Lin PS. The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study. Part 2. J Endod 1982;8:487–92.
10. Yamada R, Armas A, Goldman M, Lin P. A scanning electron microscopic comparison of a high-volume final flush with several irrigation solutions. Part 3. J Endod 1983;9:137– 42.

11. Baumgartner J, Mader C. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endod 1987;13:147–57.
12. Bystrom A, Sunqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35– 40.
13. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hyppochlorite. J Endod 1978;4:60–4.
14. Torabinejad M, Handysides R, Ali Khademi A, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:658–66.
15. Hottel T, El-Refai N, Jones J. A comparison of the effects of three chelating agents on the root canals of extracted human teeth. J Endod 1999;25:716 –7.
16. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indication for their use. Int Endod J 2003;36:810 –30.
17. White R, Goldman M, Lin P. The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. J Endod 1984;10:558–62.
18. Pallares A, Faus V, Glickman GN. The adaptation of mechanically softened gutta-percha to the canal walls in the presence or absence of smear layer: a scanning electron microscopic study. Int Endod J 1995;28:266 –9.
19. Kouvas V, Liolios E, Vassiliadis L, Parissis-Messimeris S, Boutsioukis A. Influence of smear layer depth of penetration of three endodontic sealers: a SEM study. Endod Dent Traumatol 1998;14:191–5.
20. Kokkas AB, Boutsioukis A, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. J Endod 2004;30:100 –2.
21. Williams S, Goldman M. Penetrability of the smeared layer by a strain of Proteus vulgaris. J Endod 1985;11:385– 8.
22. Villegas JC, Yoshioka T, Kobayashi C, Suda H. Obturation of accessory canals after four different final irrigation regimes. J Endod 2002;28:534–6.
23. Ohara P, Torabinejad M, Kettering JD. Antibacterial effects of various endodontic irrigants on selected anaerobic bacteria. Endod Dent Traumatol 1993;9:95–100.
24. Delany GM, Patterson SS, Miller CH, Newton CW. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg Oral Med Oral Pathol 1982;53:518 –23.
25. Siqueira JF, Batista MM, Fraga RC, de Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. J Endod 1998;24:414–6.
26. Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J 1998;31:8 –14.
27. Basrani B, Santos JM, Tjaderhane L, et al. Substantive antimicrobial activity in chlorhexidine- treated human root dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:240 –5.
28. Basrani B, Tjaderhane L, Santos JM, et al. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against Enterococcus faecalis in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:618 –24.
29. Loe H. Does chlorhexidine have a place in the prophylaxis of dental diseases? J Periodontal Res 1973;12(Suppl):93–9.

30. Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JAP. Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. Int Endod J 2004;37:38–41.
31. Spångberg L, Engström B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1973;36:856-71.
32. Goldman LB, Goldman M, Kronman JH, Sun Lin P. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. Oral Surg 1981;52:197-204.
33. Goldberg F, Abramovich A. Analysis of the effect of EDTAC on dentinal walls of the root canal. J Endod 1977;28:461.
34. Goldman M, Kronman JH, Goldman LB, Clausen H, Grady J. New method of irrigation during endodontic treatment. J Endod 1976;2:257.
35. Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J 1998;31:8-14.
36. Grawehr M, Sener B, Waltimo T, Zender M. Interactions of ethylenediamine tetraacetic acid with hypochlorite in aqueous solutions. Int Endod J 2003;36:411.
37. Zehnder M, Lehnert B, Schonenberger K, Waltimo T. Spullosungen und medikamentose Einlagen in der Endodontie. Schweiz Monatsschr Zahnmed 2003;113:756.
38. Kanisavaran ZM. Chlorhexidine gluconate in endodontics: an update review. Int Dent J 2008;58:247-57.
39. Torabinejad M, Johnson WB, inventors. Irrigation solution and methods for use. US Patent 20030235804. December 25, 2003.
40. Bogardus JB, Blackwood RK Jr. Solubility of doxycycline in aqueous solution. J Pharmacol Sci 1979;68:188-94.
41. Torabinejad M, Shabahang S, Bahjri K. Effect of MTAD on postoperative discomfort: a randomized clinical trial. J Endod 2005;31:171-6.
42. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod 2003;29:654-7.
43. Rossi-Fedele G, Dogramaci EJ, Guastalli AR, et al. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. J Endod 2012;38: 426–31.
44. Orhan EO, Irmak O, Hur D, et al. Does para-chloroaniline really form after mixing sodium hypochlorite and chlorhexidine? J Endod 2016;42:455–9.
45. 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 using gas chromatography. J Endod 2010;36:312–4.
46. Crane AB. A practicable root canal technic. Philadelphia: Lea & Febiger; 1920;69.
47. Whitworth JM, Seccombe GV, Shoker K, et al. Use of rubber dam and irrigant selection in UK general dental practice. Int Endod J 2000;33:435–41.
48. Slaus G, Bottenberg P. A survey of endodontic practice amongst Flemish dentists. Int Endod J 2002;35:759–67.
49. Clarkson RM, Podlich HM, Savage NW, et al. A survey of sodium hypochlorite use by general dental

practitioners and endodontists in Australia. Aust Dent J 2003;48:20–6.

50. Palmer NO, Ahmed M, Grieveson B. An investigation of current endodontic practice and training needs in primary care in the north west of England. Br Dent J 2009; 206:584–5.
51. Koshy S, Chandler NP. Use of rubber dam and its association with other endodontic procedures in New Zealand. N Z Dent J 2002;98:12–6.
52. Hoffman PN, Death JE, Coates D. The stability of sodium hypochlorite solutions. In: Collins CH, Allwood MC, Bloomfield SF, Fox A, eds. Disinfectants: their use and evaluation of effectiveness. London: Academic Press; 1991:77–83.
53. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod 1998;24:472– 6.
54. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J 2009;42:288–302.
55. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int Endod J 2011;44: 583–609.
56. Homayouni H, Majd NM, Zohrehei H, et al. The effect of root canal irrigation with combination of sodium hypo-chlorite and chlorhexidine gluconate on the sealing ability of obturation materials. Open Dent J 2014;8:184–7.
57. Krishnan, U.; Saji, S.; Clarkson, R.; Lalloo, R.; Moule, A.J. Free active chlorine in sodium hypochlorite solutions admixed with octenidine, smearoff, chlorhexidine, and EDTA. J. Endod. 2017, 43, 1354–1359.
58. Grawehr M, Sener B, Waltimo T, et al. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J 2003;36:411–7.
59. Tay, F.R., Hiraishi, N., Schuster, G.S. et al, Reduction in antimicrobial substantivity of MTAD after initial sodium hypochlorite irrigation. J Endod. 2006;32:970–975.