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# Interaction of endodontic irrigants: A review

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***Abstract*---**Root canal therapy is to eliminate or at least reduce the number of microorganisms and remove inflamed or necrotic pulpal tissue. Root canal cleaning and disinfection during chemomechanical preparation relies heavily on irrigants because of the anatomic complexities of the pulp canal system. Although endodontic irrigants have been well characterized individually, combinations of the materials are not well understood. To know the interactions of different irrigants this review throws more light in depth to understand the interactions of irritants better.

***Keywords*---**endodontic irrigants, interaction, NaOCl, CHX, EDTA, MTAD, precipitate.

## Introduction

The aim of root canal treatment is to eliminate or at least reduce the number of microorganisms and pathogens which causes Inflammation, and remove inflamed or necrotic pulpal tissue. Root canal cleaning, shaping and disinfection during chemomechanical preparation relies mainly on irrigants because of the anatomic complexities of the pulp canal system. Biomechanical cleaning and shaping of the root canal system significantly decreases or reduces the number of bacteria [1], but the anatomical complexity of the root canal system, organic and inorganic residues and bacteria cannot be completely removed and may often persist [2] for which the use of irrigants plays a vital role.. Mechanical instrumentation using files usually forms a smear layer on the canal surface [3]this can be flushed out with the use of irrigants.


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**Ideal Requirements Of Root Canal Irrigants[4]**

1. It should have a broad antimicrobial spectrum.
2. It should be highly efficacy against anaerobic and facultative microorganisms organized in biofilms.
3. It should have the ability to dissolve necrotic radical at soft tissue remnants.
4. It should have the ability to inactivate endotoxin.
5. It should have the ability to reduce the formation of smear layer during instrumentation and/or to dissolve the smear layer once it has been formed.
6. It should be systemically non toxic when they come in contact with vital tissues, non caustic to periodontal tissues, and with little or no potential to cause an anaphylactic reaction.

Irrigants should contain or close to the above mentioned properties such as antimicrobial and tissue-dissolution actions as well as other properties, like lubrication, demineralisation, and the ability to remove debris and the smear layer [5].Till date no irrigation solution has been found capable of demineralising the smear layer and dissolving organic tissue simultaneously with great efficiency [6]and without causing any irritation to the surrounding tissues. Mechanical instrumentation forms a smear layer on the canal walls[3]. Thus, chemical debridement in the form of various irrigants is performed to aid in removal of residual debris, necrotic pulpal tissue, microbes and smear layer. Therefore, the adjunctive use of chelating agents such as EDTA is suggested in order to remove and prevent the formation of the smear layer associated with root canal instrumentation [4].

In general, an irrigant solution is not completely flushed out from the root canal before applying the next irrigant. As a result, endodontic irrigants routinely come into contact with each other inside the root canal and can form by products [7,8]. These by products can be solid precipitates that may occlude the dentinal tubules, forming a barrier between the filling material and dentin surface and increasing the coronal microleakage [9–11]. In addition, the by products formed can also be toxic to the periapical tissues[3].The aim of this review article is to characterize the by-products formed by the interaction between the most commonly used irrigants in endodontic practice.

**Sodium Hypochlorite**

The most common irrigant used in root canal treatment is sodium hypochlorite (NaOCl) [12–28]. Hypochlorite preparations are sporicidal and virucidal and show far greater tissue dissolving effects on necrotic than on vital tissues. This main feature lead to the use of sodium hypochlorites as gold standard irrigant as early as 1920. There has been much controversy over the concentration of hypochlorite solutions to be used in endodontics. The antibacterial effectiveness and tissue dissolution capacity of hypochlorite is based on its concentration, and so is its toxicity [4]. NaOCl is an effective tissue solvent and antimicrobial agent [12–14]. Its germicidal ability is related to the formation of hypochlorous acid when in contact with organic debris. In high concentration NaOCl is toxic and can lead to inflammation of the periapical tissues[12,13,29,30] , whereas in low concentrations it is ineffective against specific microorganisms [12]. NaOCl is not a substantive antimicrobial agent[31] ; it tends to discolour[30] and corrode surgical instruments; and it has a very unpleasant odour [31].

**Interaction of NaOCl and Chlorhexidine**

Chlorhexidine digluconate (CHX) is widely used in disinfection of root canals because of its good antimicrobial activity [32–34]. It has gained considerable popularity in endodontics as an irrigating solution and as an intracanal medicament. CHX does not possess undesired characteristics compared to sodium hypochlorite (ie, bad smell and strong irritation to periapical tissues). However, CHX has no tissue-dissolving capability and therefore it cannot replace sodium hypochlorite.The wide range of use is due to its substantivity (ie, continued antimicrobial effect), because CHX binds to hard tissue and remains antimicrobial. However, similar to other endodontic disinfecting solutions, the activity of CHX depends on the pH and is greatly decreased in the presence of organic matter[34].Several studies have compared the antibacterial effect of NaOCl and 2% CHX against intracanal infection and have shown little or no difference between their antimicrobial effectiveness [29,35–37]. CHX has the capacity to kill bacteria but unable to remove biofilm and organic debris. Residual organic tissue may have a negative effect on the quality of the seal by the permanent root filling, necessitating the use of NaOCl during instrumentation. However, CHX does not cause erosion of dentin like NaOCl hence used as the final rinse after EDTA, and therefore 2% CHX may be a good choice for maximised antibacterial effect at the end of the chemomechanical preparation [38].

Kuruvilla et al.[13] said that the antimicrobial effectiveness of 2.5% NaOCl and 0.2% chlorhexidine (CHX) used in combination was greater than used individually. The reaction between NaOCl and CHX produces a carcinogenic product, parachloroanaline (PCA), the potential leakage of which into the surrounding tissues is a concern. The precipitate formed is an insoluble neutral salt, due to the acid-base reaction between NaOCl and CHX. PCA is the main product of the interaction of NaOCl and CHX, and has the molecular formula NaC6H4Cl [7]. When mixed with NaOCl, CHX molecules become hydrolyzed into smaller fragments, each forming a byproduct. The first bonds to be broken in this reaction are those between carbon and nitrogen because of the low-bond dissociation energy between these two atoms. The presence of PCA was confirmed by the Beilstein test for the presence of chlorine and the HCl solubility test for the presence of aniline.Leaching of PCA from the insoluble precipitate formed is of concern because it has been shown to be cytotoxic in rats[39] and possibly carcinogenic in humans [40–42]. This reaction coats the canal surface and significantly occludes the dentinal tubules and affects the seal of the root canal[10].

The interaction of NaOCl and CHX solution resulted in orange-brown precipitate. Several products of chlorination were confirmed by mass spectrometry from oxidizing agent NaOCl [43–45]. The orange brown colour may be associated with guanidine oxidation [46]. Basrani et al[7] in their study using X-ray photon spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) found the presence of para-chloroaniline on combining NaOCl and CHX.

Basrani et al [47] using Gas chromatography mass spectrometer found the presence of para-chloroaniline. The orange-brown colour can be associated with the guanidine oxidation[46] .

The formation of the precipitate could be explained by the acid–base reaction that occurs when NaOCl and CHX are mixed. CHX, a dicationic acid (pH 5.5– 6.0) has the ability to donate protons. NaOCl is alkaline and can accept protons from the dicationic CHX. This proton exchange results in the formation of a neutral and insoluble substance, referred to as the “precipitate.” It is assumed that this precipitate contains PCA.

In conclusion NaOCl mixed with CHX formed PCA, and the amount of PCA directly increased with the increment in the concentration of NaOCl. Although the precipitate being insoluble raises questions about leaching of PCA, the findings may be clinically relevant because PCA has been shown to be toxic[39,48] . As an aromatic amine, the primary toxic effect is methemoglobin formation [39]. Short-term exposure of humans to PCA results in cyanosis, which is a manifestation of methemoglobin formation [39]. Toxicological studies in rats and mice have shown that the hematopoietic system is the major target for PCA[39]. Further investigations of interaction between NaOCl and CHX and it's by products should emphasise the bioavailability of PCA leaching out and cytotoxicity. In clinical scenario, it would be better to minimise its formation by washing away the remaining NaOCl with alcohol or EDTA, before using CHX.

**Interaction of NaOCl and EDTA**

Complete disinfection of the root-canal system necessitate the use of irrigants that vanish organic and inorganic material. As hypochlorite is active only against the former, other substances must be used to complete the removal of the smear layer and dentin debris. EDTA effectively dissolve inorganic material, including hydroxyapatite [3,6,49,50]. EDTA is used for 2 to 3 minutes at the end of instrumentation and after NaOCl irrigation. Removal of the smear layer by EDTA improves the antibacterial effect of locally used disinfecting agents in deeper layers of dentin [51,52].

Sodium Hypochlorite and EDTA are frequently used irrigating solutions. As they have different characteristics and tasks, it has been tempting to use them as a mixture. However, EDTA instantaneously reduces the amount of chlorine when mixed with sodium hypochlorite, resulting in the loss of NaOCl activity. Thus, these solutions should not be mixed [53].

EDTA is used commonly at concentrations of 15% or 17%. Aqueous solutions are prepared by dissolving the di- or trisodium salts, resulting in a neutral to slightly alkaline pH [3,54]. At this pH, the reaction between NaOCl and EDTA is exothermic [55], and mixing NaOCl with EDTA results in a rapid and dramatic decrease of free available chlorine [53,55,56]. Gas formation has been observed from this combination [57], and low level chlorine gas emissions have been measured [3]. This interaction has been attributed to an acid/base neutralization reaction between NaOCl and EDTA, with the formation of HOCl, which then subsequently decomposes, releasing chlorine and oxygen.

A solutions of increased alkalinity can be produced by mixing the tetrasodium salt of EDTA with NaOCl. This combination results in the longer maintenance of both free available chlorine and desirable pH conditions [58], although concentrations of both EDTA and NaOCl need to be reduced to obtain this result [59]. The pH of a 1:1 mixture of 5% NaOCl and 10% Na4EDTA falls from 11.9 at 10 min after mixing to 9.0 at one hour after mixing, during which period the remaining free available chlorine declines from 90% to 62% [58].

For mixtures of EDTA and NaOCl that have a low pH, the loss of free available chlorine significantly reduces the ability of NaOCl to dissolve organic tissue [3,52]. Thus, these irrigants should neither be mixed, nor come into contact with one another. The chelating ability of EDTA [60] and the disinfecting capacity of NaOCl are, however, maintained[3,60] .A case report attributing a subcutaneous emphysema to gas formation, resulting from the interaction of RC-Prep with NaOCl, has been reported [61]. RC-Prep contains both EDTA and urea peroxide and reactions of these components with NaOCl is possible.

**Interaction of EDTA and CHX**

When CHX and EDTA interact, a precipitate is formed that contains 90% CHX and EDTA, with less than 1% of the potential decomposition product, p-chloroaniline. The high recovery indicates that CHX is not degraded by EDTA under normal conditions. The precipitate is most likely a salt formed by electrostatic neutralisation of cationic CHX by anionic EDTA. A white milky precipitate produced in the association of EDTA with CHX solution and gel was analysed by ESI(+)-MS and found to be related to the acid-base reactions. Rasimick et al[8], who analysed the precipitate formed after mixing 17% EDTA with 2% or 20% CHX by using reversed phase high-performance liquid chromatography and observed that more than 90% of the precipitate mass was either EDTA or CHX salt. The clinical significance of the EDTA/CHX precipitate is largely unknown. There are no published measurements of how much precipitate adheres to the root canal dentin. Furthermore, it is unknown if any adhering precipitate interferes with the apical seal.

**Interaction of NaOCl and MTAD (Mixture of Tetracycline, an Acid, And a Detergent)**

The recent arrival of MTAD [61–69], an endodontic irrigant commercially known as BioPure MTAD (Dentsply TulsaDental, Tulsa, OK) containing 3% doxycycline hyclate, 4.25% citric acid, and 0.5% polysorbate 80 detergent[70], represents an ingenious approach in simultaneous removal of endodontic smear layers and disinfection of root canals. MTAD has been shown to be a clinically effective [62,68,69] and biocompatible[67] endodontic irrigant with potential antibacterial substantivity [63,64]. It is notable that, this doxycycline-containing irrigant was found to be effective against E. faecalis in refractory root canal infections [6]. This experimental irrigant is now commercially known as BioPure MTAD (Dentsply TulsaDental, Tulsa, OK)[70]. For unintentional or accidental erosion of the intraradicular dentin and ideal removal of endodontic smear layers, an updated clinical regime has been proposed that involves the use of an initial rinse with 1.3% sodium hypochlorite (NaOCl) for a approximate period of 20 minutes, followed by the use of MTAD as the final rinse for a approximate period of 5 minutes [62–69]. Using this protocol for in vitro cleaning of root canals, it has recently been reported that intrinsic staining of dentin occurred after light exposure of the NaOCl- and MTAD irrigated coronal and intraradicular dentin [71]. This phenomenon occurred irrespective of whether the root canals were filled. A similar reaction between NaOCl and MTAD with the formation of a brown solution was also reported in few studies , in the absence of light exposure, when MTAD was employed as the initial rinse followed by the use of different concentrations of NaOCl as final rinses.

Bench-top reproductions of the phenomenon revealed that the interaction between NaOCl and MTAD [71] resembled the mechanism of tetracycline tooth staining and it is caused due to the redox reaction occurring between NaOCl and MTAD , and adult onset pigmentation of teeth and bone [72,73].The cause of the observed pigmentation has been studied previously, in which a red purple degradation product is produced as a by-product product of photo-oxidation of tetracycline. This by-product has a high affinity towards hydroxyapatite.[74,75]. It is known that reactive oxygen species (ROS) derived from oxygen, hydroxyl radical and hydrogen peroxide can oxidise different types of tetracycline to yield quinone derivatives [76,77], with reductions in the antimicrobial potency in this class of substituted hydronaphthacene antibiotics [78]. In the context of endodontic irrigation, as the phenomenon may be prevented by treating the NaOCl-irrigated dentin with ascorbic acid, an anti-oxidant, before the application of MTAD[78] , it was hypothesised that the photo-oxidative degradation process is triggered by the NaOCl functioning in the capacity of an oxidising agent [78,79]. Iatrogenic doxycycline staining of endodontically treated teeth are usually rare. As root canals are not directly exposed to light , concern over the potential iatrogenic doxycycline staining of endodontically treated teeth should not take precedence over the well-documented advantages of the NaOCl-MTAD irrigation regime in disinfecting bacteria-infected root canals [71]. Peroxidation of tetracycline leads to partial loss of its antimicrobial potency and its clinical implications and have not been fully investigated in the field of endodontics.Our team has extensive knowledge and research experience that has translate into high quality publications[80–89],[90–93],[94–98][99]

## Conclusion

The aim of root canal therapy is to eliminate or at least reduce the number of microorganisms and remove inflamed or necrotic pulpal tissue. Root canal cleaning and disinfection during chemomechanical preparation relies heavily on irrigants because of the anatomic complexities of the pulp canal system.Although endodontic irrigants have been well characterized individually, combinations of the materials are not well understood.Irrigation plays a vital role in successful endodontic treatment. Available literature and studies demonstrate advantages and limitations of each irrigant under consideration, and none of them completely satisfy the requirements of the ideal root canal irrigant. Although NaOCl is the most significant irrigating solution, no single irrigant can accomplish all the tasks required by irrigation. Understanding the interaction of various available and commonly used solutions is important for optimal irrigation.

**Reference**

1. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981 Aug;89(4):321–8.
2. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004 Aug;30(8):559–67.
3. Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridement. J Endod. 1987 Feb;13(2):47–51.
4. Zehnder M. Root canal irrigants. J Endod. 2006 May;32(5):389–98.
5. Gutmann JL, Lovdahl PE. Chapter 11 - Problem Solving in Cleaning and Disinfecting the Root Canal System. In: Gutmann JL, Lovdahl PE, editors. Problem Solving in Endodontics (Fifth Edition). Saint Louis: Mosby; 2011. p. 209–17.
6. Baumgartner JC, Brown CM, Mader CL, Peters DD, Shulman JD. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. J Endod. 1984 Nov;10(11):525–31.
7. Basrani BR, Manek S, Sodhi RNS, Fillery E, Manzur A. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod. 2007 Aug;33(8):966–9.
8. Rasimick BJ, Nekich M, Hladek MM, Musikant BL, Deutsch AS. Interaction between chlorhexidine digluconate and EDTA. J Endod. 2008 Dec;34(12):1521–3.
9. Akisue E, Tomita VS, Gavini G, Poli de Figueiredo JA. Effect of the combination of sodium hypochlorite and chlorhexidine on dentinal permeability and scanning electron microscopy precipitate observation. J Endod. 2010 May;36(5):847–50.
10. 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 Feb;34(2):181–5.
11. Vivacqua-Gomes N, Ferraz CCR, Gomes BPFA, Zaia AA, Teixeira FB, Souza-Filho FJ. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root fillings. Int Endod J. 2002 Sep;35(9):791–5.
12. Leonardo MR, Tanomaru Filho M, Silva LA, Nelson Filho P, Bonifácio KC, Ito IY. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endod. 1999 Mar;25(3):167–71.
13. 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 Jul;24(7):472–6.
14. Ohara P, Torabinejad M, Kettering JD. Antibacterial effects of various endodontic irrigants on selected anaerobic bacteria. Endod Dent Traumatol. 1993 Jun;9(3):95–100.
15. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. Pesqui Bras Odontopediatria Clin Integr [Internet]. 2019;19. Available from: https://www.scielo.br/scielo.php?pid=S1983-46322019000100364&script=sci_arttext
16. Nandakumar M, Nasim I. Comparative evaluation of grape seed and

cranberry extracts in preventing enamel erosion: An optical emission spectrometric analysis. J Conserv Dent. 2018 Sep;21(5):516–20.
17. Rajakeerthi R, Ms N. Natural Product as the Storage medium for an avulsed tooth--A Systematic Review. Cumhuriyet Dental Journal. 2019;22(2):249–56.
18. Manohar MP, Sharma S. A survey of the knowledge, attitude, and awareness about the principal choice of intracanal medicaments among the general dental practitioners and nonendodontic specialists. Indian J Dent Res. 2018 Nov 1;29(6):716.
19. Azeem RA, Sureshbabu NM. Clinical performance of direct versus indirect composite restorations in posterior teeth: A systematic review. J Conserv Dent. 2018 Jan;21(1):2–9.
20. Teja KV, Ramesh S, Priya V. Regulation of matrix metalloproteinase-3 gene expression in inflammation: A molecular study. J Conserv Dent. 2018 Nov;21(6):592–6.
21. Siddique R, Sureshbabu NM, Somasundaram J, Jacob B, Selvam D. Qualitative and quantitative analysis of precipitate formation following interaction of chlorhexidine with sodium hypochlorite, neem, and tulsi. J Conserv Dent. 2019 Jan;22(1):40–7.
22. Poorni S, Srinivasan MR, Nivedhitha MS. Probiotic Streptococcus strains in caries prevention: A systematic review. J Conserv Dent. 2019 Mar;22(2):123–8.
23. Jenarthanan S, Subbarao C. Comparative evaluation of the efficacy of diclofenac sodium administered using different delivery routes in the management of endodontic pain: A randomized controlled clinical trial. J Conserv Dent. 2018 May;21(3):297–301.
24. Malli Sureshbabu N, Selvarasu K, V JK, Nandakumar M, Selvam D. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. Case Rep Dent. 2019 Jan 22;2019:7046203.
25. Govindaraju L, Neelakantan P, Gutmann JL. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. Clin Oral Investig. 2017 Mar;21(2):567–71.
26. Khandelwal A, Palanivelu A. Correlation Between Dental Caries And Salivary Albumin In Adult Population In Chennai: An In Vivo Study. BDS. 2019 Apr 30;22(2):228–33.
27. Siddique R, Nivedhitha MS. Effectiveness of rotary and reciprocating systems on microbial reduction: A systematic review. J Conserv Dent. 2019 Mar;22(2):114–22.
28. Sathyanarayanan U, Ramarao S. CRA Grid - A preliminary development and calibration of a paper-based objectivization of caries risk assessment in undergraduate dental education [Internet]. Vol. 22, Journal of Conservative Dentistry. 2019. p. 185. Available from: http://dx.doi.org/10.4103/jcd.jcd_389_18
29. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod. 1994 Jun;20(6):276–8.
30. Ferguson JW, Hatton JF, Gillespie MJ. Effectiveness of intracanal irrigants and medications against the yeast Candida albicans. J Endod. 2002 Feb;28(2):68–71.
31. White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal

irrigation with chlorhexidine. J Endod. 1997 Apr;23(4):229–31.
32. Russell AD. Activity of biocides against mycobacteria. Soc Appl Bacteriol Symp Ser. 1996;25:87S – 101S.
33. Shaker LA, Dancer BN, Russell AD, Furr JR. Emergence and development of chlorhexidine resistance during sporulation of Bacillus subtilis 168. FEMS Microbiol Lett. 1988 Jun 1;51(1):73–6.
34. Russell AD, Day MJ. Antibacterial activity of chlorhexidine. J Hosp Infect. 1993 Dec;25(4):229–38.
35. Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J. 1998 Jan;31(1):8–14.
36. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro. Endod Dent Traumatol. 1993 Dec;9(6):243–8.
37. Buck RA, Eleazer PD, Staat RH, Scheetz JP. Effectiveness of three endodontic irrigants at various tubular depths in human dentin. J Endod. 2001 Mar;27(3):206–8.
38. Zamany A, Safavi K, Spångberg LSW. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Nov;96(5):578–81.
39. Chhabra RS, Huff JE, Haseman JK, Elwell MR, Peters AC. Carcinogenicity of p-chloroaniline in rats and mice. Food Chem Toxicol. 1991 Feb;29(2):119–24.
40. Organization WH, Others. International Agency for Research on cancer: IARC monography on the evaluation of carcinogenic risks to human. Lyon, France. 2006;86:1–25.
41. Barbin LE, Saquy PC, Guedes DFC, Sousa-Neto MD, Estrela C, Pécora JD. Determination of para-chloroaniline and reactive oxygen species in chlorhexidine and chlorhexidine associated with calcium hydroxide. J Endod. 2008 Dec;34(12):1508–14.
42. Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine. J Endod. 2010 Jul;36(7):1154–7.
43. Zhang C, Reiter C, Eiserich JP, Boersma B, Parks DA, Beckman JS, et al. L-arginine chlorination products inhibit endothelial nitric oxide production. J Biol Chem. 2001 Jul 20;276(29):27159–65.
44. Thomas EL, Jefferson MM, Grisham MB. Myeloperoxidase-catalyzed incorporation of amines into proteins: role of hypochlorous acid and dichloramines. Biochemistry. 1982 Nov 23;21(24):6299–308.
45. Grisham MB, Jefferson MM, Melton DF, Thomas EL. Chlorination of endogenous amines by isolated neutrophils. Ammonia-dependent bactericidal, cytotoxic, and cytolytic activities of the chloramines. J Biol Chem. 1984 Aug 25;259(16):10404–13.
46. Micklus MJ, Stein IM. The colorimetric determination of mono- and disubstituted guanidines. Anal Biochem. 1973 Aug;54(2):545–53.
47. Basrani BR, Manek S, Mathers D, Fillery E, Sodhi RNS. Determination of 4-chloroaniline and its derivatives formed in the interaction of sodium hypochlorite and chlorhexidine by using gas chromatography. J Endod. 2010 Feb;36(2):312–4.
48. Burkhardt-Holm P, Oulmi Y, Schroeder A, Storch V, Braunbeck T. Toxicity of 4-Chloroaniline in Early Life Stages of Zebrafish (Danio rerio): II. Cytopathology and Regeneration of Liver and Gills After Prolonged Exposure to Waterborne 4-Chloroaniline. Arch Environ Contam Toxicol. 1999 Jul

1;37(1):85–102.
49. Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. Dent Clin North Am. 1990 Jan;34(1):13–25.
50. Loel DA. Use of acid cleanser in endodontic therapy. J Am Dent Assoc. 1975 Jan;90(1):148–51.
51. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res. 1987 Aug;66(8):1375–9.
52. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Dent Traumatol. 1990 Aug;6(4):142–9.
53. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod. 2005 Nov;31(11):817–20.
54. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014 Mar;216(6):299–303.
55. 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 Apr;37(4):538–43.
56. Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J. 2003 Jun;36(6):411–7.
57. Prado M, Santos Júnior HM, Rezende CM, Pinto AC, Faria RB, Simão RA, et al. Interactions between irrigants commonly used in endodontic practice: a chemical analysis. J Endod. 2013 Apr;39(4):505–10.
58. Tartari T, Oda DF, Zancan RF, da Silva TL, de Moraes IG, Duarte MAH, et al. Mixture of alkaline tetrasodium EDTA with sodium hypochlorite promotes in vitro smear layer removal and organic matter dissolution during biomechanical preparation. Int Endod J. 2017;50(1):106–14.
59. Biel P, Mohn D, Attin T, Zehnder M. Interactions between the Tetrasodium Salts of EDTA and 1-Hydroxyethane 1,1-Diphosphonic Acid with Sodium Hypochlorite Irrigants. J Endod. 2017 Apr;43(4):657–61.
60. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J. 2001 Sep;34(6):424–8.
61. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J. 2003 Dec;36(12):810–30.
62. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endod. 2003 Apr;29(4):233–9.
63. Beltz RE, Torabinejad M, Pouresmail M. Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite, and EDTA on bovine pulp and dentin. J Endod. 2003 May;29(5):334–7.
64. Torabinejad M, Shabahang S, Aprecio RM, Kettering JD. The antimicrobial effect of MTAD: an in vitro investigation. J Endod. 2003 Jun;29(6):400–3.
65. Shabahang S, Pouresmail M, Torabinejad M. In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. J Endod. 2003 Jul;29(7):450–2.
66. Shabahang S, Torabinejad M. Effect of MTAD on Enterococcus faecalis-contaminated root canals of extracted human teeth. J Endod. 2003 Sep;29(9):576–9.

67. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod. 2003 Oct;29(10):654–7.
68. Park DS, Torabinejad M, Shabahang S. The effect of MTAD on the coronal leakage of obturated root canals. J Endod. 2004 Dec;30(12):890–2.
69. Torabinejad M, Shabahang S, Bahjri K. Effect of MTAD on postoperative discomfort: a randomized clinical trial. J Endod. 2005 Mar;31(3):171–6.
70. García-Godoy F, Loushine RJ, Itthagarun A, Weller RN, Murray PE, Feilzer AJ, et al. Application of biologically-oriented dentin bonding principles to the use of endodontic irrigants. Am J Dent. 2005 Aug;18(4):281–90.
71. Tay FR, Mazzoni A, Pashley DH, Day TE, Ngoh EC, Breschi L. Potential iatrogenic tetracycline staining of endodontically treated teeth via NaOCl/MTAD irrigation: a preliminary report. J Endod. 2006 Apr;32(4):354–8.
72. Sánchez AR, Rogers RS III, Sheridan PJ. Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity. Int J Dermatol. 2004;43(10):709–15.
73. Eisen D, Hakim MD. Minocycline-Induced Pigmentation. Drug Saf. 1998 Jun 1;18(6):431–40.
74. Wiebe JA, Moore DE. Oxidation photosensitized by tetracyclines. J Pharm Sci. 1977 Feb;66(2):186–9.
75. Davies AK, McKellar JF, Phillips GO, Reid AG. Photochemical oxidation of tetracycline in aqueous solution. J Chem Soc Perkin Trans 2. 1979;(3):369–75.
76. Gilio VE, Cilento G. Light emission accompanies oxygen uptake during the peroxidative metabolism of tetracyclines. Photochem Photobiol. 1993 Sep;58(3):446–9.
77. Lau C, Lu J, Kai M. Chemiluminescence determination of tetracycline based on radical production in a basic acetonitrile–hydrogen peroxide reaction. Anal Chim Acta. 2004 Feb 9;503(2):235–9.
78. Miskoski S, Sánchez E, Garavano M, López M, Soltermann AT, Garcia NA. Singlet molecular oxygen-mediated photo-oxidation of tetracyclines: kinetics, mechanism and microbiological implications. J Photochem Photobiol B. 1998 May 15;43(2):164–71.
79. Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM, et al. Reversal of compromised bonding to oxidized etched dentin. J Dent Res. 2001 Oct;80(10):1919–24.
80. Muthukrishnan L. Imminent antimicrobial bioink deploying cellulose, alginate, EPS and synthetic polymers for 3D bioprinting of tissue constructs. Carbohydr Polym. 2021 May 15;260:117774.
81. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of Vertical Root Fractures by Cone-beam Computed Tomography in Root-filled Teeth with Confirmation by Direct Visualization: A Systematic Review and Meta-Analysis. J Endod. 2021 Aug;47(8):1198–214.
82. Chakraborty T, Jamal RF, Battineni G, Teja KV, Marto CM, Spagnuolo G. A Review of Prolonged Post-COVID-19 Symptoms and Their Implications on Dental Management. Int J Environ Res Public Health [Internet]. 2021 May 12;18(10). Available from: http://dx.doi.org/10.3390/ijerph18105131
83. Muthukrishnan L. Nanotechnology for cleaner leather production: a review. Environ Chem Lett. 2021 Jun 1;19(3):2527–49.

84. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? J Dent Sci. 2020 Dec;15(4):562–3.
85. Narendran K, Jayalakshmi, Ms N, Sarvanan A, Ganesan S A, Sukumar E. Synthesis, characterization, free radical scavenging and cytotoxic activities of phenylvilangin, a substituted dimer of embelin. ijps [Internet]. 2020;82(5). Available from: https://www.ijpsonline.com/articles/synthesis-characterization-free-radical-scavenging-and-cytotoxic-activities-of-phenylvilangin-a-substituted-dimer-of-embelin-4041.html
86. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. Oral Health Prev Dent. 2020 Apr 1;18(1):379–86.
87. Sawant K, Pawar AM, Banga KS, Machado R, Karobari MI, Marya A, et al. Dentinal Microcracks after Root Canal Instrumentation Using Instruments Manufactured with Different NiTi Alloys and the SAF System: A Systematic Review. NATO Adv Sci Inst Ser E Appl Sci. 2021 May 28;11(11):4984.
88. Bhavikatti SK, Karobari MI, Zainuddin SLA, Marya A, Nadaf SJ, Sawant VJ, et al. Investigating the Antioxidant and Cytocompatibility of Mimusops elengi Linn Extract over Human Gingival Fibroblast Cells. Int J Environ Res Public Health [Internet]. 2021 Jul 4;18(13). Available from: http://dx.doi.org/10.3390/ijerph18137162
89. Karobari MI, Basheer SN, Sayed FR, Shaikh S, Agwan MAS, Marya A, et al. An In Vitro Stereomicroscopic Evaluation of Bioactivity between Neo MTA Plus, Pro Root MTA, BIODENTINE & Glass Ionomer Cement Using Dye Penetration Method. Materials [Internet]. 2021 Jun 8;14(12). Available from: http://dx.doi.org/10.3390/ma14123159
90. Rohit Singh T, Ezhilarasan D. Ethanolic Extract of Lagerstroemia Speciosa (L.) Pers., Induces Apoptosis and Cell Cycle Arrest in HepG2 Cells. Nutr Cancer. 2020;72(1):146–56.
91. Ezhilarasan D. MicroRNA interplay between hepatic stellate cell quiescence and activation. Eur J Pharmacol. 2020 Oct 15;885:173507.
92. Romera A, Peredpaya S, Shparyk Y, Bondarenko I, Mendonça Bariani G, Abdalla KC, et al. Bevacizumab biosimilar BEVZ92 versus reference bevacizumab in combination with FOLFOX or FOLFIRI as first-line treatment for metastatic colorectal cancer: a multicentre, open-label, randomised controlled trial. Lancet Gastroenterol Hepatol. 2018 Dec;3(12):845–55.
93. Raj R K, D E, S R. β-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. J Biomed Mater Res A. 2020 Sep;108(9):1899–908.
94. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441–8.
95. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species [Internet]. Vol. 94, Archives of Oral Biology. 2018. p. 93–8. Available from: http://dx.doi.org/10.1016/j.archoralbio.2018.07.001
96. Uma Maheswari TN, Nivedhitha MS, Ramani P. Expression profile of salivary micro RNA-21 and 31 in oral potentially malignant disorders. Braz Oral Res.

2020 Feb 10;34:e002.

97. Gudipaneni RK, Alam MK, Patil SR, Karobari MI. Measurement of the Maximum Occlusal Bite Force and its Relation to the Caries Spectrum of First Permanent Molars in Early Permanent Dentition. J Clin Pediatr Dent. 2020 Dec 1;44(6):423–8.
98. Chaturvedula BB, Muthukrishnan A, Bhuvaraghan A, Sandler J, Thiruvenkatachari B. Dens invaginatus: a review and orthodontic implications. Br Dent J. 2021 Mar;230(6):345–50.
99. Kanniah P, Radhamani J, Chelliah P, Muthusamy N, Joshua Jebasingh Sathiya Balasingh E, Reeta Thangapandi J, et al. Green synthesis of multifaceted silver nanoparticles using the flower extract of Aerva lanata and evaluation of its biological and environmental applications. ChemistrySelect. 2020 Feb 21;5(7):2322–31.