Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies *NLM ID: 101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI) Index Copernicus value = 100

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Review Article

Root Canal Cleanse: Science Behind the Rinse - Irrigation in Endodontics

[1]Gauri Sachdeva, [2]Vansh Vardhan Madahar, [3]Aakriti Bharbutta, [4]Harsimran Kaur

[1,3,4]BDS, [2]BDS MDS, Genesis Institute Of Dental Science And Research, Ferozepur, India

ABSTRACT:
Root canal irrigation is an indispensable component of endodontic therapy, complementing mechanical instrumentation to eliminate microbial biofilms, pulp remnants, and smear layers. Despite advancements in shaping techniques, anatomical complexities hinder complete debridement, making chemical irrigation crucial. Sodium hypochlorite remains the gold standard for its tissue-dissolving and antimicrobial properties, while agents like chlorhexidine, EDTA, and modern combination solutions such as QMix and MTAD offer enhanced cleaning efficacy. The integration of ultrasonic activation further improves irrigant penetration and biofilm disruption, optimizing root canal disinfection and clinical success.
Keywords: Root canal irrigation, Sodium hypochlorite, Biofilm removal, Endodontic disinfection

Received: 22 June, 2025 Accepted: 25 July, 2025 Published: 30 July, 2025

Corresponding author: Vansh Vardhan Madahar, BDS MDS, Genesis Institute Of Dental Science And Research, Ferozepur, India

This article may be cited as: Sachdeva G, Madahar VV, Bharbutta A, Kaur H. Root Canal Cleanse: Science Behind the Rinse - Irrigation in Endodontics. J Adv Med Dent Scie Res 2025; 13(7):133-140.

INTRODUCTION

Root canal therapy is an elaborated procedure in which each step is crucial to ensure a successful debridement, disinfection and sealing of the root canal with the goal of preventing or curing of the apical periodontitis. Apical periodontitis is an inflammatory condition caused primarily by bacterial infection within the root canal system of a tooth along with peri-radicular tissues which is characterized by the formation of periapical lesions and bone resorption (1).

The removal of necrotic pulp tissue, debridement of dentinal wall and shaping of the canal for optimal obturation is done using endodontic file systems. In vitro and clinical records have suggested that mechanical instrumentation of the canal does not debride all areas of the canal walls (2) which can be attributed to anatomy of root canal, dentinal structures, constituents of dentin and biofilm (3) (4) (5) and hence it seems impossible that microorganisms can be completely eliminated from the canal by mechanical preparation solely (6). Although the cleaning and shaping of the root canal system are efficiently done using endodontic file but the biofilm removal is done by copious irrigation.

Irrigation of the root canal system plays a vital role in dissolution of the biofilm and removal of the dentinal debris produced during filing of the root canal. Variety of irrigating solution can be employed to efficiently dissolve the biofilm complex while acting as a bactericidal to the microorganisms (7).

Microbial Biofilm in Endodontics

Layers of enamel and dentine encloses the dental pulp, preventing bacterial invasion yet the entry of micro-organisms happens most commonly through caries. Microbial action on the surface of the tooth leads to cavitation, as the caries progresses through enamel then to dentin, the endotoxins seep into the pulp. Colonization of the root canal leading to necrosis of pulp and periapical lesion eventually leading to formation of bio-film (9).

A biofilm is a complex aggregation of microorganisms growing on the surface of the teeth or other oral structures. These microorganisms are embedded within a self-produced matrix of extracellular polymeric substances (EPS), which protects the bacteria from external threats and allows them to adhere firmly to the surfaces. Biofilms are responsible for pulpal and periapical diseases, due to the pathogenic activities of the bacteria within them (10) (11). Within the root canal system, bacteria adhere to the dentinal walls, apical complex area andaccessory canals (1)(8). These colonies are far from the reach of any mechanical removal techniques. Formation of the bio-film initiate by microbe attaching to the substrate and the production of the extracellular polymeric substances leaded to the matrix formation. Matrix provides the colony with a safe harbour to replicate and proliferate as described in Figure 1. The microbes communicate through quorum sensing, which regulates gene expression and enhances biofilm maturation. The diversity of the biofilm makes it a tedious to remove it efficiently. Complex matrix and various microbes resist antimicrobial action and host immune response (8). The copious irrigation along with mechanical preparation is necessary for efficient and effective removal of the biofilm complex. The list of microbes present in the biofilm in the root canal biofilm, these bacteria often present a collaboration they produce the biofilm :

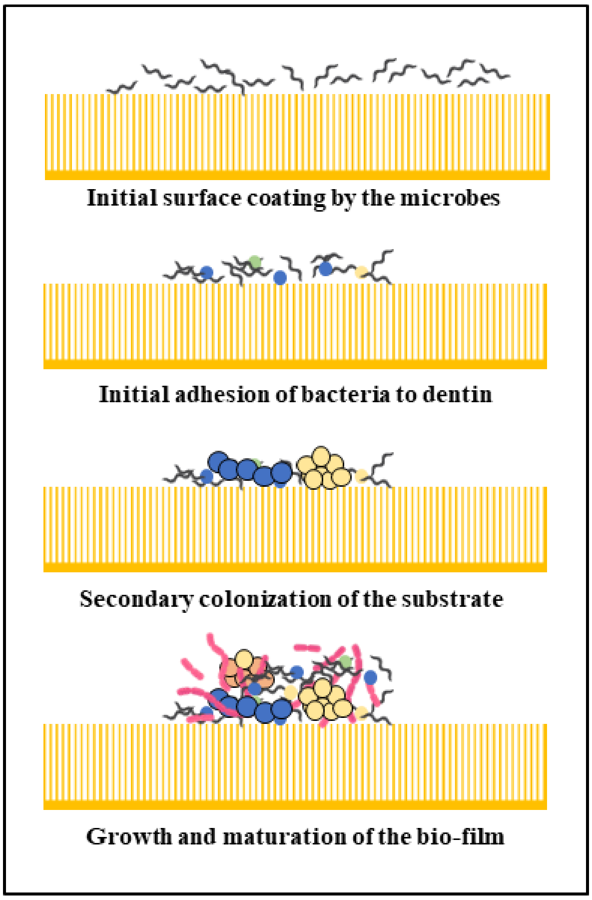
1. **Gram-negative anaerobes:**
   - Fusobacterium nucleatum
   - Porphyromonas gingivalis
   - Prevotella intermedia
   - Tannerella forsythia
   - Treponema denticola
2. **Gram-positive facultative anaerobes:**
   - Streptococcus spp. (e.g., Streptococcus sanguinis, Streptococcus gordonii)
   - Actinomyces spp.
   - Lactobacillus spp.
   - Enterococcus faecalis
   - Propionibacterium spp.
3. **Gram-positive obligate anaerobes:**
   - Parvimonas micra
   - Eubacterium spp.
   - Peptostreptococcus spp.
4. **Other notable bacteria:**
   - Dialister spp.
   - Pseudoramibacteralactolyticus
   - Olsenellauli
   - Filifactoralocis

Fungi:

1. Candida albicans
2. Candida glabrata

Archaea:

1. Methanobrevibacteroralis
2. Methanobacterium spp.



(FIGURE 1 – Schematic representation of biofilm formation)

Endodontic Irrigating Solutions

Irrigation of the root canal during the endodontic procedure is crucial to not only remove dentinal debris but also to irradicate the microbial biofilm. Each irrigating solution must meet up to following:

- Effective antimicrobial activity against a wide range of microorganisms, including both free-floating and biofilm-structured forms.
- Neutralization of bacterial virulence factors like endotoxins and lipoteichoic acids.

- Disruption or removal of biofilm structures.

Breakdown of residual pulp tissue.

- Elimination of hard-tissue debris and smear layer, or prevention of their buildup.
- No adverse effects on local tissues (such as dentine and periapical tissues) or systemic issues (like toxicity and allergic reactions).
- Easily accessible and inexpensive.

The list of the irrigating solutions available are as Sodium hypochlorite, Chlorhexidine, Chelating Agents (EDTA, HEBP), Mixtures.

Sodium Hypochlorite (NaOCl)

Sodium Hypochlorite is the most common yet the gold standard for irritating the canal system because of its exceptional antimicrobial action particularly against bacteria organized in biofilms (14) (15). NaOCl produces two strong oxidizing agents that being hypochlorite (OCl-) and hypochlorous acid (HOCl) (16) (17). It is an effective proteolytic, antimicrobial agent efficient in dissolving organic tissue. NaOCl is both an oxidizing and hydrolysing agent (18).

It is important to understand the working of sodium hypochlorite as with the pH of 11 it is a basic solution. Mechanism of action of NaOCl described by Estrela et al. as the initial reaction produced is saponification reaction, which accounts for degradation of fatty acids into fatty acid salts and glycerol. The NaOCl neutralize ammino acids produced by microbes and is refred to as Neutralization Reaction. Formation of hypochlorous acid due to chlorine coming in contact with the organic matter referred to as Hypochlorous acid formation which act as an oxidizer. Solvent action of sodium hypochlorite is efficient as it produces chlorine free radicles. As mentioned earlier high pH of the solution which is 11, this interferes with cytoplasmic membrane integrity due to irreversible enzymatic inhibition and phospholipid degeneration (19).

According to the literature, sodium hypochlorite (NaOCl) can be utilized in concentrations ranging from 0.5% to 6%. Tissue dissolving ability of the irrigating solution varies with varying concentration while disinfecting ability is efficient irrespective of the concentration (20).

Estrela's in vitro study examined the dissolution of bovine pulp tissue using sodium hypochlorite at concentrations of 0.5%, 1.0%, 2.5%, and 5.0% under various conditions. The study concluded that:

1. The dissolution speed of bovine pulp fragments rose with increased concentrations of sodium hypochlorite and was more rapid in the absence of a surfactant.
2. Changes in surface tension throughout the pulp dissolution process were directly related to the sodium hypochlorite concentration, with more pronounced variations in solutions lacking surfactant. Solutions without surfactant showed a reduction in surface tension, whereas those with surfactant exhibited an increase.
3. Heated sodium hypochlorite solutions dissolved bovine pulp tissue more swiftly.
4. Higher initial concentrations of sodium hypochlorite resulted in a smaller drop in pH.

The volume used to irrigate the canal is critical for canal disinfection. Larger amount of solution with lesser concentration is equally effective in disinfecting the root canal to that of the lesser volume of high concentration solution. While volume is set and concentration is decided time is the next important parameter to take in consideration. Bacterial killing efficacy of irrigating solution increases as the clinician increase the contact time. A lesser concentration with higher contact time will give similar results to that of solution with higher concentration but lesser contact time.

Clegg et al. (21) found that a 6% NaOCl solution was the only substance effective at both physically removing artificial biofilm and eradicating bacteria. The antibacterial efficacy of NaOCl was dose-dependent, with higher concentrations providing greater antibacterial effects. In summary, 3% and 6% NaOCl resulted in the complete absence of biofilm, 1% NaOCl led to biofilm disruption, and 2% CHX left the biofilm intact.

Despite its many ideal properties, NaOCl has several limitations. It is toxic non substantive, ineffective at removing the smear layer, and corrosive. Additionally, NaOCl can cause discoloration and has an unpleasant odor. Using NaOCl as a final rinse can also affect the bonding of the sealer to the dentin (22) (23).

Most complications with NaOCl use involve accidents where NaOCl is unintentionally injected beyond the apical foramen, causing severe tissue reactions, extreme pain, swelling, and possibly secondary infections (24). An in vitro study by Brown et al. (25) suggested that filling the coronal access cavity with NaOCl and allowing it to flow into the canal space during instrumentation can result insignificantly less extrusion of irrigation solutions from the apical foramen compared to deep irrigant distribution using needles.

Ultrasonic activation of sodium hypochlorite (NaOCl) is used to enhance its effectiveness in endodontic treatment. Here are some key effects and benefits of ultrasonic activation:

1. Improved Cleaning and Disinfection:
   a. Ultrasonic activation creates acoustic cavitation, which produces microscopic bubbles that collapse rapidly, generating shock waves and micro-streaming. This process helps in dislodging debris and improving the penetration of NaOCl into dentinal tubules and canal irregularities, leading to better cleaning and disinfection of the root canal system (26).
2. Enhanced Removal of Smear Layer:
   a. The mechanical agitation caused by ultrasonic waves helps in the effective removal of the

smear layer, a residual layer of debris that forms on the canal walls during instrumentation. This is crucial for ensuring better adhesion of root canal sealer and preventing bacterial growth [27].

3. Reduced Need for Multiple Irrigation Solutions:
   a. By improving the efficacy of NaOCl, ultrasonic activation may reduce the need for additional irrigation solutions, such as EDTA, as it enhances the ability of NaOCl to dissolve organic material and remove debris [28].
4. Better Penetration in Complex Canal Systems:
   a. Ultrasonic activation aids in the delivery of NaOCl to complex canal systems and areas that are difficult to reach with conventional irrigation techniques, leading to more thorough cleaning [29].
5. Potential for Increased Dentinal Erosion:
   a. It is important to use ultrasonic activation with caution, as prolonged or excessive use can potentially lead to dentinal erosion and weakening of the tooth structure [30].

Chlorhexidine Gluconate (CHX)

Chlorhexidine being lipophilic and hydrophobic is capable of penetrating the cell wall of bacteria. It belongs to the class of poly-biguanide which consist of two symmetrical rings. This molecule is electrostatic as it binds to negatively charge membrane of bacterial cell, while damaging the outer layer and changing the osmolarity. CHX isbacteriostatic in action at 0.2% conc. whereas at 2% conc. act as bactericidal (31).

In an in vitro study, Oncag et al. (33) found that a 2% CHX solution was more effective against *Enterococcus faecalis* than 5.25% NaOCl after both 5 minutes and 48 hours. Ercan et al. (34) reported that 2% CHX significantly reduced microbial volume in teeth with necrotic pulp, periapical disease, or both. Basson and Tait (33) demonstrated that 2% CHX was the only irrigant capable of fully disinfecting channels contaminated with *Actinomyces israelii* even after two months. However, Tanomaru et al. (35) found that 2% CHX, combined with biomechanical preparation, did not inactivate endotoxins in vivo.

As an intracanal irrigant CHX is well capable of disinfecting the root canal system but as tissue dissolution capacity is lacking. In-vivo and In vitro studies showed comparison between sodium hypochlorite and CHX, where 2.5% NaOCl was more effective than 0.2% CHX when effected root canals were irrigated for 30 mins with either of the solution (32).

Allergic reaction to CHX is uncommon, but a few allergic reactions such as anaphylactic, contact dermatitis and urticaria have been reported as CHX had a direct contact with the mucosa and open wound (36).

Clinical consideration while using CHX are as, it is a better irrigating solution in teeth with open apex or teeth with perforation. CHX is a better choice of irrigating solution in pediatric patients where using sodium hypochlorite is tedious job, considering behaviour and co operation of the patient.

When sodium hypochlorite (NaOCl) is combined with chlorhexidine (CHX), it results in a color change and the formation of a precipitate, with the extent of this precipitate increasing as the concentration of NaOCl rises, provided 2% CHX is used. This color change may raise clinical concerns, potentially leading to staining of the tooth. Moreover, the precipitate could affect the sealing of the root canal during obturation.

Basrani et al. investigated the nature of this precipitate and identified it as 4-chloroaniline (PCA). Recent TOF-SIMS analyses revealed PCA's infiltration into dentinal tubules. PCA is known to be toxic to humans and can cause cyanosis due to methemoglobin formation with short-term exposure. To avoid these issues, it is advisable to use EDTA or other irrigants following NaOCl and before applyingCHX, or alternatively, to dry the canals with paper points prior to the final rinse.

Ethylenediaminetetraacetic Acid (EDTA)

EDTA is a colourless, water soluble solid amino-poly-carboxylic acid. It is a chelating agent well capable of removing the mineralized portion of the smear layer. First described in 1935 by Ferdinand Munz as it was prepared by him, yet Nygaard-Ostby used it in calcified root canal in 1957.

EDTA extract metal ions from cell membrane and lead to cell death eventually. Chelator such as EDTA makes a stable complex with metals such as Ca+ and Fe+.

Endodontic treatment using EDTA, is efficient when used with a proteolytic agent such as sodium hypochlorite. While EDTA, act upon the matrix of the biofilm disintegrating the complexes NaOCl act upon the bacteria. 17% EDTA is normally used for efficient removal of smear layer.

A controversial opinion upon use of EDTA on the dentin, where Hulsmann et al. states that 50µm demineralization of dentin occurs with the use of EDTA whereas Qian et al. reported that no erosion was detected when demineralizing agent were used post NaOCl.

Researchers examined how EDTA interacts with sodium hypochlorite (NaOCl) and found that while EDTA retains its ability to form calcium complexes when combined with NaOCl, it impairs NaOCl's tissue-dissolving properties, leading to nearly undetectable levels of free chlorine in the mixture. This implies that EDTA and NaOCl should be used sequentially rather than together. In practice, a regimen where NaOCl is applied after EDTA is essential to ensure the removal of any EDTA residues (37) (38).

In contemporary endodontic practice, EDTA is typically used after the cleaning and shaping phase for about one minute, and it can be enhanced with ultrasonic activation to improve its penetration into dentinal tubules. However, it's important to avoid heating EDTA, as its calcium-binding efficiency diminishes when the temperature rises from 20℃ to 90℃.

Combination Solutions

In endodontics, combining irrigation solutions with detergents can further enhance the cleaning and disinfection of root canals. Here’s a summary of commonly used combinations involving detergents and their effects. Some of the latestcommercially available are SmearClear, Chlor-XTRA, CHX-Plus, Tetraclean, MTAD, QMiX.

SmearClear

SmearClear is an endodontic irrigating solution designed to effectively remove the smear layer formed during the instrumentation of root canals. Composed primarily of EDTA (ethylenediaminetetraacetic acid) along with surfactants and other agents, SmearClear works by chelating calcium ions to dissolve the inorganic component of the smear layer while the surfactants reduce surface tension, enhancing penetration and efficacy (39). This combination results in the efficient removal of the smear layer, which in turn exposes the dentinal tubules and improves the adhesion of root canal sealers (40).

Additionally, by eliminating the smear layer, SmearClear enhances the effectiveness of subsequent antimicrobial irrigants like sodium hypochlorite (NaOCl) and chlorhexidine (CHX), as these agents can penetrate more deeply into the dentinal tubules. Clinically, SmearClear is used after the cleaning and shaping phase of endodontic treatment, typically for 1-2 minutes, to ensure thorough removal of the smear layer before the final irrigation (41) (42).

It is essential to rinse thoroughly after using SmearClear to avoid any potential interaction with other irrigants. While SmearClear provides significant benefits in terms of cleaning and disinfection, care must be taken to follow recommended usage protocols, as overuse or prolonged exposure to chelating agents like EDTA can potentially weaken the dentin structure (43).

Chlor-XTRA

Chlor-XTRA is an advanced sodium hypochlorite (NaOCl) solution used in endodontic irrigation to enhance root canal cleaning and disinfection. It features a higher NaOCl concentration, typically around 6%, along with added surfactants and wetting agents. These surfactants lower the solution's surface tension, allowing it to penetrate deeper into the root canal's intricate anatomy, including lateral canals and dentinal tubules.

This improved penetration capability enhances Chlor-XTRA's ability to dissolve organic tissues and biofilms more effectively (42). The increased NaOCl concentration also provides stronger antimicrobial action, eliminating a broad spectrum of pathogens within the root canal (39).

Clinically, Chlor-XTRA is used throughout the cleaning and shaping phases of endodontic treatment, often with activation techniques like passive ultrasonic irrigation to boost its efficacy further. While it offers significant advantages in tissue dissolution and antimicrobial properties, it requires careful use to avoid potential dentin erosion and cytotoxic effects associated with high NaOCl concentrations (43).

CHX-Plus

CHX-Plus is an advanced chlorhexidine (CHX) solution used in endodontic irrigation, known for its superior antimicrobial properties and enhanced cleaning capabilities. Formulated with chlorhexidine gluconate and additional surfactants, CHX-Plus reduces surface tension, allowing deeper penetration into dentinal tubules and more effective elimination of pathogens, including bacteria, fungi, and viruses (39). The inclusion of wetting agents in CHX-Plus aids in breaking down biofilms and removing debris, ensuring a comprehensive cleaning of the root canal system (41).

It also leaves a residual antimicrobial effect, providing prolonged protection against reinfection. Typically used as a final rinse following sodium hypochlorite (NaOCl) and EDTA, CHX-Plus ensures thorough disinfection and removal of the smear layer (42). However, it is crucial to rinse out NaOCl completely before using CHX-Plus to avoid the formation of precipitates that could impact the canal seal and stain the tooth. By following the manufacturer's guidelines for concentration and duration, CHX-Plus maximizes its benefits while minimizing potential adverse effects (43).

MTAD

MTAD (Mixture of Tetracycline, Acid, and Detergent) is a specialized endodontic irrigating solution used for root canal disinfection and smear layer removal. Comprising doxycycline, citric acid, and a detergent (Tween 80), MTAD provides a comprehensive cleaning approach. Doxycycline offers broad-spectrum antimicrobial properties, effectively eliminating various bacteria, while citric acid demineralizes the smear layer, exposing dentinal tubules for enhanced cleaning (44).

The detergent component reduces surface tension, allowing the solution to penetrate the intricate anatomy of the root canal system, including lateral canals and dentinal tubules. MTAD is typically used as a final rinse after initial cleaning and shaping with sodium hypochlorite (NaOCl), ensuring thorough disinfection and smear layer removal.

Although MTAD excels in antimicrobial efficacy and smear layer removal, it is less effective at dissolving organic tissue compared to NaOCl, making it essential to use it in conjunction with other irrigants for comprehensive cleaning. Adhering to the manufacturer's guidelines is crucial to avoid potential adverse effects or interactions with other solutions (45).

QMix

QMix is an advanced endodontic irrigating solution that combines chlorhexidine (CHX), EDTA (ethylenediaminetetraacetic acid), and a detergent to provide effective smear layer removal and antimicrobial activity in a single step. The chlorhexidine component offers broad-spectrum antimicrobial properties, effectively eliminating bacteria, fungi, and viruses, while the EDTA chelates calcium ions, dissolving the inorganic component of the smear layer and exposing dentinal tubules. The detergent reduces surface tension, enhancing the solution's penetration into the complex anatomy of the root canal system, including dentinal tubules and lateral canals (41) (42).

Clinically, QMix is used as a final rinse following the initial cleaning and shaping with sodium hypochlorite (NaOCl), simplifying the irrigation protocol by combining smear layer removal and disinfection in one application. This dual action not only ensures thorough cleaning and disinfection but also saves time during the procedure. It is essential to thoroughly rinse out NaOCl before using QMix to avoid potential interactions and to follow the manufacturer's guidelines for optimal results and minimal adverse effects (44) (45).

Suggested Disinfection Protocol

In modern endodontics, several irrigation protocols are recommended for effective root canal treatment. The following steps outline a widely accepted approach:

1. Initial Irrigation:
   a. Use 2.5–5% sodium hypochlorite (NaOCl) throughout the instrumentation process until the root canal achieves its final shape, including the desired size and taper.
2. Activation and Heating:
   a. Activate and heat the fresh NaOCl solution using techniques such as ultrasonic, sonic, or laser activation for approximately 30 seconds per canal.
3. Apical Negative Pressure:
   a. Optionally employ apical negative pressure devices (e.g., Endovac) to enhance irrigation at the apex without causing extrusion.
4. 4.Smear Layer Removal:
   a. Apply a solution like EDTA or citric acid for about 1 minute to remove the smear layer. Activation and/or apical negative pressure can be used optionally during this step.
5. Final Rinse Options:
   a. Choose one of the following for the final rinse:
   b. Fresh NaOCl for approximately 1 minute, or
   c. Chlorhexidine (CHX), or
   d. QMix, or
   e. Alcohol, or
   f. Dry with paper points and proceed with obturation.

CONCLUSION

Root canal therapy is a complex process crucial for treating apical periodontitis and ensuring the longevity of endodontically treated teeth. The mechanical preparation of the root canal, though essential, often fails to completely debride all canal areas due to the intricate anatomy and biofilm formation. Therefore, irrigation plays a pivotal role in the success of root canal treatments by effectively dissolving biofilms and removing debris.

The selection of appropriate irrigating solutions is critical. Sodium hypochlorite (NaOCl) remains the gold standard due to its strong antimicrobial properties and ability to dissolve organic tissue. However, it has limitations such as potential toxicity and the risk of creating a precipitate when mixed with chlorhexidine (CHX). CHX, while excellent at reducing microbial load, lacks tissue dissolution capabilities. Ethylenediaminetetraacetic acid (EDTA) is used to remove the smear layer but should be applied sequentially with NaOCl to avoid compromising its effectiveness.

Recent advancements include the use of combination solutions like SmearClear, Chlor-XTRA, and QMix, which integrate multiple functions into a single irrigation step. These innovations enhance the cleaning and disinfection process, making it more efficient.

REFERENCES

1. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med. 2004;15(6):348-81.
2. Peters OA, Laib A, Gohring TN, et al. Changes in root canal geometry after preparation assessed by high resolution computed tomography. J Endod. 2001;27(1):1-6.
3. Paqué F, Laib A, Gautschi H, Zehnder M. Hard-tissue debris accumulation analysis by high-resolution computed tomography scans. J Endod. 2009;35(7):1044-7.
4. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004;30(6):559-67
5. Hübscher W, Barbakow F, Peters OA. Root-canal preparation with FlexMaster: canal shapes analysed by micro-computed tomography. Int Endod J. 2003;36(11):740-7.
6. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981;89(4):321- 328.
7. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J. 2009;42(4):288-302.
8. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod. 2010 Aug;36(8):1277-88.

9. Siqueira JF, Jr, Rôças IN. Microbiology of endodontic infections. In: Hargreaves KM, Berman LB. editors. Cohen's Pathways of the Pulp. 11 ed. St. Louis, MO: Elsevier; (2016). p. 599–629.
10. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623-633. doi:10.1038/nrmicro2415
11. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. J Clin Periodontol. 2017;44 Suppl 18
12. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod. 2002;28(10):689-693
13. Siqueira JF Jr, Rôças IN. Diversity of endodontic microbiota revisited. J Dent Res. 2009;88(11):969-981.
14. Ruiz-Linares, M. , Aguado-Pérez, B. , Baca, P. , Arias-Moliz, M.T. & Ferrer-Luque, C.M. (2017) Efficacy of antimicrobial solutions against polymicrobial root canal biofilm. International Endodontic Journal, 50, 77–83.
15. Yang, Y. , Shen, Y. , Wang, Z. et al. (2016) Evaluation of the susceptibility of multispecies biofilms in dentinal tubules to disinfecting solutions. Journal of Endodontics, 42, 1246–1250.
16. Baker, R.W. (1947) Studies on the reaction between sodium hypochlorite and proteins: 1. Physico-chemical study of the course of the reaction. Biochemical Journal, 41, 337–342.
17. Davies, J.M. , Horwitz, D.A. & Davies, K.J. (1993) Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radical Biology & Medicine, 15, 637–643.
18. Tejada, S. , Baca, P. , Ferrer-Luque, C.M. , Ruiz-Linares, M. , Valderrama, M.J. & Arias-Moliz, M.T. (2019) Influence of dentine debris and organic tissue on the properties of sodium hypochlorite solutions. International Endodontic Journal, 52, 114–122.
19. Estrela C, Estrela CR, Barbin EL, Spano JC, Marchesan MA, Pecora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13:113.
20. Grossman L, Meiman B. Solution of pulp tissue by chemical agent. J Am Dent Assoc. 1941;28:223.
21. Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod. 2006;32: 434–7.
22. 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.
23. Rocha AW, de Andrade CD, Leitune VC, Collares FM, Samuel SM, Grecca FS, de Figueiredo JA, dos Santos RB. Influence of endodontic irrigants on resin sealer bond strength to radicular dentin. Bull Tokyo Dent Coll. 2012;53(1):1–7.
24. Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part1.Intracanal drugs and substances. Int Endod J. 2003;36(2):75-85.
25. . Brown DC, Moore BK, Brown CE, et al. An in vitro study of apical extrusion of sodium hypochlorite during endodontic canal preparation. J Endod. 1995;21(12):587–591.
26. Haapasalo M, Shen Y, Qian W, et al. Irrigation in endodontics. Dent Clin North Am. 2010;54(2):291-312. doi:10.1016/j.cden.2010.01.002.
27. Reddy S, Suresh A, Usha K, et al. Ultrasonic activation of sodium hypochlorite in endodontics: A systematic review. J Endod. 2021;47(1):25-31. doi:10.1016/j.joen.2020.10.009.
28. Berkhoff J, de Carvalho M, Fernandes V, et al. Comparison of ultrasonic and manual agitation of sodium hypochlorite in the cleaning and disinfection of root canals: A systematic review. J Endod. 2015;41(1):27-32. doi:10.1016/j.joen.2014.10.004.
29. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-398. doi:10.1016/j.joen.2005.09.014.
30. Santos J, Silva E, Gomes R, et al. Influence of ultrasonic activation on the dentin microhardness of root canals. J Endod. 2014;40(6):844-847. doi:10.1016/j.joen.2013.12.015.
31. Gomes BPFA, Souza SFC, Ferraz CR, Teixeira FB, Zaia AA, Valdrighi L, Souza-Filho FJ. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. Int Endod J. 2003;36(4):267-275.
32. Rasimick BJ, Nekich M, Hladek MM, Musikant BL, Deutsch AS. Interaction between chlorhexidine digluconate and EDTA. J Endod. 2008;34:1521–3.
33. Basson NJ, Tait CM. Effectiveness of three root canal medicaments to eliminate Actinomyces israelii from infected dentinal tubules in vitro. South Afr Dent J. 2001;56(11):499-501.
34. Ercan E, Ozekinci 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(2):84-87.
35. Tanomaru JM, Leonardo MR, Tanomaru Filho M, Bonetti Filho I, Silva LA. Effect of different irrigation solutions and calcium hydroxide on bacterial LPS. Int Endod J. 2003;36(11):733-739.
36. Pham NH, Weiner JM, Reisner GS, Baldo BA. Anaphylaxis to chlorhexidine. Case report. Implication of immunoglobulin e antibodies and identification of an allergenic determinant. Clin Exp Allergy. 2000;30:1001–7.
37. Grawehr M, Sener B, Waltimo T, Zehnder M.Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J. 2003;36:411–7.
38. Zehnder M, Paque F. Disinfection of the root canal system during root canal re-treatment. Endod Top. 2008;19(1):58–73. Article fi rst published online: 26 MAY 2011.
39. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-398. doi:10.1016/j.joen.2005.09.014.
40. Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. J Endod. 2003;29(3):170-175. doi:10.1097/00004770-200303000-00002.
41. Niu W, Yoshioka T, Kobayashi C, et al. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. Int Endod J. 2002;35(11):934-939. doi:10.1046/j.1365-2591.2002.00591.x.
42. Haapasalo M, Shen Y, Qian W, et al. Irrigation in endodontics. Dent Clin North Am. 2010;54(2):291-312. doi:10.1016/j.cden.2010.01.002.

43. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod. 2002;28(1):17-19. doi:10.1097/00004770-200201000-00004.
44. Shabahang S, Torabinejad M. Effect of MTAD on Enterococcus faecalis-contaminated root canals of extracted human teeth. J Endod. 2003;29(9):576-579. doi:10.1097/00004770-200309000-00008.
45. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J. 2009;42(4):288-302. doi:10.1111/j.1365-2591.2008.01540.x