

A Review of Chemical Approaches Inherent to Endodontic Disinfection Protocols: Part 1

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Endodontics; chemical disinfection; endodontic irrigation.

Running title

Chemical Approaches for Endodontic Disinfection Protocols

ABSTRACT

Objectives

Endodontic treatment is a common dental procedure. This review paper discusses chemical endodontic disinfection protocols, including types of irrigation solutions, solution activation techniques, and intracanal medicaments. This comprehensive review is structured in two parts: Part one will examine the various types of endodontic irrigation solutions, while Part two will focus on adjunctive strategies to optimise irrigation efficacy, review the use of intracanal medicaments, and provide perspectives on future directions in endodontic therapy.

Materials and methods

Scientific platforms such as Pubmed and Google Scholar were (re)searched using the keywords: endodontics, endodontic disinfection, root canal disinfection, endodontic chemical debridement, endodontic irrigation, endodontic solutions, endodontic irrigant activation, and intracanal medicaments. Relevant articles were identified, screened, reviewed, and discussed.

Results

Sodium hypochlorite irrigation supplemented with EDTA

remains the most popular, effective and economical protocol for endodontic disinfection.

Conclusions

Despite advancements in the field, no single ideal irrigant is available and sodium hypochlorite in combination with EDTA, is the gold standard for endodontic irrigation. Lately, there has been a shift toward developing biocompatible disinfection protocols. Materials such as ozonated oils, morinda citrifolia juice, photo-activated disinfection, nitric oxide-releasing nano matrix, and nanoparticles have been suggested, but further research on their effectiveness is still required.

INTRODUCTION

Caries is a common disease and, when left untreated, spreads through enamel into dentine and the underlying delicate pulpal tissue, eliciting an inflammatory response. In addition, pulpal insults from trauma and periodontal infections make pulpal inflammation common, and when left untreated, pulpal tissues become necrotic, leading to apical periodontitis, which requires endodontic treatment to prevent tooth loss. Endodontic treatment is a fairly common and routine dental procedure whereby 10% of all teeth will require such treatment.¹ A review by Tibúrcio-Machado *et al.* found that half the adult population worldwide would have at least one tooth displaying signs and symptoms of disease requiring endodontic treatment.² For predictable and successful outcomes, the clinician must comprehensively understand endodontic principles.

The main goal of endodontic treatment is debridement and disinfection of the root canal system. In 1992, Chong and Pitt Ford emphasised the importance of controlled asepsis in infected root canals and the primary role of canal debridement and adequate canal preparation in successful outcomes.³ Ordinola-Zapata *et al.*, also supports this concept: what is removed from the root canal is of greater significance with regard to success than what is used to seal the root canal system.⁴ Therefore, it is vital for the clinician to have an understanding of tooth morphology, pathosis of pulp infection and an understanding of the materials and techniques available to debride and disinfect the root canal system.

Endodontic treatment is a complex multi-step procedure that aims to disinfect the root canal system to allow for the resolution of inflammation, infection and apical periodontitis. Complete disinfection is achieved with a combination of mechanical debridement, chemical irrigation and application of intracanal medicaments.⁵ Mechanical debridement is carried out with manual and motorised filing instruments. This removes infected tissue while shaping the root canals to allow for the delivery of antimicrobial and sealing materials. Studies have

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shown that mechanical instrumentation alone is insufficient to remove microbes from infected root canals, and while microbial loads decrease during instrumentation, positive cultures are still found afterwards.⁶ Combining mechanical instrumentation with chemical irrigation has effectively decreased microbial loads.^{6, 7} This paper will focus on chemical disinfection protocols including irrigation solutions, its activation methods, and intracanal medicaments.

The first step to effective disinfection is understanding the pathosis of pulp infection. Microorganisms and their endotoxins are responsible for the initiation of root canal infections and persistent endodontic-induced apical periodontitis.^{8, 9} The oral cavity is known to have more than 700 bacterial species,¹⁰ but not all of these species are found in root canal infections. Understanding the specialised root canal microbial environment will help determine which biocides will be effective disinfectants. Studies have shown that a core group of microbes is responsible for root canal infections and of the hundreds of bacteria in the oral cavity, just twenty to thirty species have been consistently isolated in endodontic infections and are known as the core microbiome.¹¹ The root canal system is an interesting environment as it is a closed system which limits bacterial interactions, nutrient availability and produces low oxygen potentials restricting the number of bacterial species in endodontic infections. These conditions allow for mostly facultative and anaerobic microorganisms to dominate the microflora in infected root canals.¹² Of these, the most isolated species are gram-negative: *Fusobacterium nucleatum*, *Porphyromonas* species, *Prevotella* species, *Treponema* species and *Tannerella forsythia* and gram-positive bacteria: *Pseudoramibacter alactolyticus*, *actinomyces* species and *Streptococcus* species.^{2, 11, 13-17}

To further complicate disinfection, these endodontic pathogens are in a coagulated mass or a polymicrobial biofilm.¹⁸ Bacteria in biofilm are more resistant to antibacterial agents than the same bacteria in planktonic form,¹⁹ making it difficult for antimicrobials to penetrate its structure.²⁰ Therefore, a two-prong approach is standard practice: mechanical disruption of the biofilm combined with a chemical biocide attack. An additional challenge to successful outcomes is the formation of a smear layer during mechanical debridement, which can be a nidus for secondary infections²¹ and can compromise the final obturating seal.

The variegated anatomy of the root canal system adds further complexity to endodontic treatment. Complex and intricate anatomical variations such as isthmuses and ramifications often make mechanical irrigation impossible in these spaces. Chemical irrigation solutions are able to flow into these spaces, augmenting mechanical debridement for more thorough disinfection.⁷

Therefore, in addition to its bactericidal effects, chemical irrigation should eliminate the smear layer, organic and inorganic components, and necrotic debris and disinfect the difficult-to-reach anatomy within the root canal system.^{7, 22} This paper will focus on chemical disinfection irrigant solutions. The materials and methods of mechanical instrumentation are not discussed and are beyond the scope of this paper.

IRRIGATION SOLUTIONS

An essential part of endodontic therapy is the chemical disinfection protocol. Chemical solutions augment

mechanical debridement by removing or inactivating microbes, removing the smear layer, and disinfecting the hard-to-reach anatomical spaces within the root system. Many different chemical irrigants can be used to this end.

Each provides different benefits, but none provide complete disinfection with a one-step solution. Combination irrigant protocols are standard to overcome the individual limitations of each irrigant. An ideal irrigant should fulfil the following requirements:^{21, 23-26}

1. Have broad-spectrum antimicrobial properties with rapid antimicrobial action
2. Have the ability to penetrate biofilms and dentine tubules
3. Dissolve organic, inorganic, necrotic tissue and smear layer
4. Inactivate endotoxin and lipoteichoic acid
5. Low surface tension for optimal irrigant penetration
6. Should not weaken or stain tooth structure
7. Easy to use and easy to remove from the root canal
8. Should be innocuous to dental materials
9. Be biocompatible with the adjacent oral tissues
10. Be non-toxic to local and systemic tissues
11. Have minimal ability to evoke an anaphylactic reaction
12. Low cost and easy to store

A practical way to classify these solutions is by their mechanism of action, which enables the clinician to choose an irrigant based on their usefulness and appropriate clinical application (see Figure 1):

- Antibacterial agents
- Chelating agents
- Combination of antibacterial and chelating agents
- Adjunct solutions
- A new subclass of natural irrigants with regenerative potentials

Some irrigants have a dual purpose and may not strictly fall into the sub-classification but cross over into other categories. We have used their most common purpose for classification purposes.

Antimicrobial Irrigants

Microorganisms and their endotoxins are responsible for the initiation of root canal infections and persistent endodontic-induced apical periodontitis.⁸⁻⁹ Mechanical debridement alone is not sufficient to eradicate these microorganisms. A two-prong approach is standard, whereby mechanical instrumentation disrupts the microbial biofilm, enabling chemical biocides to attack the microbes. Furthermore, complex root anatomies make it impossible for mechanical instruments to navigate into all areas of the root system. Antimicrobial irrigant solutions can reach these complex areas, augmenting disinfection for improved results.

Sodium Hypochlorite

Sodium hypochlorite (NaOCl) has an extensive history in endodontic therapy and is still the most widely used irrigant today.²⁷⁻³⁰ NaOCl is a proteolytic agent that works as an oxidiser and hydrolysing agent and is effective against bacterial biofilms, removal of organic tissue remnants and necrotic debris.^{27, 31} It plays a minor role in lubricating the canal for easier instrumentation and cooling the tooth when higher-powered instruments are used.

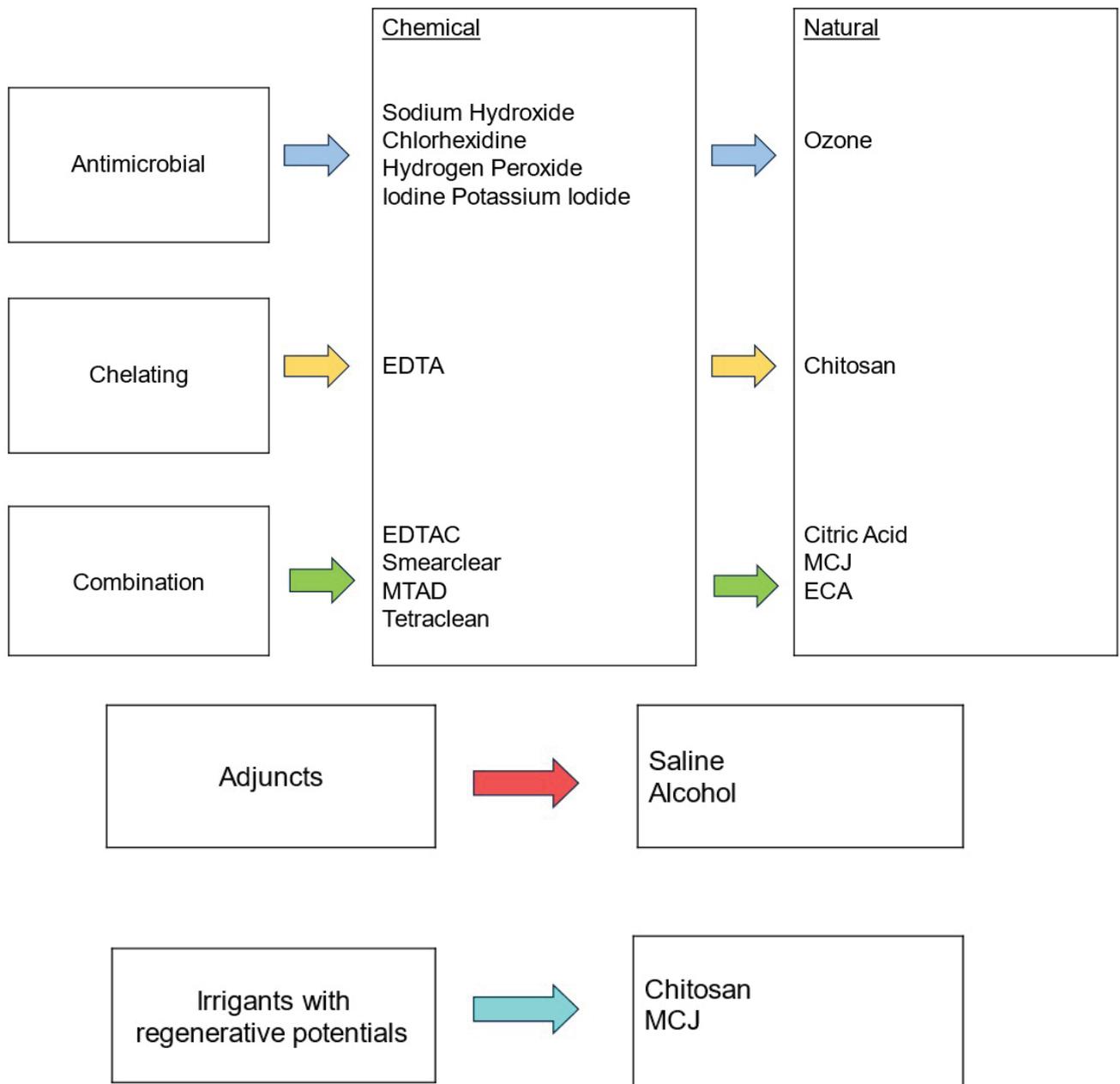


Figure 1: Classification system for irrigation solutions.

NaOCl is a strong bactericidal base commonly used in concentrations of 0.5% to 6%. NaOCl ionises in water into sodium (Na^+) and hypochlorite ions, OCl^- , and establishes a dynamic balance with hypochlorous acid (HOCl) as depicted in the equation below.³²



NaOCl has multiple modes of action. Firstly, the high pH of the hydroxyl ion groups compromises the integrity of cytoplasmic membranes and irreversibly inhibits enzymatic functions. Secondly, NaOCl dissolves organic tissue through saponification reactions where NaOCl dissolves fatty acids, converting them into fatty acid salts (soap) and glycerol (alcohol). Thirdly, NaOCl neutralises amino acids forming water and salt, removing the hydroxyl group and lowering the pH. Lastly, when HOCl comes into contact with organic tissue, it acts as a solvent, releasing chlorine.³² Chlorine is a

strong oxidant that not only inhibits bacterial enzymes but also interacts with amino groups and forms chloramines, affecting the cellular functions of microbial cells and causing rapid cell death.²⁷

NaOCl toxicity can induce severe adverse reactions if the irrigant accidentally extrudes into the surrounding tissues. At high concentrations, NaOCl damages vital tissues making it extremely cytotoxic, while at lower concentrations, it induces an inflammatory reaction in vital tissues.⁷ Therefore, care must be taken to prevent spillage and extrusion, which can cause severe adverse reactions. There are a few ways to limit this risk, from the use of a dental rubber dam, which protects the oral cavity and throat, to using specially designed endodontic needles without exerting pressure or using negative pressure irrigation systems. Heating NaOCl to between 45° - 60° increases its efficacy, and it can be more effective at lower, safer concentrations.⁷ Despite its limitations and toxicity

potential, NaOCl is the most widely used irrigant because of its effectiveness, low cost and ease of availability.^{30, 33}

Chlorhexidine

Chlorhexidine (CHX) is an antimicrobial agent with many uses in dentistry. It was first used as a general antiseptic. It is effective against a wide variety of micro-organisms, making it effective as a periodontal and endodontic irrigant, typically in a 2% solution.³⁴

CHX is a cationic bis-biguanide antiseptic with broad spectrum qualities. CHX is bacteriostatic at low concentrations and bactericidal at high concentrations.³⁵ As a cationic molecule, CHX is strongly attracted to the negatively charged components of the biofilm, i.e., bacterial cell wall, glycoproteins and phospholipids.³⁶⁻³⁸ This results in adsorption to the phosphate-containing components of the cell, allowing CHX to passively diffuse into the cell towards the cytoplasmic membrane. The CHX molecule attaches to the inner cell membrane changing the membrane's osmotic equilibrium increasing its permeability.^{39, 40} This results in the loss of low-molecular-weight molecules and cytoplasmic components, which in turn inhibits cytoplasmic enzyme activity.³⁷ This action occurs at low concentrations of CHX (0.2%) and is bacteriostatic; if CHX is removed at this stage, the processes will reverse. If CHX concentration increases to 2% or is sustained for an extended period of time, the process becomes bactericidal³⁶ where cytoplasmic coagulation and precipitation occurs with irreversible cellular damage and cell death ensues.^{35, 36, 38, 41}

Multiple studies have shown that CHX exhibits antimicrobial action dependent on the solution's type, concentration and presentation.^{39, 41-44} CHX is also an effective anti-fungal agent, particularly against *Candida Albicans*, and is clinically relevant as secondary endodontic infections tend to have increased levels of fungi within their biofilm.

CHX has been shown to have a sustained antimicrobial effect - a phenomenon known as substantivity.⁴⁵ The cationic molecules of CHX binds to the negative components of the biofilm and the hydroxyapatite of dental tissue, and is released slowly thereby prolonging its antimicrobial effects.^{41, 44, 46} CHX on its own does not dissolve necrotic tissue and is less effective on gram-negative than gram-positive bacteria and, therefore, cannot be used as a single endodontic irrigant. The substantivity properties of CHX make it an ideal final irrigant, as its effects are prolonged from 48 hours up to 12 weeks, depending on the concentration used and contact time.⁴¹

To further support this idea of CHX as a supplementary final irrigant, studies have shown that dentine, dead microorganisms and inflammatory products from the apical exudate exhibit some buffering effect on CHX and can decrease or inhibit its antibacterial effects.^{39, 47, 48} Using CHX in cleaned, debrided and disinfected root canals will improve its efficacy.

CHX demonstrates low toxicity. The worst reported adverse effects are contact dermatitis and dysgeusia. When used for prolonged periods, it causes brown staining of teeth, but this is usually reversible upon cessation. Its genotoxicity has been shown to be irrelevant on its own,⁴⁹ but when combined with NaOCl or calcium hydroxide, it can produce oxidative and pro-oxidant reactions.⁴⁵ However, this is concentration-dependent, and care should be taken

to ensure that the irrigant remains within the root canal space.⁴⁵

The main disadvantage of CHX is its inability to dissolve tissue. In an attempt to overcome this drawback, Kuruvilla and Kamath combined 0.2% CHX with 2.5% NaOCl and found this combination to be effective in reducing microbial load.⁴⁹ The active mechanism in this combination is thought to be due to the formation of CHX chloride. CHX chloride increases the ionising capacity of CHX, increasing the effectiveness of CHX alone but not NaOCl.⁴⁹ Despite the increased potential efficacy, NaOCl and CHX combination is known to produce a brown precipitate that can cause tooth discolouration and potentially interfere with the final obturation seal,⁵¹⁻⁵³ and care must be taken when this protocol is used.

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is an active biocide against viruses, bacteria, fungi and spores.⁵⁰ H_2O_2 is routinely used for sterilisation and disinfection. In dentistry, it is used in concentrations of between 1 and 30%. According to McDonnell and Russell, H_2O_2 is most effective at higher concentrations (10%-30%) and when used over longer periods of time.⁵¹ As an endodontic irrigant, it is used in concentrations of 3-5%.⁵² Its mechanism of action involves the formation of free hydroxyl radicals, which leads to oxidation of proteins, membrane lipids, and DNA, disrupting cellular structure and function.⁵³ At higher concentrations, H_2O_2 is bactericidal as it causes damage to the microbial cell wall, resulting in loss of intracellular material and cell death.^{54, 55}

H_2O_2 dissolves organic tissue, which may allow for deeper dentine penetration of the irrigants that follow, and is often used in combination with other irrigant solutions, thereby increasing the overall effectiveness of these irrigants. When 3% H_2O_2 was combined with CHX, the efficacy of each irrigant improved and in lower concentrations.⁵⁶ This mechanism of action is poorly understood, but it is thought that once CHX causes damage to the cytoplasmic membrane, thereby changing its osmotic permeability, H_2O_2 is able to penetrate more easily and cause irreversible damage.⁵²

In the medical concentrations where H_2O_2 is used, it is non-toxic and safe as H_2O_2 degrades to water and oxygen, making it biocompatible. However, H_2O_2 has effervescent properties and there is a risk of air embolism if seepage occurs into the surrounding tissue. Case reports have shown that extrusions of H_2O_2 into the periapical tissues cause intense pain, erythema and oedema, and treatment involves antibiotic cover with symptomatic relief.⁵²

Ozone

Ozone is a natural gas in the stratosphere consisting of three oxygen atoms. Ozone has high oxidant properties and is a strong bactericide.²⁴ It reacts with the hydrocarbons in cell membranes destroying bacterial cell walls and cytoplasmic membranes increasing cell membrane permeability allowing ozone molecules to enter the cell causing cell death.^{5, 57} Ozone is effective in multiple forms: ozone gas, ozonated water or ozonised oils. As a gas, ozone is unstable and lasts only a few minutes. Ozonated water and ozonised oil have a longer shelf life of a few days and months to years, respectively. Side effects of long-term exposure include epiphora, irritation to the upper airways, bronchoconstriction, rhinitis, coughing and vomiting. Ozone toxicity treatment is mainly supportive with oxygen, ascorbic acid, vitamin E, and N-acetylcysteine.⁵⁷

In endodontics, ozonated water has been used as an irrigant while ozonised oil has been used as an intracanal dressing. In a study by Nagayoshi *et al.*, the effects of ozonated water against *Streptococcus* infections in vitro in bovine dentin were evaluated. They found that the viability of both bacteria was significantly decreased in the dentine tubules. The authors also compared the cytotoxicity between ozonated water and NaOCl against L-929 mouse fibroblasts. They found that the metabolic activity of fibroblasts was high when the cells were treated with ozonated water. In contrast, when the cells were treated with 2.5% NaOCl, fibroblast activity was significantly decreased. The authors suggest that ozonated water can be useful in endodontic therapy.⁵⁸ Interestingly, Reddy and co-workers found that when sonification was combined with ozone water, the antimicrobial activity was almost the same as 2.5% NaOCl.⁵⁹ In another study by Nagayoshi *et al.*, it was found that in concentrations of 0.5–4 mg/L, ozonated water killed pure cultures of *Porphyromonas* endodontics and *Porphyromonas gingivalis* effectively.⁶⁰

Despite this promising potential, Hems *et al.* discovered that ozone was only effective on planktonic bacteria and not on bacterial biofilms, which is the predominant state of bacteria within the root canal system.⁶¹ Estrela *et al.*, studied the antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine against *Enterococcus faecalis* in human root canals. The authors found that after twenty minutes of exposure, none of the irrigants were effective against *Enterococcus faecalis*.⁶² These contradictory results limit the antibacterial activity of ozone as part of a root canal disinfection protocol.

Iodine potassium iodide

Iodine potassium iodide (IKI) is a combination of potassium iodide and iodine dissolved in distilled water. IKI is a potent antimicrobial agent with quick antiseptic action against microorganisms while displaying low toxicity. It is used in various concentrations ranging from 1% to 5%.^{63, 64} It has shown excellent elimination of bacteria, especially *Enterococcus faecalis*, within a short time. Iodine is an oxidising agent that reacts with free sulfhydryl groups of bacterial enzymes. This causes disulphide linkages and destroys bacterial cell walls. Vapour evaporation and sublimation expands the reach of its antibacterial effects. As a 2% root canal irrigant, IKI has sufficient antimicrobial effects, a more pleasant odour and taste with lower toxicity compared to NaOCl and can penetrate deep into the dentine walls.⁶⁵

However, IKI does not exert any effects on organic and necrotic tissue. Despite IKI demonstrating promising antimicrobial effects, it is less effective against bacterial biofilms. Biofilms are inherently more complicated in structure and more resistant to attack than planktonic bacteria. Abbaszadegan *et al.* state that to improve the antimicrobial effects of IKI, exposure time needs to increase. The authors state that a five-minute final rinse with IKI may be insufficient to kill the microorganisms in the extensive biofilms of necrotic pulps. They recommend using it as part of the irrigation protocol from the beginning or increasing the exposure time at the end.⁶⁶ As yet, there is no clear recommendation on the application time of IKI, with suggestions ranging between 10 and 15 minutes,^{67, 68} which can be impractical in the clinical setting.

In an attempt to improve microbial elimination, IKI has been added to conventional disinfection protocols.^{67, 69}

Studies have shown that if IKI is used as a final rinse after mechanical instrumentation and NaOCl irrigation, it can produce negative culture tests. Peciulienė *et al.* reported a 95% culture-negative test in retreatment cases.⁶⁹ Tello-Barbaran *et al.* reported that a 15-minute rinse with 2% IKI after instrumentation and NaOCl irrigation produced 95% negative cultures.⁶⁷ However, other studies have found no additional benefit to adding IKI to the disinfection protocol. Molander *et al.* found no added antimicrobial benefit when IKI was used as an intracanal medicament for 3-7 days. The authors postulated that the organic tissue and fluids within the root canal inactivate the medicament.⁷⁰ CaOH demonstrates better antimicrobial effects over a longer time than IKI. Kvist *et al.* stated that a final rinse with IKI had the same antibacterial effects as calcium hydroxide and found no added benefit.⁷¹ The long working time and contradictory reports on its antimicrobial effectiveness make the addition of IKI to standard disinfection protocols controversial with no clear advantage.

Enzymatic Irrigation

Niazi and co-workers proposed using enzymatic irrigation to kill and disrupt the biofilm. They developed a nutrient-stressed multispecies biofilm model from refractory endodontic infections to test the effectiveness of trypsin and proteinase K against 0.2% CHX and 1% NaOCl with or without ultrasonic activation.⁷² Despite NaOCl demonstrating the best antibacterial properties, trypsin and proteinase K displayed biofilm-disrupting capability. The exact mechanisms at work are unclear, but the protease is thought to act by degrading the protein components of the bacterial cell walls and membranes, causing cellular damage and, ultimately, cell lysis and death. In addition, the proteolytic enzymes might disrupt the extracellular matrix, which is also made up of proteins secreted by bacteria, thus reducing the cohesion of the biofilm.⁷²

Chelating agents

During mechanical instrumentation, a smear layer is formed on the walls of the root canal, occluding dentine tubules. The smear layer comprises dentine, remnants of pulp tissue, odontoblastic processes and bacteria. This smear layer can prevent effective irrigation penetration into dentine and the biofilm and interfere with the adhesion and penetration of sealing materials.⁷³ Chelating agents supplement chemical disinfection by removing the smear layer, exposing the root canal surface for improved irrigant penetration, and are used alternately with antimicrobial solutions.

EDTA

Ethylenediaminetetraacetic acid, or EDTA, is a polyaminocarboxylic acid. It is a biocompatible chelating agent that binds to minerals and metals. It is commonly used to dissolve lime scale and, systemically to treat heavy metal toxicity. EDTA reacts with calcium ions forming soluble calcium chelates.⁷⁴ As an endodontic irrigation solution, EDTA reacts with calcium ions in the dentine, helping to remove the smear layer to improve irrigation penetration while also delivering some antimicrobial activity by detaching biofilms from the walls of the root canal.^{24, 75} Therefore, an alternating regimen of NaOCl and EDTA is recommended to effectively lower bacterial loads in the root canal system.²¹ Jaju *et al.*, state that a combination of NaOCl and EDTA may be more efficient in reducing bacterial loads in root canal systems than NaOCl alone (24). EDTA reduces the available chlorine in the solution and thus can reduce the effectiveness of NaOCl.²¹

and therefore, the two solutions should not be mixed, but rather irrigation with each solution should occur separately using different syringes.

EDTA has been used in varying concentrations; the most used concentration in dentistry is 17%. EDTA is known to cause dentine demineralisation by liberating phosphorus from dentine. A study by Serper and Calt demonstrated that EDTA used at higher concentrations with increased exposure time caused increased amounts of phosphorus to be released, increasing the rate of dentine demineralisation⁷⁶. Calt and Serper tested 10 ml 17% EDTA irrigation for 1 minute and found it effective in removing the smear layer. However, it was observed that a 10-minute application of 17% EDTA caused excessive dentine demineralisation.⁷⁷ These studies concluded that increasing contact time and EDTA concentrations increases dentine demineralisation and advised against rinsing with EDTA for more than 1 minute.^{76,77} Nakashima and Terata tested 3% and 15% EDTA concentrations. They observed that both concentrations were effective in smear layer removal allowing for irrigant permeability, and concluded that 3% EDTA is sufficient for clinical application.⁷⁸

Citric Acid

Citric acid (CA) is a weak acid with antibacterial and chelating properties and has demonstrated effective smear layer removal. As an antibacterial agent, Yamaguchi *et al.* showed that CA is able to neutralise twelve different types of root canal bacteria.⁷⁹ A recent study by Scelza *et al.* showed that a combination of 10% citric acid and 1% CHX has promising antibacterial effects in root canal irrigation.⁸⁰

As a chelating agent, CA is reported to be slightly more potent than EDTA at a similar concentration.^{74,79,81} A study by Prado *et al.* showed that CA can be more effective than EDTA in short durations of 30 seconds (82). It provides the same benefits as a chelator, where it may detach biofilm from root canal walls, allowing for improved irrigant penetration. CA can also be useful to remove NaOCl/CHX precipitate should it form during irrigation.⁸³ Additional studies have also demonstrated that 10% CA is more cytocompatible and safer than NaOCl and EDTA.^{80,82,84} Machado and co-workers have shown that the effectiveness of CA is comparable to EDTA while being less toxic and may retain the highest rate of cell viability, and CA appears to offer more advantages than EDTA⁸⁵.

CA has also been used in combination with other irrigants. Scelza *et al.* tested 10% CA combined with 1% CHX (CACHX). This combination was tested against 2.5% NaOCl, 1% CHX, 10% CA and sterile water. They found that CACHX was the only irrigant to sustain a total absence of *E. faecalis* for a longer time after treatment. The authors concluded that this CACHX combination contributes to biofilm-free root walls. They postulate that the reason for this total elimination is the combination of chelating and antimicrobial action. With continuous irrigation, there is less possibility of smear layer and dentine debris formation, allowing for CHX substantivity.⁸⁰ Dewi *et al.*'s study support these findings where they found that the CA/CHX combination is more effective at removing the smear layer than CHX or 17% EDTA.⁸⁶

CA is biocompatible and the least toxic of root canal irrigants, with the highest rate of cell viability.^{82,87} Scelza and co-workers tested the biocompatibility of CA/CHX on Human

Periodontal Ligament Fibroblasts (HPdLF). HPdLF are used to test irrigant toxicity as root canal irrigants should be harmless to peri radicular tissues. To stimulate the clinical scenario, HPdLF were exposed to a 0.1% solution for 15 minutes, and no significant toxicity was observed when compared to unexposed cells in either cell metabolism, membrane integrity or cell proliferation.⁸⁰ CA is not only a promising adjunct to root canal disinfection, but the antimicrobial properties combined with biocompatibility and protection of cell viability, could be valuable in regenerative endodontics and the development of biocompatible root canal disinfection protocols.

Combination solutions

Chelating solutions with additives for antimicrobial effects

EDTA has limited antibacterial properties, and in the quest to simplify disinfection, antimicrobial substances have been added to EDTA to create a new class of EDTA-combination alternatives (75). Antiseptics such as quaternary ammonium compounds, usually cetrimide, have been added to EDTA, forming EDTAC to improve its bactericidal effects and clinical performance.⁸⁸ EDTAC solutions have lower surface tension than traditional EDTA, increasing irrigant penetrability.⁷⁴ Another EDTA variant is Smearclear, a 17% EDTA solution with cetrimide and an anionic surfactant to reduce its surface tension and increase its wettability properties. However, Khedmat *et al.*, demonstrated that there was no significant difference in smear layer removal between Smearclear, 17% EDTA and 10% CA.⁸⁹ Dunavant *et al.* and Wu *et al.*, on the other hand, found that traditional irrigation solutions of 1 and 6% NaOCl and 17 % EDTA, respectively outperformed Smearclear in removing root canal biofilms.^{90,91} There is no clear indication of whether Smearclear offers any added advantage over traditional EDTA.

EDTA is also available in paste form, usually in combination with urea peroxide. Urea peroxide allows debris to float out of the root canal, but a residue may be left behind, which can compromise the final seal.⁹² There are several different brands of these preparations available. The use of these pastes is controversial as some studies have shown that it is not as efficient as 17% EDTA solution in removing the smear layer.^{74,93,94} A common use of EDTA paste is as a lubricant during mechanical instrumentation, and it is particularly important when using nickel-titanium (Ni-Ti) rotary files. Manufacturers of Ni-Ti rotary files recommend using these pastes to reduce the risk of file separation in the root canal.⁹⁵ To overcome the limitations of EDTA paste preparations, it is recommended that NaOCl must be used before using the paste and regularly during root canal disinfection to be most effective.

Antibiotics have also been added to EDTA solutions to improve their antibacterial properties, and examples of these types of EDTA-antibacterial solutions are MTAD and Tetraclean. Both these irrigants have similar ingredients: an antibiotic, an acid and a detergent. In 2004, Torabinejad *et al.* developed MTAD containing doxycycline (a tetracycline isomer), CA and Tween 80 detergent.²⁴ Tetraclean was developed by Luciano Giardino in 2008, and the active ingredients of Tetraclean are doxycycline, CA, cetrimide and polypropylene glycol. MTAD has a higher doxycycline concentration than Tetraclean: 150mg/ml and 50mg/ml, respectively.

The tetracycline family of antibiotics is a broad-spectrum antibiotic effective against a wide variety of infections. It

works by inhibiting protein synthesis and interferes with normal cell functions, exerting bacteriostatic effects. It has a low pH and acts as a calcium chelator, causing enamel and dentine demineralisation. Tetracyclines have a strong affinity to dentine and are able to bind quickly to dentine, prolonging its effects and demonstrating substantivity properties.^{24, 96} As discussed above, CA is antibacterial and a chelating agent and effectively removes the smear layer.⁷⁹ The antibacterial effects of these EDTA-antibacterial solutions are due to the synergistic combination of doxycycline and CA. The detergent decreases the surface tension of the irrigant, allowing for better wettability and deeper penetration of the irrigant into the dentine tubules.⁹⁷

MTAD effectively dissolves organic and inorganic debris.⁹⁸ It is less cytotoxic than other irrigants and medicaments such as EDTA, hydrogen peroxide and CaOH paste.^{100, 101} Hence, MTAD provides some advantages over conventional intracanal irrigants and medicaments. Shabahang and Torabinejad found that MTAD is useful in eliminating microbes resistant to conventional endodontic irrigants and medicaments.¹⁰² MTAD is recommended as a final rinse before sealing.

Another advantage of these chelating solutions is their substantivity effects. Neglia *et al.* tested Tetraclean effectiveness and demonstrated no negative effect on bacteria immediately after application, but its action progressively increases for 72 hours until the bacterial load is wholly eliminated.¹⁰³ The behaviour is partly due to the synergistic effects of the combination of its ingredients. The CA removes the smear layer, allowing direct access of the irrigant to the dentine wall. At the same time, the reduced surface tension causes wetting of the dentine walls and allows for deeper penetration of the irrigant into the dentine.¹⁰³ Finally, the doxycycline binds to the dentine and now has time to exert its gradual and prolonged antibacterial effects on the bacteria.⁹⁶ It is as if an antibacterial reservoir is created to continuously release its antibacterial agents, thereby prolonging its effects. In comparison to NaOCl, tetraclean, with its low surface tension, can penetrate the dentine. NaOCl, on the other hand, cannot penetrate dentine, and its effects are observed immediately with obliteration of bacteria. However, without the combination of additional irrigants and medicaments, root canals irrigated with NaOCl quickly become recolonised with bacteria. In this way, tetraclean and other substances with substantivity properties are advantageous in limiting or preventing bacterial recolonisation in root canals and are recommended as a final rinse.⁷

Electrochemically Activated Solutions

Electrochemically Activated (ECA) Solutions are produced by tap water and low-concentration salt solutions.⁵ Two types of ECA solutions are produced: anolyte and catholyte. Anolyte solutions have a pH of between 2 and 9 with oxidative properties and are antibacterial.⁷ In contrast, catholyte solutions are alkaline and have shown strong detergent properties.⁵

As a root canal irrigant, ECA solutions have shown to have broad-spectrum antimicrobial activity and can be effective against 99.999% of microorganisms making it potentially bactericidal.²⁴ Studies have also demonstrated promising results, with some studies showing ECA solutions to be as effective as NaOCl at eliminating bacteria.¹⁰⁴⁻¹⁰⁶ A study by Jaju and Jaju, 2011 has shown that ECA solutions can also be effective at removing the smear layer.²⁴ In

addition, ECA is nontoxic and harmless to human cells,^{24, 107} and this characteristic makes ECA solutions a potentially biocompatible irrigant in a step towards dental pulp regeneration.

Etidronic acid/NaOCl combination

Etidronic acid is a weak chelator that can be combined with NaOCl, simplifying irrigation with a single solution, delivering a novel concept: continuous chelation.^{108, 109} Etidronic acid, also known as 1-hydroxyethylidene-1,1-bisphosphonate or HEBP, is a non-nitrogenous bisphosphonate with various medicinal uses. Etidronic acid is a biocompatible chelator, and when combined with NaOCl, it does not consume the free available chlorine molecules, unlike other chelators. In 2005, Zehnder *et al.* proposed its use as a root canal irrigant. This combination solution allows for continuous chelation throughout instrumentation instead of using a chelator as a post-instrumentation rinse. Continuous chelation allows continuous removal of tissue debris and smear layer (or prevention of its formation), thus enhancing NaOCl's penetration and improving antimicrobial effects.^{110, 111} Other possible solutions in this category are tetrasodium EDTA and clodronate.

Adjunct solutions

Adjunct solutions are mentioned as supplement irrigants and not as antimicrobial irrigants. Its purpose is usually as a final rinse to remove residual chemicals before obturation. The use of these final rinses is debatable without any clear evidence of its benefits. These solutions are mentioned here for the sake of completeness.

Alcohol

Alcohol irrigation is mainly intended as a final rinse to remove irrigants and medicaments. As mentioned, the combination of CHX and NaOCl results in the formation of a brown precipitate¹¹²⁻¹¹⁴ and can cause tooth discolouration and prevent adequate sealing of the root canal.¹¹⁵⁻¹¹⁷ It is thought that rinsing intermittently with alcohol or as a final rinse will remove this precipitate.⁸³ However, results from studies have been contradictory. Krishnamurthy and Sudhakaran tested the ability of isopropyl alcohol in removing debris from the root canal and found that as an intermediate flush between NaOCl and CHX, it prevents precipitate formation and completely cleaned the canals.¹¹⁴ However, Magron *et al.* found no significant difference in debris removal and precipitate formation between isopropyl alcohol, distilled water and saline.⁸³

If intracanal dressings are not completely removed, it can compromise the final obturation seal. Over time, root canal medicaments can be dimensionally unstable and soluble, allowing bacteria to infiltrate from the coronal, lateral or apical environments.^{118, 119} Various irrigating solutions have been used to remove intracanal medicaments with limited success. These irrigants include NaOCl, EDTA, CA and phosphoric acid and their combinations but with limited success.^{119, 120} Studies have shown that when alcohol is used as a final rinse, the wettability of dentine improves,¹²² and sealer penetration increases.¹²¹ De Lima Dias-Junior *et al.*, compared the effectiveness of 2.5 % NaOCl, 17% EDTA + 1.25% sodium lauryl ether sulfate (EDTA-T), 37% phosphoric acid and 70 % ethanol in removing CaOH from the root canal. They found that 70% ethanol was more effective at cleaning and penetrating root canals than NaOCl and EDTA-T in the apical third of the canal with no difference in

both outcomes between 70% ethanol and 37% phosphoric acid.¹¹⁹ In addition, studies by Ramírez-Bommer showed that 70% ethanol does not affect the inorganic content in dentine after CaOH removal.¹²² Therefore, alcohol can be beneficial as a final irrigant rinse.

An added advantage of an alcohol irrigation protocol is its fast-acting antimicrobial action. In concentrations of between 60–90%, ethyl alcohol and isopropyl alcohol have broad-spectrum activity against bacteria, viruses and fungi.⁵¹ Krishnamurthy and Sudhakaran add that since alcohol is volatile, it can assist in drying the canals in preparation for obturation.¹¹⁴ Alcohol as a final root canal rinse may have benefits in removing debris, residual irrigants and medicaments, supplementary antimicrobial effects and aid in drying the canals.

Saline

Saline is another adjunct endodontic irrigant and helps with lubrication and flushing of the canals. It does not exert significant antimicrobial effects and is usually the final rinse after chemical disinfection to remove any residual chemicals to allow for optimal canal sealing. In experiments, saline is often used as the negative control in bacterial elimination and smear removal (8).

Natural irrigants

The modern world is moving towards natural organic solutions that not only mimic nature but also preserve that which is natural. From the destruction of our natural world to the increase in antibiotic-resistant infections, there is the constant search for natural alternatives. Modern medicine follows suit by looking at these natural alternatives for contemporary solutions. The field of endodontics is no different. Natural irrigants are being tested, and some of these solutions have the added potential for tissue regeneration.

Morinda Citrifolia

Morinda citrifolia juice (MCJ) is derived from the Morinda citrifolia or noni plant native to Southeast Asia. It has been used as an herbal remedy for centuries and all parts of the plant from the roots, bark, stems, leaves, fruit, juice and seeds are used. The Morinda citrifolia fruit has gained interest in recent years in the medical world for its diverse range of medicinal properties, some of which are immunostimulatory, antiviral, antifungal, antibacterial, anti-inflammatory, antiseptic, analgesic, antitumor and periodontal regeneration.^{8, 123-125} Morinda citrifolia has been effective against several microorganisms, some of which are Salmonella typhimurium, Escherichia coli, Staphylococcus aureus and Candida Albicans.¹²⁶⁻¹²⁹

With these promising properties, it is no surprise that MCJ has been suggested as a root canal irrigant. In 2008, Murray *et al.* designed the first study to compare the effectiveness of MCJ against NaOCl and CHX in smear layer removal. The authors determined that the minimum inhibitory concentration of MCJ was 6%. They tested combinations of MCJ with EDTA flushing, MCJ/CHX combination with EDTA flushing, and MCJ with saline flushing; the positive control groups were NaOCl with EDTA flushing and 2% CHX, and the negative control was plain saline rinsing. The most effective smear layer removal occurred with MCJ and NaOCl, both with EDTA flushing. The effectiveness of MCJ and NaOCl was comparable, with both irrigants removing up to 80% of the smear layer. MCJ was more effective

than CHX. The authors concluded that these results were revolutionary because they suggest that root canal irrigants and disinfectant solutions can be formulated from fruit juice. Using MCJ as a root canal irrigant might be advantageous because in vitro observations are promising and are not likely to cause adverse reactions unlike NaOCl.⁸

Morinda citrifolia leaf extract has been shown to have regenerative properties. Boonanantanasarn and co-workers evaluated the potential of Morinda citrifolia on cell proliferation, mineralisation and protein synthesis in the periodontal ligament of premolars and molars. Their results show that the Morinda citrifolia extract was effective at inducing cellular proliferation, protein synthesis, alkaline phosphatase activity and in vitro matrix mineralisation, thus having osteoinductive effects on periodontal regeneration.¹³⁰ Further, Al Moghazy and co-workers reported that MCJ (in combination with EDTA) can promote survival and attachment of DPSC to the root canal walls.¹³¹ These regenerative properties of the Morinda citrifolia have promising implications in the progress towards biocompatible root canal disinfection protocols. Peer-reviewed research on MCJ is lacking, but it has promising and exciting potential. Further studies are needed to determine the effectiveness, safety, and biocompatibility of MCJ conclusively before general use.

Chitosan

Chitosan is an abundant natural polysaccharide obtained by the deacetylation of chitin from crab and shrimp shells. It is an attractive biomaterial as it fulfils many of the pre-requisites of a biomaterial: biocompatibility, biodegradability, bioadhesiveness, non-toxic, relatively low cost, good adsorption, and demonstrates tissue regenerative properties.^{132, 133} Chitosan has also displayed high chelating and antibacterial properties and, therefore, has been proposed as a root canal irrigant.¹³⁴⁻¹³⁸

Chitosan is polycationic and can attach to the negatively charged components of bacterial cell walls, causing their disruption and loss of intracellular components and ultimately cell death. Chitosan is also capable of disrupting the extracellular matrix of biofilms, increasing the vulnerability of bacteria within the biofilm complex.¹³⁹ Chitosan nanoparticles have already been combined with endodontic sealers to provide antibacterial effects.¹⁴⁰⁻¹⁴² Chitosan can also enhance photodynamic therapy by attaching to photosensitisers.¹⁴³

Silva *et al.* compared the efficacy of 0.2% chitosan against more common root canal irrigants; 15% EDTA, 10% CA, and 1% acetic acid. They observed that smear layer removal of 0.2% chitosan was comparable to 15% EDTA and 10% CA. In addition, 0.2% chitosan and 15% EDTA demonstrated the greatest effect on root dentine demineralisation.¹³⁴ In a similarly designed study by Pimenta *et al.*, where 0.2% chitosan was compared to 15% EDTA and 10% CA in its effects on root dentine microhardness. All three solutions showed similar effects on dentine root microhardness, and SEM micrographs showed that all three solutions removed the smear layer from the middle third of the root canal.¹³⁶ Rath *et al.* undertook investigation of the effect of chitosan nanoparticles as a final irrigation solution on smear layer removal, micro-hardness, and surface roughness of root canal dentin. It was shown that 0.2% chitosan nanoparticles and 17% EDTA were similarly effective in smear layer removal and better than 2.5% NaOCl. Further, 0.2% chitosan produced higher micro-hardness and lower surface roughness of root

canal dentin than 17% EDTA but the same as 2.5% NaOCl.¹³⁵ Darrag *et al.* compared smear layer removal of 17% EDTA, 10% CA, MTAD and 0.2% chitosan as the final irrigant. All tested irrigants were effective at smear layer removal, but more so in the coronal two-thirds than the apical third of the root canal. However, 0.2% chitosan demonstrated increased efficiency at removing the smear layer than the other three test irrigants.¹³⁷

These studies demonstrate that chitosan is an effective chelating agent. This establishes chitosan as a potential root canal irrigant, but further studies are needed to investigate all its benefits, particularly in regenerative endodontics. With its biocompatible, biodegradable, antimicrobial, and tissue regeneration properties, chitosan may provide a biocompatible disinfection protocol.

Berberine

Berberine is an antimicrobial plant alkaloid, and in combination with other substances, it has shown antibacterial activity. In combination with 1% CHX, the antibacterial activity of berberine is comparable to 5.25% NaOCl and 2% CHX.¹⁴⁴ Berberine, in combination with miconazole, has demonstrated favourable biofilm activity in a non-endodontic model.¹⁴⁵ Further studies are needed to determine the potential clinical effectiveness of these irrigants.

Despite the variety of endodontic irrigant solutions, NaOCl combined with EDTA still remains the gold standard for chemical disinfection. This combination fulfils many requirements for an ideal solution: high efficacy against most endodontic pathogens, good penetrability and dissolvability against organic, inorganic and necrotic tissue, cost efficiency and long shelf life. Other solutions tend to be used as an adjunct to the gold standard. With medicine evolving into preservation and tissue regeneration, there has been a shift to discover more natural and biocompatible disinfection protocols. *Morinda Citrifolia*, chitosan and berberine are just some chemicals that have been suggested, but further research is still required in this field.

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Data availability statement

No datasets were generated or analysed during the current study.

Competing Interests

The Authors declare no Competing Financial or Non-Financial Interests.

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CPD questionnaire on page 392

The Continuing Professional Development (CPD) section provides for twenty general questions and five ethics questions. The section provides members with a valuable source of CPD points whilst also achieving the objective of CPD, to assure continuing education. The importance of continuing professional development should not be underestimated, it is a career-long obligation for practicing professionals.

