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Review Article

Optimization of the Irrigation Protocol in Endodontics. A Review on Continuous Chelation

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Abstract

The gold standard in endodontic irrigation is sodium hypochlorite (NaOCl), followed by a strong chelating agent, ethylenediaminetetraacetic acid (EDTA), which allows antimicrobial and proteolytic action, in addition to eliminating smear layer and dentin debris. However, EDTA causes significant demineralisation, which increases the risk of fractures and affects the biomechanics of the tooth. In addition, when used with NaOCl, EDTA inhibits the antimicrobial action of NaOCl, requiring an additional step in the clinical process. To overcome these limitations, soft chelators have been investigated, with etidronate (also named 1-Hydroxyethylidene-1,1-diphosphonic acid or HEDP) being the most studied. This bisphosphonate is compatible with NaOCl, allowing its use in an all-in-one mixture, a procedure known as continuous chelation. NaOCl and HEDP solutions have been shown to maintain the antimicrobial properties of hypochlorite, as well as being effective in removing smear layers and hard debris, simplifying the clinical protocol, and a significant number of studies suggest that this technique outperforms the standard NaOCl followed by EDTA protocol in terms of biological and mechanical properties. Other chelators, such as clodronate, another bisphosphonate, and Triton®, a commercial product that combines NaOCl at low concentration with mild chelators and surfactants, have also shown promising results. However, although the preliminary findings are encouraging, there is a need to standardise research methods in laboratory studies and further *in vivo* research to validate the results obtained *in vitro*. This review aims to provide a comprehensive overview of continuous chelation in endodontics, highlighting the efficacy and compatibility of these agents with NaOCl and helping to clarify best practices for irrigation and disinfection of the root canal system.

Keywords: Continuous Chelation; Etidronate; Clodronate; Triton; HEDP; HEBP; Endodontic Irrigants

Abbreviations

NaOCl: Sodium Hypochlorite (NaOCl); EDTA: Ethylenediaminetetraacetic Acid; HEDP: 1-Hydroxyethylidene-1,1-diphosphonic acid; RCS: Root Canal System; SEM: Scanning Electron Microscopy

Introduction

Treatment of the root canal system (RCS) primarily aims to eliminate the bacteria present and prevent reinfection by adequate obturation of the RCS space [1]. Although mechanical instrumentation removes a significant portion of the microorganisms adhering to the dentin walls, up to 35% of the RCS surface may remain untreated [1]. To achieve complete disinfection, an active irrigation sequence facilitates the dissolution of organic and inorganic matter produced during instrumentation [2].

Sodium hypochlorite (NaOCl) is the irrigant of choice because of its proteolytic properties that allow the dissolution of organic matter [3]. However, NaOCl does not remove the smear layer, which can result in the accumulation of debris within the RCS, preventing the irrigant from reaching all areas of the system [4-6]. In addition, the presence of a smear layer reduces the antimicrobial efficacy of NaOCl in dentin [6].

To address this limitation, chelating agents are employed that remove calcium ions from dentin hydroxyapatite due to their acidic nature. Since 1957, EDTA has been used as the chelator of choice, usually at 15-17% concentrations for 1-2 minutes [7]. However, EDTA can reduce the tissue-dissolving capacity and antimicrobial efficacy of NaOCl, which has led to the development of the "sequential protocol" for irrigation. While effective in removing the smear layer, this protocol may weaken dentin and increase the risk of fractures due to deep demineralization [8,9].

In response to these limitations, "continuous chelation" was introduced in 2005, allowing the combination of mild chelating agents with NaOCl. Etidronic acid (HEDP) has emerged as a viable alternative due to its high compatibility with NaOCl, maintaining its antimicrobial and proteolytic properties without significantly altering dentin structure, in addition to simplification of the clinical procedure and improved compatibility with dental materials [10-13]. HEDP, a first-generation non-nitrogenous bisphosphonate used in the treatment of postmenopausal osteoporosis, offers several benefits, such as simplification of the clinical procedure, improved residue removal [7], good compatibility with some dental materials [13], and maintenance of NaOCl properties [10,12].

Several alternatives to etidronate have been tested in the laboratory. Among these, one chelator of interest is clodronate. This bisphosphonate is more stable than etidronate in combination with NaOCl, maintaining greater free chloride availability and similar antimicrobial capacity against *E. faecalis* [14,16] although with less dentinal tubule opening [15]. Similarly, Triton, another recently developed endodontic irrigant, combines chelators, surfactants and NaOCl in an all-in-one solution that promises to simplify the clinical procedure and improve the efficiency of root canal cleaning, although its efficacy in removing biofilms and smear layers remains to be confirmed [18,38].

This review aims to provide an overview of continuous chelation in endodontic treatment, highlight the efficacy and compatibility of these agents with NaOCl, and help clarify best practices in RCS irrigation and disinfection.

Materials and Methods

A comprehensive online search was conducted using the electronic search engines Pubmed, Scielo and Google Scholar. The search was restricted to studies published in English between 2005 and August 2024, using the keywords Continuous chelation, etidronate, clodronate, triton, HEDP, HEBP, and endodontic irrigants. In addition, a manual search was performed in the references of relevant articles, which led to the inclusion of articles published before the search date. Studies that were not published in high-impact journals focused on related topics, as well as opinions, editorials, and social media sources, were excluded. After the selection process, a total of 48 articles were included.

Results and Discussion

Etidronate

Smear layer removal

Etidronate and sodium hypochlorite (NaOCl) have been found to be highly effective in removing the smear layer, particularly in the apical third of root canals. Aoun., *et al*. [19] confirmed that the combination of etidronate and 3% NaOCl (Dual Rinse) led to a reduction in detritus and smear layer in the apical third, surpassing the traditional sequential approach.

The pH influences calcium chelation and, thus, the ability of HEDP and EDTA to remove the smear layer. Deari., *et al*. [20] noted that although EDTA has a higher chelating capacity than HEDP and removes more calcium, HEDP still provides effective smear layer removal. In a study comparing different concentrations of HEDP, the recommended optimal concentration of HEDP for optimal smear layer removal in the clinical setting is 18% in conjunction with an oxidizing agent [21]. Ulusoy., *et al*. [22] demonstrated that the use of 9% and 18% HEDP resulted in more efficient smear layer removal in the apical third compared to other chelators, including 17% ethylenediaminetetraacetic acid (EDTA) and 0.5% and 1.2% peracetic acid. Despite this, some studies, such as that of Patil., *et al*. [48], have suggested that the traditional sequential protocol with 5.25% NaOCl and 17% EDTA, both combined with surfactants, may be more effective in removing the smear layer in the apical third than the combination of NaOCl with HEDP. However, none of the chelating solutions could remove the smear layer and debris from the root canal walls [1]. On the other hand, heating NaOCl, either alone or combined with HEDP, improves its ability to dissolve organic matter and remove the smear layer. However, mixing with HEDP requires frequent renewals to maintain these effects [23].

While etidronate-based solutions and continuous chelation have shown to be equally or more effective in cleaning root canals than traditional chelating agents, it is crucial to note that no irrigant alone can ensure complete root canal cleanliness. Therefore, the use of additional activation methods is imperative to enhance debris removal. Existing studies present significant methodological limitations, especially in the use of scanning electron microscopy (SEM). These limitations, such as operator bias, evaluation of non-representative areas and the introduction of artefacts during sample processing, affect the reliability and reproducibility of the results. Alternative methods, such as 3D laser microscopy and micro-CT, offer promising avenues for future research [1].

Antimicrobial activity of etidronate

HEDP, in combination with NaOCl, is highly effective in antimicrobial activity during endodontic irrigation. The continuous chelation protocol, such as NaOCl/HEDP, has been equally or more effective than the traditional sequential protocol in terms of antimicrobial activity [12,24-29]. Although the addition of HEBP may delay the antibiofilm action of NaOCl, it does not compromise its overall antimicrobial efficacy. However, none of the solutions eliminated bacteria from root canals [1].

Clinical studies have supported the efficacy of NaOCl combined with HEDP, showing that this combination does not alter the clinical efficacy of NaOCl. A non-inferiority clinical trial found that HEDP had no adverse impact on the clinical efficacy of NaOCl, and no significant differences were observed in the microbiota recovered after irrigation with NaOCl alone or NaOCl/HEDP [30].

Compared to the decrease observed with EDTA, the pH stability in NaOCl solutions with HEDP suggests that higher pH preserves the antimicrobial activity of sodium hypochlorite during irrigation [31]. In *in vitro* studies, the combination of NaOCl with 9% HEDP stood out as one of the most effective irrigants against *Candida albicans* and *Enterococcus faecalis*. However, these studies were performed in a planktonic environment and not in biofilms, which is more representative of endodontic infections [31].

In addition, the NaOCl/HEDP combination has been reported to be effective in dissolving and removing bacteria in the root canal, outperforming the NaOCl/EDTA combination in antibacterial efficacy [24,25,32], although no significant differences in smear layer removal were found between the two strategies [32-34]. Morago., *et al*. [32] confirmed that both NaOCl/HEDP and NaOCl/EDTA showed significant antimicrobial activity within dentinal tubules, reducing bacterial biovolume and removing the smear layer, with higher efficacy observed in the NaOCl/HEDP group.

However, Giardino., *et al*. [25] highlighted that the addition of HEDP to NaOCl could potentially increase the surface tension of the solution, which might hinder its penetration into the root canal for thorough cleaning. Despite this, the NaOCl/Dual Rinse HEDP mixture demonstrated superior antibacterial action to NaOCl/EDTA, possibly because HEDP does not significantly decrease the available chlorine in the solution within the first 60 min. Furthermore, the combination of etidronate powder with NaOCl creates a hypertonic solution, which could enhance the antimicrobial efficacy through an osmotic effect, promoting bacterial cell death and decreasing biofilm cohesion [1].

It's important to note that while studies on endodontic irrigants typically focus on Enterococcus faecalis and employ culture methods, which are considered the gold standard, these methods have limitations when applied to the complex anatomy of the root canal. The use of confocal laser microscopy has significantly improved the assessment of antimicrobial activity, providing more accurate results. Despite these limitations, the continuous chelation protocol has been shown to be equally or more effective than the traditional sequential protocol in terms of antimicrobial activity [1].

Effects on dentin

The use of HEDP is a milder method compared to the use of EDTA, resulting in reduced or even no dentin erosion, as well as minimal modification of dentin surface roughness [20,33-37]. However, Ulusoy., *et al*. [22] reported that final irrigation with etidronic acid, either alone or combined with NaOCl, structurally altered root canal dentin. On the other hand, Girard., *et al*. [38] found that HEDP gel showed a higher calcium chelating capacity compared to commercial paste-type products containing EDTA and hydrogen peroxide. In addition, Tartari., *et al*. [37] demonstrated that NaOCl alone did not affect dentin surface roughness, whereas only irrigation protocols that included chelating agents modified dentin surface roughness.

HEDP appears to be a milder chelating agent than EDTA, with less impact on erosion and dentin roughness when used in continuous chelation. However, methodological differences between studies and the use of two-dimensional techniques limit the reliability of the results, especially in smear layer removal and erosion assessment. Future studies with three-dimensional techniques and high-quality clinical trials are needed to obtain more consistent and clinically relevant results [1].

Biocompatibility

The use of etidronate as a chelator in endodontic irrigation, especially in combination with sodium hypochlorite (NaOCl), has shown promising biocompatibility. According to Ballal., *et al*., combining HEDP with NaOCl does not increase the toxicity of sodium hypochlorite, maintaining the same cytotoxicity as NaOCl alone [39]. However, these findings are from *in vitro* studies and, therefore, cannot be directly extrapolated to clinical practice [39]. In a clinical study, it was observed that the use of HEDP together with 2.5% NaOCl did not cause significant inflammatory effects in periapical tissues, such as postoperative pain or increased MMP-9, an enzyme related to neutrophil activity [30]. However, these results should be interpreted cautiously, as the long-term clinical effects still need further investigation [30].

Furthermore, future studies should compare different concentrations of sodium hypochlorite, especially those with higher antimicrobial efficacy, to assess whether the addition of HEDP maintains its effectiveness without increasing toxicity [30]. Also, the importance of including EDTA in these studies as a control group is highlighted, given that the NaOCl-EDTA sequence is the gold standard in root canal irrigation.

Effects on adhesion of endodontic sealers

The combined use of etidronate (HEDP) and sodium hypochlorite (NaOCl) has shown beneficial effects on the adhesion of endodontic sealants to dentin. This combination improves the bond strength of epoxy resin sealants compared to the traditional NaOCl and EDTA irrigation method due to its chelating and antimicrobial properties, simplifying the irrigation process [40,41]. Ulusoy., *et al*. [22] observed that using 18% HEDP in ultrapure water improved marginal adaptation, reducing gaps detected with scanning electron microscopy and suggesting better integration of the sealer to the treated dentin.

Similarly, Ballal [42] reported that the highest bond strength was obtained in canals irrigated with 2.5% NaOCl combined with Dual Rinse HEDP, attributing this improvement to the soft chelating action of HEDP, which exposes the amino groups of collagen, facilitating adhesion of the AH Plus sealer. In the case of the BioRoot RCS, the combination of 5.25% NaOCl and HEDP also increased the micromechanical retention of the sealer, thanks to the continuous dissolution of the organic and inorganic components of the dentin.

On the other hand, the group treated with saline and HEDP showed the lowest bond strength, possibly due to poor dissolution of dentin components, which limited the interaction between BioRoot RCS and dentin. Furthermore, Ballal [42] highlighted that treatment with NaOCl and HEDP generates an optimal surface for the adhesion of calcium silicate-based hydraulic sealants, as HEDP causes collagen fibres to partially degrade and mineralize, creating a homogeneous dentin surface of organic and inorganic components, thus optimizing the adhesion of both epoxy resin- and calcium silicate-based sealants, when applied at concentrations of 2.5% and 5.25% NaOCl, respectively [42].

This suggests that both the deproteinizing agent and the chelating agent play fundamental roles in endodontic sealant adhesion, being key factors to consider in irrigation and obturation strategies to optimize marginal adaptation [42]. Fernandez Zancan [43] and De-Deus [44] have emphasized the importance of dentin conditioning on sealant adhesion and performance.

While some studies found that chelating solutions increased sealant bond strength, the differences were not always statistically significant [41]. However, chelating solutions generally improved sealer penetration into dentinal tubules [45]. The research also found that bioceramic sealers, such as iRoot SP and EndoSequence BC Sealer, demonstrated higher bond strength and penetration compared to resin-based sealers, regardless of the irrigation protocol used [41,45].

Clodronate

Clodronate, a non-nitrogenous phosphorus-containing chelator, has begun to be studied for use in continuous chelation. At alkaline pH, clodronate is compatible with sodium hypochlorite (NaOCl) due to its chemical structure, making it less reactive with chlorine than nitrogenous chelators. Furthermore, since clodronate already contains two chlorine atoms in its chemical structure bonded to the central carbon, it is unlikely to react with NaOCl. Clodronate is synthesized using NaOCl, which enhances its stability in this combination [15,17]. This stability, together with its anti-inflammatory and anti-osteoclastic ability, makes it a promising irrigant for endodontics, in addition to possessing antibacterial activity against Pseudomonas aeruginosa and not being associated with osteonecrosis of the jaw [17].

Soft chelators, such as etidronic acid (HEBP) and clodronate, have minimal impact on dentin walls, although they are still effective in reducing the smear layer [33]. However, due to their limited ability to dissolve large amounts of detritus, they are not recommended as final irrigants. However, they are suggested for combined use with NaOCl in the continuous chelation protocol. This combination is advantageous as it allows NaOCl to maintain a higher tissue-dissolving capacity and, at the same time, facilitates smear removal, which optimizes canal cleaning [14,17].

Clodronate offers additional benefits in continuous chelation due to its analgesic and anti-inflammatory properties, which are used in the treatment of osteoarthritis. These characteristics could help reduce post-endodontic pain, a complication that affects up to 58% of patients after root canal treatment [14,46]. Furthermore, its inclusion in the continuous chelation protocol maintains the antimicrobial efficacy of NaOCl, avoiding the need to reduce the concentration of this irrigant, which preserves its antibacterial effects [14]. Another important aspect is its potential to offer greater safety in cases of accidental extrusion of NaOCl beyond the root canal since clodronate could minimize the pain, swelling and inflammation that usually occur in these cases [14], thus reducing the need for post-treatment analgesic medication.

Triton

Triton is a newly developed all-in-one endodontic irrigant that combines NaOCl, surfactants, and soft chelators, allowing simultaneous dissolution of organic and inorganic tissues with a lower concentration of sodium hypochlorite. Its design optimizes the irrigation process by eliminating the need for multiple solutions, reducing clinical times. The formula consists of two parts: Part A includes chelators, surfactants, pH modifiers, and stabilizers, while Part B contains 8% NaOCl and pH modifiers, resulting in a final solution of 4% NaOCl [18,38].

Studies have shown Triton to effectively remove *E. faecalis* and multispecies biofilms in dentinal canals, outperforming NaOCl or NaOCl+EDTA in antibiofilm activity and smear layer removal [38]. However, some studies report that Triton fails to remove the smear layer in all root regions [18]. A recent study indicated that Triton was more effective than Dual Rinse HEDP and NaOCl/EDTA in removing debris in the thirds of the root canal, except in the coronal third with NaOCl/EDTA [47]. On the other hand, no significant differences were found in antimicrobial efficacy between Triton and sodium hypochlorite when used with mechanical instrumentation. In contrast, a lower efficacy of Triton in removing the smear layer was reported, which could be due to the exposure time used in the studies [18].

Furthermore, the differences observed in the present study could also be due to a difference in the solutions' surface tension and penetration capacity. However, further studies are needed to investigate Triton's surface tension. Long-term treatment with Triton showed superior antimicrobial effects, especially when combined with 4% NaOCl, outperforming even 6% NaOCl with 17% EDTA. This could be due to the chelating agent (CA) promoting hypochlorite's antibiofilm efficacy in deep dentin layers. Another study showed that compared to Dual Rinse HEDP and NaOCl/EDTA, Triton had similar efficacy in reducing *E. faecalis* and was more effective against *C. Albicans* [47].

Furthermore, Triton's surfactants enhance hypochlorite's antimicrobial and cartridge activity [38]. Despite possible variability in results depending on tissue concentrations and types, using pH modifiers in Triton maintains a beneficial alkaline environment to counteract microbial acidic byproducts. Incorporating chelating agents also facilitates the dissolution of inorganic materials such as dentin debris and apatite crystals [38].

Although Triton's all-in-one design effectively combines NaOCl, chelating agents, surfactants, and pH modifiers, simultaneously achieving antimicrobial and tissue dissolution activities without compromising the efficacy of its components. Studies to date have been conducted primarily under *in vitro* conditions. Therefore, Triton's performance in clinical settings could vary. Further research and standardization of studies are essential to obtain comparable and practical results that reflect its efficacy under real-world clinical conditions.

Conclusion

The continuous chelation procedure, where a soft chelator is combined with NaOCl to achieve antibacterial and proteolytic activities simultaneously with removing the smear layer and debris, has shown promising results since its introduction in 2005. Many studies, mainly focused on the etidronate agent mixed with NaOCl, suggest that this technique outperforms the standard protocol of NaOCl followed by EDTA regarding biological and mechanical properties. Continuous chelation aims to overcome sequential chelation's limitations and optimize clinical treatment times. However, most current studies are laboratory-based and further clinical research is needed to validate these results. Furthermore, methodological differences between studies make it difficult to compare and interpret the effectiveness of the continuous protocol. Further standardization in research is required to obtain more meaningful conclusions.

Conflict of Interest

The author declares that he has no financial interests or conflicts of interest.

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