

Ethylenediaminetetraacetic acid in endodontics

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ABSTRACT

Ethylenediaminetetraacetic acid (EDTA) is a chelating agent can bind to metals via four carboxylate and two amine groups. It is a polyamino carboxylic acid and a colorless, water-soluble solid, which is widely used to dissolve lime scale. It is produced as several salts, notably disodium EDTA and calcium disodium EDTA. EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. A review of the literature and a discussion of the different indications and considerations for its usage are presented.

Key words: Chelator, ethylenediaminetetraacetic acid, endodontics

INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) refers to the chelating agent with the formula $(\text{HO}_2\text{CCH}_2)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$. This aminoacid is widely used to sequester di- and trivalent metal ions. EDTA binds to metals through four carboxylate and two amine groups. EDTA forms especially strong complexes with Mn (II), Cu (II), Fe (III) and Co (III). It is mostly synthesized from 1, 2-diaminoethane (ethylene diamine), formaldehyde, water and sodium cyanide.^[1] This yields the tetrasodium salt, which can be converted into the acidic forms by acidification.

EDTA is a polyaminocarboxylic acid and a colorless, water-soluble solid. It is widely used to dissolve lime scale. Its usefulness arises because of its role as a hexadentate ligand and chelating agent, i.e. its ability to sequester metal ions such as Ca^{2+} and Fe^{3+} .^[2] After being bound by EDTA, metal ions remain in solution, but exhibit diminished reactivity. EDTA is produced as several salts, notably disodium EDTA and calcium disodium EDTA. The compound was first described in 1935 by Ferdinand Munz, who prepared the compound from ethylene diamine and chloroacetic

acid.^[3] Nowadays, EDTA is mainly synthesized from ethylene diamine, formaldehyde and sodium cyanide.^[4]

EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. It has been reported that EDTA decalcified dentin to a depth of 20-30 μm in 5 min.^[5]

This review will address the different indications and considerations for EDTA.

SMEAR LAYER REMOVAL

Wu *et al.*^[6] showed that the smear layer removal ability of 17% EDTA was significantly better than 20% of citric acid and MTAD (Biopure™ mixture of tetracycline isomer, acid and detergent). According to Fabiani *et al.*^[7] orthophosphoric acid was more effective than EDTA in removing surgical smear layer even with less time of action. Prado *et al.*^[8] compared the effectiveness of 37% phosphoric acid with that of 17% EDTA and 10% citric acid in the removal of smear layer. Findings revealed that phosphoric acid was comparable with EDTA in removing the smear layer.

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Dai *et al.*^[9] revealed that Q-Mix was as effective as 17% EDTA in removing canal wall smear layers after the use of 5.25% NaOCl as the initial rinse. Rödiger *et al.*^[10] confirmed the efficacy of EDTA in removing the smear layer. Caron *et al.*^[11] revealed that although 17% EDTA 3% NaOCl in removing the smear layer, sonic and ultrasonic activation improved the efficacy of the mentioned combination in removing the smear layer. Using scanning electron microscope (SEM), Zand *et al.*^[12] indicated that the use of NaOCl gel could be as effective as NaOCl solution along with EDTA in smear layer removal in the three parts of root canal walls. In another SEM study, Mello *et al.*^[13] demonstrated that a continuous rinse with 5 ml of EDTA for 3 min could remove the smear layer from root canal walls efficiently. Uroz-Torres *et al.*^[14] showed that EndoActivator did not enhance the efficacy of NaOCl/EDTA in removing the smear layer. The efficacy of EDTA in removing the smear layer was revealed by Mancini *et al.*^[15] as well as da Silva *et al.*^[16] Using atomic absorption spectroscopy and SEM, Spanó *et al.*^[17] revealed that the use of 15% EDTA resulted in the greatest concentration of calcium ions compared with other chelating agents. In addition, 15% EDTA was the most efficient solution for removal of smear layer. Gu *et al.*^[18] showed that EDTA performed significantly better than NaCl and NaOCl in smear layer removal and dentinal tubule opening. Additional ultrasonic irrigation did not improve smear layer removal significantly. Kuah *et al.*^[19] demonstrated that 1-min application of combined use of EDTA and ultrasonics was efficient for smear layer and debris removal in the apical region of the root canal. Saito *et al.*^[20] revealed that root canal irrigation with 17% EDTA for 1 min was more effective than 30 s in removing the smear layer after root canal instrumentation. According to Teixeira *et al.*^[21] canal irrigation with EDTA and NaOCl for 1, 3 and 5 min were equally effective in removing the smear layer from the canal walls of straight roots. Guerisoli *et al.*^[22] revealed that under ultrasonic agitation, sodium hypochlorite (NaOCl) associated with ethylenediaminetetraacetic acid plus Cetavlon (EDTAC) removed the smear layer. Di Lenarda *et al.*^[23] confirmed the effectiveness of EDTA in removing the smear layer. Adiguzel *et al.*^[24] indicated that the self-adjusting file operation with continuous irrigation using EDTA resulted in canal walls that were free of smear layer in 85%, 60% and 50% and of debris in 95%, 90% and 85% of the cervical, middle and apical thirds of the root canals, respectively. Sen *et al.*^[25] demonstrated that there was no significant difference between the smear layer removing the ability of different concentrations of EDTA (15%, 10%, 5% and 1%). Perez and Rouqueyrol-Pourcel^[26] evaluated, *in vitro*, the ability

of an 8% EDTA solution to remove smear produced during the canal preparation and found that 3 min 8% EDTA irrigation was as effective as 1 min 15% EDTA. Scelza *et al.*^[27] evaluated the effect of EDTA-T, 17% EDTA and 10% citric acid on the smear layer removal after final irrigation for 3, 10 and 15 min. Results revealed that there were significantly better results when irrigation with EDTA for 3 min was compared with 15 min.

ANTIMICROBIAL ACTIVITY

According to Patterson,^[28] EDTA had limited antibacterial activity. It seems that the antibacterial activity of EDTA is due to the chelation of cations from the outer membrane of bacteria. Russell^[29] revealed that 10% EDTA produced a zone of bacterial growth inhibition similar to Creosote. However, lower concentrations of EDTA produced little to non-inhibition zone. Kotula and Bordácová^[30] indicated that the antimicrobial effect of Na-EDTA was maintained as long as the chelators have not formed bonds with metal ions. Yoshida *et al.*^[31] assessed the antibacterial activity of EDTA combined with ultrasonic activation clinically. After 7 days, without placing any intracanal medicament, most cases were bacteria-free. According to Heling and Chandler,^[32] RC-Prep was more effective against gram-negative bacteria than Gram-positive ones. According to Heling *et al.*^[33] increasing the temperature of RC-Prep from 10°C to 45°C increased its efficacy against *Staphylococcus aureus*. A study investigated the effect of components of RC-Prep on *Streptococcus sobrinus*. Findings revealed that minimum concentration for a bactericidal effect was 0.25% for EDTA and 50% for glycol.^[34] On the other hand, Orstavik and Haapasalo^[35] putted the antibacterial activity of 17% EDTA under question. Ordinola-Zapata *et al.*^[36] revealed that EDTA had no significant effect on the biofilm viability and architecture. Ballal *et al.*^[37] indicated that efficacy of EDTA against *Enterococcus faecalis* was equivalent to maleic acid. Arias-Moliz *et al.*^[38] showed that EDTA had no efficacy against *E. faecalis* even after 60 min contact. Bystrom and Sundqvist^[39] demonstrated that combination of EDTA and 5% NaOCl had better antibacterial activity of NaOCl alone. Using the agar diffusion technique, Sen *et al.*^[40] revealed that EDTA was effective against *Candida albicans*.

EFFECTS ON DENTINE MICROHARDNESS

Pawlicka^[41] reported that chelators can reduce the root dentine microhardness, whereby the greatest differences are to be found in dentine immediately

adjacent to the root canal lumen. The effect of the chelator is already apparent after 5 min and cannot be significantly increased by extending the working time to 24 h.

Cruz-Filho *et al.*^[42] evaluated the effect of different chelating solutions on the microhardness of the most superficial dentin layer from the root canal lumen. Findings revealed that EDTA and citric acid had the greatest overall effect, causing a sharp decrease in dentin microhardness without a significant difference from each other. In another study, Ballal *et al.*^[43] found that there was no significant difference between EDTA and maleic acid in the reduction of microhardness of dentine.

Eldeniz *et al.*^[44] assessed the effect of citric acid and EDTA solutions on the microhardness and the roughness of dentine. Findings revealed that there was a significant difference in microhardness among the test groups, citric acid group being the least hard. In another study, Ari *et al.*^[45] as well as Cruz-Filho *et al.*^[46] confirmed decreasing dentine microhardness after using EDTA. De-Deus *et al.*^[47] assessed the effect of EDTA, EDTAC and citric acid on dentine microhardness and found that microhardness decreased with increasing time of application of chelating solutions. There were no significant differences between initial microhardness and after 1 min. After 3 min, EDTA produced a greater reduction in microhardness. However, there was no difference between EDTA and EDTAC after 5 min.

INTERACTION BETWEEN EDTA AND NaOCl

The addition of chelators to NaOCl reduces its pH in a ratio and time-dependent manner. This affects the forms of free chlorine in the solution and causes an increase in hypochlorous acid and chlorine gas, which subsequently reduces the amount of the hypochlorite ion.^[48] According to Zehnder *et al.*^[49] when 1% NaOCl was mixed with 17% EDTA (pH = 8) in ratios of 1:1, 1:5 and 5:1, the pH of the solutions ranged between 8.0 and 8. Furthermore, they showed that the addition of 10% citric acid to 1% NaOCl in the same ratios resulted in pH values between 1.8 and 4.3.

Irala *et al.*^[50] mixed 1-2% NaOCl with 17% EDTA in equal proportions, resulting in a final pH value of 8 from an initial value of 10 after an elapse of 48 h. However, when mixed in 1:3 ratio and although with a larger volume of EDTA, the pH value was stable during the 48 h experimental time, probably because

of an immediate interaction between the solutions. According to Baumgartner and Ibay^[51] the reduction of pH values in the NaOCl solution caused the release of chlorine gas, which has potentially hazardous effects on humans. When EDTA is added to NaOCl, chlorine gas can be detected at relatively low levels. When citric acid is used, significantly more chlorine is detectable and present at a further distance. This is according to a laboratory-based investigation that studied the reactions between NaOCl (5.25%, pH = 12.12) and citric acid (50%, pH = 1.28) or EDTA (15%, PH = 7.51). Portions of the chelator were added to the NaOCl at regular time intervals for a total time period of 2 h; the release of chlorine gas was measured at 6 inches and 6 feet from the container.^[51]

The consequences of chemical interactions between chelating agents and NaOCl result in a loss in the free available chlorine of the mixtures. Zehnder *et al.*^[49] indicated that when NaOCl was mixed with citric acid, free available chlorine decreased to 0 in less than a minute, whereas EDTA required between 1 and 60 min decreasing the free available chlorine to the same level. Clarkson *et al.*^[52] confirmed the findings of Zehnder *et al.*^[49] and found that the available chlorine loss was up to 80%.

Using the spectroscopy, Girard *et al.*^[53] assessed the interactions of gel-type preparations of chelators containing 15% EDTA and 10% urea peroxide with 1% NaOCl. Findings revealed that both compounds depleted the solution from its chlorine content after 5 min.

The dramatic reduction of free available chlorine in NaOCl mixtures caused by chemical interactions appears to explain the inability of NaOCl and EDTA mixtures to dissolve soft-tissues.^[48] Irala *et al.*^[50] evaluated tissue dissolving ability of NaOCl (1-2.5%) alone and combined with 17% EDTA in different ratios (2:2 and 1:3). Findings indicated that after 48 h only unmixed NaOCl was able to completely dissolve the tissue. Grawehr *et al.*^[54] confirmed the findings of Irala *et al.*^[50] NaOCl does not reduce the calcium chelating or smear layer ability of EDTA and citric acid.^[48] Using standardized dentin disks, Saquy *et al.*^[55] assessed the calcium chelation ability of a combination of 17% EDTA and distilled water and a combination of 17% EDTA and 0.5% NaOCl and found that greater calcium chelation occurred in the solution containing NaOCl. Another study indicated that NaOCl had little effect on EDTA's calcium chelating ability.

Saquy *et al.*^[55] revealed that the addition of NaOCl to EDTA did not alter EDTA's ability to decalcify human dentin. According to Grawehr *et al.*^[54] as well as Zehnder *et al.*^[49] if the original free available chlorine values were modest, chelators could eliminate the antimicrobial efficacy of NaOCl, whereas EDTA and CA performance did not seem to be affected because of interactions with NaOCl. Using agar diffusion test, Grawehr *et al.*^[54] assessed the antimicrobial activity of EDTA, NaOCl and their combination against *E. faecalis* and *C. albicans*. According to their findings, NaOCl produced smaller zones of inhibition compared to EDTA or mixture of EDTA/NaOCl.

INTERACTION BETWEEN EDTA AND CHLORHEXIDINE

There are only two studies on the interaction between CHX and chelating agents.^[56,57] Akisue *et al.*^[56] as well as González-López *et al.*^[57] indicated that CHX is easily mixed with citric acid and no modification of its demineralizing ability or precipitation occurs. Using atomic absorption spectroscopy, González-López *et al.*^[57] evaluated the effect of adding 1% CHX and 10% to 20% citric acid on the demineralizing capacity of the citric acid. Results indicated no alteration of the decalcifying effect of citric acid. They further revealed that due to the formation of a highly insoluble pink powdery precipitate, obtaining a homogenous solution impossible by mixing % CHX and 17% EDTA 1.^[57] Akisue *et al.*^[56] showed that 15% citric acid followed by 2% CHX caused the formation of a milky solution, which could be easily removed by using further CHX.

Using reverse-phase high-performance chromatography, Rasimick *et al.*^[58] analyzed the precipitate that formed after the combination of 17% EDTA with 2% or 20% CHX in equal volumes and three different mixing conditions. Findings indicated that over 90% of the precipitate mass was either EDTA or CHX.

González-López *et al.*^[57] suggested that the precipitate was most likely a salt formed by neutralization of the cationic CHX by anionic EDTA

EFFECT ON THE ADHESION OF ROOT CANAL SEALERS

Adhesion is defined as a process in which two surfaces of different molecular compositions are bonded by chemical, physical or mechanical attraction forces.^[59] Mechanical adhesion occurs by entrapment

of a material into another body, within the natural or artificial cavities. Chemical adhesion may result from primary valence forces, such as covalent and metallic bonds. Physical adhesion, in turn, relies on secondary valence forces, such as Van der Waals forces, London dispersion forces and hydrogen bonds.^[60] For adhesion to occur, it is necessary that the materials to be adhered are sufficiently close to each other. Therefore, a primary condition is the wet ability of the liquid in a solid material,^[61] which will provide the required proximity between the materials, facilitating molecular attraction and promoting adhesion.^[59]

Adhesion of an endodontic sealer is defined as its capacity to adhere to the root canal walls and promote the union of Gutta-percha cones to each other and to the dentin.^[62,63] Some variables may interfere with the outcome and understanding of sealer adhesion to root canal walls, namely the employed methodology, treatment of dentin surface and type of material.

Several resin-based sealer materials have been developed in an attempt to minimize leakage by improving the effectiveness of the seal between the filling material and the root canal walls.^[64,65] Different monomers were used in the development of resin-based sealers. AH Plus (De Trey, Konstanz, Germany) is a two-component sealer based on an epoxy resin; it is used in combination with gutta-percha points. Epiphany SE self-etch (Pentron Clinical Technologies, Wallingford, USA) is a dual-curable self-etching methacrylate resin sealer that is used in association with Resilon points (Resilon Research LLC, Madison, USA), a thermoplastic synthetic polyester polymer-based material that replaces Gutta-percha. A related advantage of the Epiphany system could be its ability to seal the canal, creating a monoblock between the sealer and point materials.^[66-68] Nevertheless, chemical irrigants used during the root canal preparation may alter the chemical composition of the dentin surface as well as the interaction between the dentin and resin-based sealer. In another study, Nunes *et al.*^[69] showed that treating dentine with a combination of 1% NaOCl and 17% EDTA produced stronger adhesion of AH-Plus sealer compared to 1% NaOCl alone.

BIOCOMPATIBILITY

Nygaard-Ostby^[70] assessed the effect of 15% EDTA on the human pulpal and periapical tissues in teeth with vital and necrotic pulps. Findings revealed that even though EDTA was forced through the apical foramen into the periapical tissues, no periapical

tissue damage could be detected after 14 months. Furthermore, he showed that placement of EDTA for 28 days after pulpotomy produced no pulpal tissue necrosis. Patterson^[28] assessed the effect of intramuscular injection of EDTA and EDTAC and found that EDTAC caused much greater tissue irritation than EDTA. Lindemann *et al.*^[71] showed that EDTA was not capable of destroying collagen.

In an investigation of the tissue reaction in rats after intramuscular implantation and injection of EDTA and EDTAC, Patterson^[28] showed that 15% EDTAC caused much greater tissue irritation after implantation and after injection than 10% EDTA. No periapical tissue irritation or damage of any kind occurred in 200 clinical cases where EDTA was used as an irrigant.

Segura *et al.*^[72] showed that extrusion of even a low concentration of EDTA solution through the apical constriction resulted not only in an irreversible decalcification of periapical bone but can also have consequences for neuroimmunological regulatory mechanisms. Segura *et al.*^[73] investigated the effect of EDTA and EGTA on the binding of vasoactive intestinal peptides (VIPs) to macrophages. VIPs act not only as vasoactive substances, but also play an important role as neuropeptides in the communication between nerves and immune cells in the pulp and periapical tissue by modifying the macrophage function. EDTA inhibits vasoactive interstitial peptides binding to macrophages even in lower concentrations than those used in endodontics (10%). EDTA can prevent the adhesion of macrophages to substrate; this is time and concentration dependent.^[73] EDTA concentrations measurable in the periapical tissues are capable of reducing binding by 50%.

However, changes in macrophage activity can cause the inflammatory reaction to be more easily initiated, but reduced capacity of phagocytosis can result. Furthermore, it has been discovered that EDTA improves plasma extravasation and mediator action. In an investigation of the effects of dental etchants and chelators on nerve compound action potentials,^[74] RC-Prep and File-EZE were shown to reduce the compound action potentials after an application time of 160 min by 61% and 62%, respectively.

ABILITY TO REMOVE CALCIUM HYDROXIDE FROM THE ROOT CANAL

Rödig *et al.*^[75] assessed the efficacy of 1% NaOCl, 10% citric acid and 20% EDTA in the removal of

calcium hydroxide from root canals. According to their findings none of the irrigants or their respective combinations was able to completely remove the calcium hydroxide. Chelating agents such as citric acid and EDTA showed the best results. The combination of chelators and NaOCl did not result in significant improvement of calcium hydroxide removal. da Silva *et al.*^[76] showed that irrigation with 17% EDTA-T and 37% phosphoric acid is more effective than NaOCl and citric acid in the removal of calcium hydroxide from the apical third. Salgado *et al.*^[77] revealed that recapitulation of master apical file in combination with irrigants improved the removal of calcium hydroxide medication better than an irrigant flush alone.

Margelos *et al.*^[78] revealed that using 15% EDTA or NaOCl alone as irrigants did not remove calcium hydroxide from the root canal, but combining these two irrigants with hand instrumentation improved the effectiveness of the removal.

ETHYLENE GLYCOL TETRAACETIC ACID

EGTA is a polyaminocarboxylic acid, a chelating agent that is related to the better known EDTA, but with a much higher affinity for calcium than for magnesium ions. It is useful for making buffer solutions that resemble the environment inside living cells^[79] where calcium ions are usually at least a thousand fold less concentrated than magnesium.

The pKa for binding of calcium ions by tetrabasic EGTA is 11.0, but the protonated forms do not significantly contribute to binding, so at pH 7, the apparent pKa becomes 6.91. Qin *et al.*^[80] for an example of a pKa calculation.

Calt and Serper^[81] indicated that the action of EDTA is stronger than that of EGTA for removal of smear layer. However, EGTA did not cause erosion of the intertubular and peritubular dentine. Cruz-Filho *et al.*^[46] reported that 1% EGTA and 15% EDTAC reduced root dentine microhardness similarly. In a SEM study Viswanath *et al.*^[82] demonstrated that both EGTA and EDTA completely removed the smear layer. De Sousa and Silva^[83] reported that that EDTA and EGTA presented the same effect on dentine Ca²⁺ extraction. Tripod *et al.*^[84] demonstrated that EGTA solubilized more than 60% of dentine while EDTA solubilized about 20% of it.

EFFECT ON THE QUALITY OF OBTURATION

The dentine adhesion of root canal sealers can be improved by dentine pre-treatment with EDTAC; although, this effect is more pronounced after Er:YAG laser pre-treatment. The highest increase in adhesiveness was found for Sealer 26. For calcium hydroxide-based sealers only a slight increase was found.^[85] Morris *et al.*^[86] found that both NaOCl and EDTA significantly reduced the bond strength of resin cement to root dentine. Perdigão *et al.*^[87] showed that this reduction can be completely reversed by application of 10% ascorbic acid or 10% sodium ascorbate. It has been revealed that dentine adhesives bound significantly better to calcified dentine than to decalcified dentine pretreated with EDTA. Michiels *et al.*^[88] showed that the reduction in through-and-through leakage was significantly higher with the Nd:YAG laser as smear-layer modifier than when smear layer was removed with an EDTA rinsing solution.

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