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Review Article

Which Irrigant Should be used for Vital Pulp Therapy in Permanent Teeth? : A Review of the Literature

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Abstract

Management of deep carious lesions has come to a more conservative approach. Vital pulp therapy (VPT) has been proposed as an alternative treatment to traditional root canal treatment with the primary goal to stimulate the pulpal repair process. The materials used for VPT must be able to provide antimicrobial effect, biocompatibility, and bioactive property to achieve successful treatment. Moreover, other minor properties such as not causing adverse effects on the remaining tooth structure, and/or on bond strength of the restoration, and any discoloration of the tooth after treatment can also improve the quality of VPT. Nowadays, there seems to be an agreement on the appropriate pulp dressing materials for VPT. However, the appropriate irrigant for VPT remains questionable. This article reviews irrigants commonly used in endodontics with an emphasis on their properties relevant to the objectives of VPT.

Keywords: chlorhexidine, ethylenediamine tetra-acetic acid, irrigant, saline solution, sodium hypochlorite, vital pulp therapy

Introduction

With the growth of advanced pulp biology and bioactive materials today, management of deep carious lesions has come to a more conservative approach. Vital pulp therapy (VPT), comprising pulp protection with liner and base, direct pulp capping, partial pulpotomy, and coronal pulpotomy, has been proposed as an alternative treatment to the traditional root canal treatment.[1] As the name implies, the objective of VPT is to keep the vitality of the tooth[1,2] when it is challenged by traumatic, mechanical, or carious insults. Therefore, the function of the pulp such as dentinogenesis, apexogenesis, pulpal immunity, along with its reparative, defensive, pain and proprioceptive mechanisms must be maintained.[3,4] Moreover, the more conservative approach can lessen the degree of overtreatment and reduce the undesirable effects of the restorative cycle, thus making the treatment more cost-effective.[3]

The choice of pulp dressing material is essential for successful VPT. The required biological and physical properties of a pulp dressing material consists of having good biocompatibility, bioactivity, no toxicity, high strength, and adherence to tooth structure.[5] Currently, the desirable biological properties can be achieved with calcium silicate-based cement (CSC) and there is abundant evidence of its success in VPT.[6-9] The common CSC products include ProRoot® MTA (Dentsply-Tulsa Dental, Johnson City, USA) and Biodentine™ (Septodont, Saint-Maur-des-Fosses, France). However, although there seems to be an agreement on the appropriate pulp dressing materials, the recommended irrigant for VPT in permanent teeth is less studied.

According to the American Association of Endodontists[10], an ideal irrigant for endodontic procedures such as root canal treatment must have the ability to effectively clean and disinfect the root canal system. To achieve this purpose, the irrigant should be able to penetrate dentinal tubules and eliminate smear layers, while its antibacterial effect should have substantivity with a broad spectrum but do no harm to the surrounding tissues and the instruments used during treatment. The agent should be active in the presence of blood and serum, and have low surface tension. Moreover, the irrigant should be inexpensive, easily manipulated, able to eliminate necrotic pulp tissue and inactivate endotoxins, and should not diminish the success outcome or cause adverse effects in the aspect of restoring the tooth after endodontic treatment. Unfortunately, there is still no such irrigant that holds all the qualities as mentioned above, and the topic of the most efficient irrigant for root canal treatment is still surrounded by controversies.[11]

As for VPT, the aim of the treatment differs from that of root canal treatment. Instead of debriding all vital and necrotic tissues, microorganisms, and their by-products from the root canal system[12], irrigants for VPT require antimicrobial effect but must also be biocompatible to aid in preserving the vital tissues and maintain the tooth's vitality. Moreover, the bioactive property and the ability of irrigant to stimulate the pulpal repair process, which is the primary goal of VPT, has recently been discussed.[10,12] Aside from these main desirable properties, other minor properties such as not causing adverse effects on the remaining tooth structure, bond strength of the restoration, or any discoloration of the tooth after treatment can improve the quality of VPT.

Recent information search was insufficient in extracting a precise conclusion from systematic reviews or meta-analyses regarding the most appropriate irrigant for VPT.[13] There are currently various irrigants which are commonly used in endodontics including sodium hypochlorite (NaOCl), chlorhexidine (CHX), normal saline solution (NSS), and ethylenediamine tetra-acetic acid (EDTA). While their properties relating to the root canal treatment procedure are typically described in most studies[10,12], their desired properties relevant to the objectives of VPT are further elaborated in this review, and summarized in Table 1.

Sodium hypochlorite

Sodium hypochlorite (NaOCl) is one of the most commonly used irrigants in endodontics because it is an antimicrobial and hemostatic agent with the ability to dissolve organic substances in an affordable price.[10,11] However, these properties mentioned above may not be essential for VPT as much as they are for root canal treatment. Moreover, NaOCl contains undesirable properties for VPT such as cyto-toxicity[14,15], which affects the remaining tooth structures and bond strength of restorations[16-18], and there are existing reports of inducing tooth discoloration when it is used in combination

Table 1: *Irrigants and their properties relevant to vital pulp therapy*

Desired properties for vital pulp therapy	NaOCl	CHX	NSS	EDTA
Antibacterial property	✓	✓	×	Very low to none
Biocompatibility	$\propto \frac{1}{\text{dose \& time}}$	$\propto \frac{1}{\text{dose \& time}}$	✓	✓
Bioactive property	×	×	×	✓
No adverse effect on tooth structure	×	?	✓	×
No adverse effect on restoration	?	?	Less to none	?
No discoloration after treatment	Higher discoloration	Higher discoloration	Lower discoloration	Lower discoloration

CHX, chlorhexidine; EDTA, ethylenediamine tetra-acetic acid; NaOCl, sodium hypochlorite; CHX, chlorhexidine; NSS, normal saline solution; ✓ indicates that there is evidence that the irrigant has this property and; × indicates that the irrigant does not have this property; while ? indicates that there is lack of evidence or controversies regarding the irrigant and this property.

with CSCs.[19-21]

The antimicrobial action of NaOCl is caused by the hypochlorous acid within the solution. It interferes the microbial cellular metabolism through amino acid degradation and hydrolysis, along with inhibiting bacterial enzymes that are needed for vital functions.[10,12] Moreover, the alkalinity of the solution is also accounted for its antimicrobial effect.[12] The antimicrobial efficacy of NaOCl is dose-dependent[22], meaning that it becomes more effective when the concentration of the solution is higher. However, once the concentration increases, NaOCl has more cytotoxic effect to tissue and cells.[15,23]

Regarding cytotoxicity, an *in vitro* study tested 0.04%, 0.08%, 0.16%, and 0.33% NaOCl compared to physiologic saline buffer on human pulp cells.[14] The cell survival was increased when the concentration and exposure time of NaOCl was decreased and a significant decrease in cell viability was found at the concentration of 0.16% and 0.33% NaOCl. Furthermore, 0.33% NaOCl produced cell death after exposure durations of 10 and 15 minutes. Heggers *et al*.[24] concluded that 0.025% NaOCl was "therapeutically efficacious as a fluid dressing" as the concentration was able to maintain its antimicrobial properties and did not interfere with the incisional wound healing process of rats. It is interesting that the concentrations in these *in vitro* studies are lower than the concentration of 0.5-6.0%, commonly used in clinical practice.[10,12] An *in vivo* study on subcutaneous rat tissue reaction with different irrigants found that 2.5% NaOCl had significantly higher inflammatory cells after 48 hours and 14 days, compared to the NSS group and the control group with no irrigant, and 5% NaOCl displayed the most toxicity.[23] Another study by Rosenfeld *et al.* found that aside from necrotic tissues, 5.25% NaOCl also affected healthy vital pulp tissue.[25] Moreover, predentin — unmineralized dentin that contains collagen fibers, nerve fibers and odontoblastic processes — were significantly eliminated in almost all NaOCl treated teeth, compared to the NSS group that had remaining predentin in all the specimens.

To accomplish successful healing, biocompatibility could be one of the most important properties that impacts the healing process in VPT. Therefore, the choice of a material that produces no harm to cells and provides an appropriate environment that can promote repair should be considered. CSC, the currently recommended pulp dressing material, not only is highly biocompatible but also provides antibacterial effect.[5] The prolonged contact of CSC to the pulp and surrounding tissue in VPT procedure may reduce the importance of antimicrobial effect provided shortly by NaOCl irrigation. Moreover, it may be assumed that this short duration use of NaOCl may have also lessened the degree of harm that NaOCl could cause by its cytotoxicity as clinical VPT studies using NaOCl in combination with CSC resulted in high success.[9,13,26] For hemostatic property, a study in monkeys demonstrated that 3% NaOCl was able to obtain complete hemostasis, eliminate dental chips and blood clots, which can facilitate healing of the pulp tissues.[27] The use of NaOCl has been suggested for hemostasis during VPT procedures[3,4,28,29]; however, the concentration and duration used to stop bleeding varied considerably, from a couple of minutes to up to 10 minutes.[6-9,26,30] Moreover, in VPT procedure, hemostasis should be easily obtained in teeth with healthy pulp, without the aid of any materials or solutions. Therefore, the use of NaOCl for this purpose may be debatable. In addition, NaOCl also has the ability to dissolve organic tissues such as necrotic pulp tissues and collagen within the dentin. The saponification reaction and chloramination reaction of NaOCl with fatty acids, lipids, and organic matters are responsible for NaOCl's organic tissue dissolving property.[31] The increase of many factors such as contact time, exposed surface area, concentration, frequency of mechanical agitation, and temperature were demonstrated to increase the efficacy of NaOCl's tissue dissolving property.[32,33] The addition of surfactants to the solution was also shown to enhance the efficacy as a study demonstrated that NaOCl that contained a surfactant had the highest tissue dissolution in various concentrations and temperatures.[33] Regarding the cleaning ability of NaOCl, Svec and Harrison[34] demonstrated that instrumentation and irrigation with 5.25% NaOCl combined with 3% hydrogen peroxide (H_2O_2) had better efficiency in cleaning the root canal when compared to instrumentation and irrigation with NSS at the level of 1- and 3- mm from root apex. However, no difference was found at the level of 5 mm from root apex suggesting that the value of using NaOCl to clean the more coronal portions of the tooth may be questionable. Root canal treatment has operation sites that are located at a more apical and narrower field which makes it difficult to be cleaned thoroughly mechanically. On the other hand, VPT is

commonly performed within the coronal portion and the common methods used for pulp tissue removal (i.e., high speed bur or spoon excavation) should be sufficient in eliminating the infected and severely inflamed pulp tissue if performed by experienced dentists. Nonetheless, the tissue dissolving ability and antimicrobial effect of NaOCl may be more beneficial for direct pulp capping, the type of VPT procedures that does not involve pulp amputation.

One of the most concerning problems when using NaOCl is NaOCl accident.[12,35] This adverse event can occur when irrigating the root canal with excessive pressure or going beyond its working length. Accidental spillage can damage clothing when it contacts with fabric and even more difficulties can occur if it contacts with the other parts of the body, i.e. the skin, oral mucosa and eyes.[36] NaOCl accidents in clinical settings have been reported and the incident can cause minor to severe complications such as severe pain, intra- and extra-oral swelling of the tissues, hematoma formation, and necrotic destruction of the oral tissues.[37,38] The management of these conditions include medical prescription for pain control, and antibiotics to prevent spread of infection from the treated tooth and secondary infection from the large amount of tissue destruction, debridement of necrotic tissues, surgical treatment for drainage or surgical correction in cases with tissue defects after the accident.[35,37,38] In addition, rehabilitation may be required for cases that encountered nerve damage which may impair the patient's speech and swallowing ability.[35] Although the incidence may be rare[35], clinicians must always practice with awareness and precaution while handling NaOCl.

Another drawback of NaOCl is its effect on the remaining tooth structure. The different concentrations of NaOCl can have different effects on the remaining tooth structure. Experimental studies demonstrated that compared to NSS, 3-5.25% NaOCl decreased the elastic modulus and flexural strength of dentin significantly[16,39], while 0.5% NaOCl showed no significant difference.[16] In addition, it was reported that dentin microhardness had a significant reduction when the concentration of NaOCl increased from 2.5% to 6%.[17] Moreover, there was a significant decline in dentin microhardness when irrigating with both 2.5% and 6% NaOCl after 10 minutes, suggesting that the effects of NaOCl on the tooth structure is also time-dependent. Results from a systematic review also concluded that there was strong evidence which suggested that NaOCl had adverse effects on the tooth structure.[40] The alteration of tooth structure by NaOCl can influence the interaction between the tooth surface and restoration material.[18] Nonetheless, studies have shown controversial results on the effect of NaOCl on bond strength. Studies which found increased bond strength after the use of NaOCl explained that it was a result from the elimination of dentin collagen which in turn will lessen the bonding technique sensitivity and provide a fresh mineralized dentin surface for the bonding agent.[41,42] In addition, it also creates porosities by solubilizing fibrils in the mineralized matrix, which will enhance the infiltration of the bonding agent.[43] On the other hand, NaOCl is said to be responsible for the reduction of bond strength due to its oxidizing property, which along with the remaining chemicals of the irrigant within the dentinal tubules interfere with polymerization of resin composites.[44] Furthermore, the elimination of organic substance in the dentin, caused by the use of NaOCl, results in an inconsistent hybrid layer.[45] The reduction of bond strength can adversely affect the quality of the coronal restoration, thus leading to microleakage that may eventually compromise the VPT outcome.

Discoloration of the VPT treated teeth is another problem reported both in *in vitro* and clinical studies when NaOCl is used along with MTA. The cause is said to be from the reaction between NaOCl and bismuth oxide – the radiopacifier contained in MTA.[46] Laboratory studies have demonstrated that CSCs can display discoloration over time because the materials containing bismuth oxide will display a greater significance in discoloration when contacted with NaOCl[19,46] and CHX.[19,21] Blood contamination with CSCs was reported as another factor that contributed to significant discoloration.[47] Parinyaprom *et al.*[26] found that 55% of direct pulp capping treated teeth using 2.5% NaOCl and MTA exhibited discoloration while none were found in the Biodentine™ group. The result in the MTA group was consistent with Uesrichai *et al.*[9], who found up to 80% of tooth discoloration in partial pulpotomy treated teeth with MTA. However, discoloration also occurred in 27% of the group treated with Biodentine™, suggesting that besides the oxidative reaction between bismuth oxide and NaOCl, blood contamination may also influence discoloration of the VPT treated tooth as reported in *in vitro*.[47]

Until now, the most appropriate concentration of

NaOCl for VPT is still unknown. Various concentrations of NaOCl, ranging from 0.5-5.25%, have been used for VPT.[8,9,30,48] In addition, a great diversity regarding protocols for NaOCl irrigation and hemostasis in clinical studies was found. Examples of the protocols include using NSS to achieve hemostasis before NaOCl application[49], using more than one concentration of NaOCl for irrigation and hemostasis[8], and spraying the cavity with water after NaOCl.[30] Although the rationales behind these protocols were not explained, it may be assumed that the authors proposed these conducts to avoid the previously mentioned drawbacks that can supposedly result from using NaOCl.

Chlorhexidine

Chlorhexidine (CHX) is a synthetic bisguanide antimicrobial agent, as the cationic property of CHX provides its antimicrobial mechanism.[50] The positive charge of CHX interacts with the negatively charged phosphate groups on microbial cell walls. Thus, the cell permeability of the microorganism will become dysfunctional, allowing CHX penetration into the cells. In addition, its cationic property is also responsible for its substantive antimicrobial effect[12], which is one of the distinctive and beneficial advantages for VPT. On the contrary, the shortcomings of CHX regarding VPT includes lack of tissue dissolving property, and reports of discoloration with CSCs in *in vitro* studies. The most common concentration of CHX used in endodontics is 2%.[10]

One of the unique properties of CHX is its substantivity. The positive charge of the solution enables it to bind to the anionic oral mucosa and tooth structure, allowing it to further exhibit its antibacterial effect after application.[12] A clinical study on a two-visit indirect pulp treatment, which is a procedure that did not encounter pulp exposure, in immature permanent teeth found that 2% CHX had greater significance in microorganism reduction compared to ozone application group and the control group (no disinfectant) after 4 months.[51]

A clinical trial on coronal pulpotomy in primary teeth proved that the efficacy of CHX prepared in a polymer scaffold was comparable to that of MTA.[52] Meanwhile, researchers had an idea to incorporate CHX's antibacterial activity into CSCs to enhance its properties.[53,54] An *in vitro* study showed a significant rise of antibacterial effect when 0.12%, 0.2%, and 2% CHX was incorporated with both MTA and calcium enriched mixture (CEM).[53] However, in an animal study, pulp capped canine teeth treated with MTA mixed with 0.2% CHX had significantly lower dentin bridge formation compared to those treated with pure MTA.[54] Consequently, further research is required for material development that can achieve both antibacterial and bioactive properties.

Despite the advantages mentioned above, CHX's drawbacks include taste alteration, formation of calculus, staining of teeth, oral soft tissue, dental restorations, and absence of tissue dissolving property.[55-57] Additionally, in the same manner as NaOCl, CHX's cytotoxicity is also dose- and time-dependent. Concentrations of 0.06%, 0.12%, 0.2%, 1% and 2% CHX had significantly higher cytotoxicity on odontoblast-like cells compared to 3% H_2O_2 and the cytotoxicity increased along with the concentration and contact duration.[58] Moreover, CHX was demonstrated to hold a significantly higher cytotoxicity compared to NaOCl on Human Lung Fibroblasts (MRC5) cells.[59] Conflicting results from another study stated that 5.25% NaOCl had a more cytotoxic effect on human periodontal ligament fibroblasts when compared to 2% CHX.[60]

Regarding the effect of CHX on tooth structure, diverse protocols were conducted, and controversial results were found. Some studies have reported adverse alterations in teeth mechanical properties after CHX application[61,62], while conflicting results from other studies showed no significant difference compared to other irrigants.[63,64] Nonetheless, the substantivity of CHX was also mentioned to be taken into account since exposure durations in *in vitro* studies could not mimic actual clinical situations.[65] Studies about CHX and its effect on bond strength had diverse methodology and designs, leading to conflicting results.[66-69] A systematic review reported bond strength reduction from 0–84.9% with most of the studies showing lower bond strength reduction when compared to the control groups that did not use CHX as a pretreatment solution or as a composition in the adhesive system.[70] In other words, CHX was shown to have a lower to no deteriorating effect on bond strength in this study. Moreover, Mohammadi and Abbott[71] concluded that CHX improved the resin-dentin bond stability by maintaining integrity of the hybrid layer, since CHX was able to inhibit endogenous metalloproteinases (MMPs), which are responsible for dentin collagen fibrils degrada-

tion. Nevertheless, further studies should be conducted to verify this matter especially in clinical settings.[70]

Few randomized controlled studies using CHX in VPT have been done. In 2010, Mente *et al*.[72] conducted a clinical study on direct pulp capping after carious exposures in permanent teeth using up to 5 minutes of 0.12% CHX irrigation with calcium hydroxide or MTA. The success rates for direct pulp capping with calcium hydroxide and MTA were 60% and 78%, respectively. Later in 2014, another clinical trial with the same protocol was conducted with a longer follow-up time.[73] They found that when using 0.12% CHX as an irrigant, MTA had a significantly higher success rate (80.5%) compared to calcium hydroxide (59%), leading to the conclusion that MTA held a more favorable long-term outcome in direct pulp capping treated teeth. It may be assumed that the type of pulp dressing material used might have a greater influence on the treatment's long-term outcome rather than the brief effects of irrigants during treatment. However, furthers studies comparing different irrigants are needed to confirm this assumption.

Although clinical studies did not provide any information about tooth discoloration after treatment with CHX[51,72,73], *in vitro* studies have found that discoloration occurs when CSCs come into contact with CHX.[19-21] CHX was found to cause significant discoloration when used with Biodentine.[20] This was consistent with findings by Sobhnamayan[21], which showed the highest discoloration after CHX was mixed in all three materials groups as follows: MTA Angelus, CEM, and Biodentine.

Normal saline solution

Normal saline solution (NSS) contains 0.9% sodium chloride in sterile water, thus making it isotonic to human body fluids. It has been used in wound irrigation due to its safe, isotonic and nontoxic properties that do not interfere with the healing process.[74] In endodontic practices, NSS is commonly used in between each irrigant to prevent chemical interactions of the residual components.[75] Despite its lack of chemical properties compared to other irrigants, its main feature as having good biocompatibility may be its major contribution to VPT.

Regarding biocompatibility, a preliminary study showed that NSS, 5.25% NaOCl, and 2% CHX did not affect the expression of extracellular matrix glycoproteins in pulp repair (tenascin and fibronectin) after calcium hydroxide pulp capping.[76] However, another *in vitro* study demonstrated that NSS had a significantly higher dental pulp stem cells (DPSCs) attachment and cell survival than 5.25% NaOCl, 25% tetracyline solution, and the control group with no treatment.[77] The higher survival rate in the NSS group was associated to the isotonic property of the solution, which provides equivalent osmotic pressure within the cells. Therefore, it did not cause cell shrinkage or expansion that would affect the cell's survival rate. The good biocompatibility of NSS was also demonstrated in an animal study which found no significant difference of inflammation compared to the control group after 14 days, which is concurrent with the period of tissue reparation of 14-30 days.[23] The results suggested that NSS had promoted a favorable outcome in subcutaneous connective tissue repair in rats and can be regarded as a biocompatible material. Biocompatibility is considered as an essential and desirable advantage for VPT, as its primary aim is to preserve the vitality and promote pulpal healing of the affected tooth.

In contrast to its biocompatibility, NSS has a disadvantage of not providing antibacterial effect. NSS had a significantly higher amount of bacterial colony-forming units after irrigation compared to 2% CHX and 5.25% NaOCl.[78] Nonetheless, Özgür *et al*.[7] showed that success rates after partial pulpotomy using calcium hydroxide or MTA with NSS, which is in an irrigant without antibacterial effect, were not different from groups that were treated with 2.5% NaOCl. Antibacterial effect provided by CSCs, the current recommended pulp dressing material, together with sufficient removal of infected pulp tissue may compensate the lack of antimicrobial effect in NSS. Conversely, Ballal *et al.*[79] demonstrated significantly higher early failures in carious teeth treated with direct pulp capping with MTA using NSS compared to 2.5% NaOCl. The reason may attributed to the lack of pulp amputation in direct pulp capping which may have resulted in insufficient elimination of infection and inflammation; thus, an irrigant with tissue dissolving ability and antibacterial effect may be beneficial in this scenario as mentioned earlier.

Another drawback of NSS for VPT procedure is its lack of bioactive property. An *in vitro* study found that DPSCs attachment and survival after irrigation with NSS was significantly lower than using chelating agents like

17% EDTA and 10% citric acid.[77] Nevertheless, the results were significantly higher than the control group with no irrigation due to its biocompatibility.

Numerous studies have been conducted to investigate the effects of irrigants on mechanical properties of the tooth. While NSS was commonly used as a control in *in vitro* studies[65], the other tested irrigants were more likely to show significant adverse effects on the tooth structure.[16,39,64] Regarding NSS's effect on bond strength, a study compared bond strength after irrigation with tap water (control), sterile water, filtered water and NSS[80], showing that bond strength was dependent on the dentin bonding agent. A significant reduction of bond strength was found in only some of the tested bonding agents irrigated with NSS.

Regarding discoloration, NSS was used as a control group to test discoloration from CSCs in *in vitro* studies. There was significantly higher discoloration in groups that were in contacted with blood when compared to those with the NSS control groups; however, discoloration in the NSS group still occurred over time.[47] In studies that compared the effect of irrigants on CSCs, NSS groups displayed significantly less discoloration to unnoticeable discoloration by the visual observation.[20,21] Nevertheless, clinical trials that compare tooth discoloration after VPT of different irrigants have not been reported.

Regardless of controversies in NSS use for VPT as mentioned above, there have been clinical studies which used NSS as an irrigant for VPT with CSC in permanent teeth with high success.[13,81] Most of the studies did not elaborate on the duration of irrigation and hemostasis but the procedures were carried on if the time to achieve hemostasis did not exceed 10 minutes.[7,81,82]

Ethylenediamine tetra-acetic acid

Ethylenediamine tetra-acetic acid (EDTA) is an aminopolycarboxylic acid which acts as a strong chelating agent. The advantage of using EDTA in endodontics is mainly due to its chelating ability. Seventeen percent EDTA is the concentration that is widely used in endodontics[12,75] and commonly used for smear layer elimination in root canal treatment. However, for treatments with a more biological approach like regenerative endodontics and VPT, its bioactive property seems to be more favorable.

With its chelating property, EDTA reacts with cationic metal ions such as calcium ion (Ca^{2+}) and can cause demineralization of the dentin and elimination of smear layer, which can aid in root canal preparation and cleaning in narrow and calcified roots.[12] The method introduced by Nygaard-Østby in 1975[12,83] showed that its demineralizing effect was self-limiting when the chelating reaction is in equilibrium. The results also showed that demineralization did not exceed 50 μμm after 48 hours. On the contrary, Patterson *et al.*[84] stated that EDTA did not have a self-limiting demineralizing effect. However, the process continued up to 5 days but only showed a 0.28 mm. decrease of dentin thickness. As a consequence of demineralization, a release of cytokines and growth factors enclosed in the dentin matrix could be found.[12] Transforming growth factor betas (TGF-βs) are known to play a crucial role in regulating mesenchymal cells for dentinogenesis.[29,85] An *in vitro* study showed a significant release of TGF-β1 after irrigation of 17% EDTA over the negative control and NSS, and with a significantly higher cell survival than the 37% phosphoric acid group.[86] This bioactive effect of EDTA can stimulate pulp repair and regeneration, which is essential for healing and maintaining pulp vitality after VPT.

The biocompatibility of 17% EDTA was shown as it significantly achieved the most DPSCs attachment and cell survival over 25% tetracycline solution, 5.25% NaOCl, 10% citric acid, and NSS in an *in vitro* study.[77] Moreover, 17% EDTA does not harm the cells and also promotes cell proliferation by enhancing cell attachment through elimination of smear layers. In agreement, another study also demonstrated that EDTA induced cell attachment and odontoblastic/osteoclastic differentiation of the DPSCs.[87] Moreover, 17% EDTA was able to enhance stem cells of the apical papilla proliferation and viability, whereas NaOCl caused the opposite effect in a dose-dependent manner with a drastic reduction at the concentration of 6% NaOCl.[15] Furthermore, an *in vitro* study showed that EDTA had significantly lower cytotoxicity compared to NaOCl and CHX.[59] In other words, EDTA can be suggested as a more biocompatible solution than NaOCl and CHX. Owing to the advantages of EDTA mentioned above, 17% EDTA is routinely used in regenerative endodontics. Following the American Association of Endodontists Clinical Considerations for a Regenerative Procedure[88], it is used as a final rinse before inducing bleeding into the root canal. In the same

manner, Bahcall *et al.*[89] suggested using 17% EDTA in VPT after application of cryotherapy (sterile-water ice at 0°C) instead of NaOCl.

Aside from the suggestion by Bahcall *et al.*[89] who used cryotherapy along with EDTA, there are no clinical studies using EDTA as an irrigant in VPT. Nonetheless, there has been a clinical study which used autogenous treated dentin matrix (TDM), fabricated by soaking and conditioning dentin chips from one of the patient's own third molar in EDTA, with MTA as a pulp dressing material for partial pulpotomy.[90] The patients had partial pulpotomy with NSS as an irrigant in two of their third molars in a split mouth manner, one tooth using only MTA while the other used MTA with TDM. Although clinical and radiographic outcomes of both groups did not show any significant difference after 6 weeks, the dentin bridge formation of the MTA with TDM group was significantly higher. This study demonstrated the beneficial application of EDTA's bioactive properties and biocompatibility in clinical use regarding VPT. Nevertheless, the study also has its limitations as the treated teeth had no inflammation because the exposure sites were intentionally prepared by the operator.

Unfortunately, the chelating property of EDTA also has its setbacks. Seventeen percent EDTA was shown to cause disruption in blood clot formation in an *in vitro* study.[91] The reason was due to its chelation reaction with the calcium ions in blood, resulting in lower and shorter fibrin networks in the blood clot. These characteristics were not found in groups that used NSS as a final rinse. Therefore, NSS was suggested to be used as the final irrigant to improve fibrin network in the blood clot which serves as a scaffold for cell regeneration. Furthermore, as cryotherapy has been shown to assist in providing hemostasis in VPT[89], it may be possible that its use can also lower the anticoagulation effect of EDTA.

Moreover, another concern was the interaction between EDTA and pulp dressing materials, which are materials that contain calcium ions. The results from a study by Lee *et al.*[92] showed decreased crystalline structures and microhardness of the material when exposed to EDTA, compared to the distilled water and NSS groups. Furthermore, there was lower cell adhesion on the surface of MTA in the EDTA group compared to the group stored in distilled water. All the findings mentioned before indicated that EDTA deteriorates the properties of MTA. In addition to improving the blood clot quality, final irrigation with NSS might also be suggested for this reason.

The chelating ability of EDTA is also demonstrated to alter mechanical properties of the tooth. With heterogenicity in study protocols, controversial results are inevitable. Nevertheless, a recent review showed that most studies on surface roughness considered EDTA as harmful.[65] The increased concentration and exposure time of the solution also enhanced its effect on the tooth structure.[93] Additionally, a study found significantly lower bond strength when EDTA was used as a dentin conditioner compared to 37% phosphoric acid.[94] The effect of EDTA on bond strength is still controversial as conflicting results of increased bond strength have also been reported.[95]

The bactericidal effect of EDTA is caused by the chelating reaction with metal ions on the bacterial cell membrane.[83] Compared to NSS, 17% EDTA is more, but not significantly, effective in bacterial reduction[96] and was demonstrated to have a higher antibacterial effect than citric acid and 0.5% NaOCl.[97] However, its antibacterial effect on E. faecalis was significantly lower than 3% H_2O_2, 1% NaOCl and 0.2% CHX, suggesting that the antibacterial effect of EDTA was weak.[96] Torabinejad *et al.*[98] also founded that 17% EDTA had limited antibacterial effect due to its significantly lower effectiveness in bacterial inhibition compared to NaOCl and a mixture of a tetracycline isomer, an acid, and a detergent (MTAD). Moreover, when 17% EDTA was diluted at the ratio of 1:5 or 1:10 it did not display any antibacterial effect. Therefore, they concluded that EDTA was non-antibacterial.

In regards of discoloration, there was a significant difference of color alteration when EDTA was used with MTA Angelus compared to specimens in the NSS group, and the material in dry condition with no contact with any irrigant (control group).[21] Another recent study found that there was a significant difference in color measurement, by using a spectrophotometer, before and after various types of CSCs were immersed in EDTA, but was unnoticeable by visualization.[20] Currently, there is still a lack of clinical studies using EDTA despite its advantages and potential as an irrigant for VPT.

To conclude, each irrigant possesses its own qualities, advantages, and drawbacks that should be considered when choosing the appropriate irrigant for VPT procedure. Antibacterial effect can be obtained by using NaOCl or

CHX, with additional tissue dissolving effect by using NaOCl. Nevertheless, both irrigants have disadvantages of having cytotoxic effects, and high tooth discoloration after treatment, with adverse effects on the remaining tooth structures by using NaOCl. Furthermore, controversies still surround the topics of CHX's adverse effects on the remaining tooth structure, and the adverse effects on resin composite bond strength of both CHX and NaOCl. On the other hand, the more biocompatible irrigants which cause less tooth discoloration, such as NSS and EDTA, have very low to no antibacterial effect. While NSS does not have bioactive property, it has no adverse effects on the tooth structure. In contrast, EDTA is the only irrigant that provides bioactive property, although it can cause negative effects on the remaining tooth structure. Since there is currently no ideal irrigant that holds all the desired properties for VPT, clinicians may require to balance the benefits and risks of each irrigant while also taking the treatment objective of VPT into account. The usage of more than one irrigant in the procedure may be another solution to this matter. The best irrigant, its concentration, and method of its use for VPT are subjects awaiting to be determined.

Conclusions

The main objective of VPT, a conservative and biologically based treatment, is to maintain vitality of the tooth. Unfortunately, no sole irrigant is able to attain all the ideal properties required for the treatment. Clinical studies can be the foundation of systematic reviews and meta-analyses on this matter. Therefore, further well-designed studies are necessary to find the most proper irrigant that should be used for VPT.

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