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Evaluation of Smear Layer Dissolution by Alternative Chelating Solutions

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

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Abstract

EVALUATION OF SMEAR LAYER DISSOLUTION BY ALTERNATIVE CHELATING SOLUTIONS

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Purpose: To evaluate in vitro smear layer dissolution using alternative chelating solutions in comparison to EDTA.

Methods: Nine extracted human anterior teeth were sectioned into dentin discs. Test solutions included: peracetic acid (PAA) at various concentrations (0.1%, 0.2%, 0.3%, 1%, 2%) for 60sec, 17% EDTA for 60sec, 18% 1-hydroxyethane 1,1-diphosphonic acid (HEDP)+NaOCl for 5min, HEDP alone for 5min and QMix for 60sec. SEM and a 4-point scale were used to evaluate smear layer removal.

Results: EDTA for 60sec had significantly better scores in dissolving the smear layer than all other groups. There was no significant difference between HEDP and HEDP+NaOCl for 5min, but both had significantly better scores in dissolving the smear layer than PAA at all concentrations and QMix at 60sec.

Conclusion: EDTA for 60sec was the most effective at dissolving the smear layer, followed by HEDP and HEDP+NaOCl for 5min. PAA at all tested concentrations and QMix for 60sec were not effective.

Introduction

Success in endodontics is dependent on the quality of debridement, disinfection and obturation of the root canal system (1). Modern advancements in technology and rotary instruments have improved the predictability and efficiency of endodontic therapy. Current treatment methods include the mechanical instrumentation of root canals in conjunction with the use of antimicrobial irrigants. However, these methods inevitably continue to result in the formation of a smear layer on instrumented root canal walls (2). The clinical significance of the smear layer in endodontics has been the subject of much research. Arguments have been made for and against the removal of the smear layer. Some argue that the smear layer may act as a barrier to bacterial penetration, while others state removal of the smear layer enhances intracanal disinfection and obturation procedures (3)(4)(5). Despite conflicting views in the literature, removal of the smear layer is considered to be an important step in endodontic therapy today (6).

Early scanning electron microscopy (SEM) studies conducted by Brannstrom & Johnson on dentinal surface characteristics following cavity preparation illustrated the presence of a thin, smeared layer of debris up to 2-5um thick with extension into dentinal tubules (7). Similarly, mechanical root canal instrumentation also generates a smear layer. In 1975, McComb & Smith were the first researchers to describe the smear layer in endodontics. They found that instrumentation using hand files and reamers formed a smeared layer on root canal walls, which was similar in appearance to that of coronal smear layers in cavity preparations. This layer

consisted not only of cut dentin, but also remnants of odontoblastic processes, pulp tissue and bacteria (2).

The scanning electron microscope was used to investigate the morphological characteristics of the smeared layer on instrumented root canal walls. The smear layer was described as two confluent components: 1) the thin layer of smeared material on the surface of the canal wall (~1-2um thick) and 2) the smeared material packed into the dentinal tubules (up to a depth of 40um) (8). The appearance of the surface layer was typically amorphous, irregular and granular with some areas more pronounced than others. The dentinal tubule layer appeared as finger-like projections in densely packed areas, while loosely packed in other areas. Areas inadvertently left untouched through instrumentation had little to no smeared material (8). This appearance was consistent with the belief that endodontic instruments translocate and burnish the superficial components (organic and inorganic) of the canal walls during root canal instrumentation (9).

Several researchers have reported that the smear layer can act as a physical barrier for dentinal tubules against penetration of bacteria and their byproducts. In vitro studies created on dentin discs found that maintenance of the smear layer established a protective diffusion barrier and had less bacterial penetration into dentinal tubules in comparison to when the smear layer was removed (10)(11). Drake et al. conducted a more clinically relevant in vitro model through conventional chemomechanical debridement of extracted human canines followed with bacterial inoculation. They found teeth which had the smear layer removed had dentinal tubules with significantly higher levels of bacterial penetration, which suggested that the smear layer produced during root canal therapy may inhibit bacterial colonization of root canals (3). However, other studies found that the smear layer only delayed passage of the bacteria through

the dentinal tubules and did not inhibit its penetration (12). Bacteria such as *Streptococcus sanguinis*, *Actinomyces viscosus* and *Coryneabcterium spp.* were able to digest the smear layer, which allowed their penetration into dentin (13).

A suggestion made in favor of removing the smear layer was that it could improve the efficacy of intracanal disinfectants. It was well known that the root canal system can harbor bacteria, their toxins and byproducts when pathologic changes occur in the dental pulp (14)(15). The use of SEM to examine extracted human teeth with necrotic pulps and found bacterial penetration into dentinal tubules up to 150um deep in the apical two-thirds of the roots (16). Sjogren et al. studied the presence of bacteria and their importance in outcomes of root canal therapy and reported a success rate of 94% when there was a negative bacterial culture prior to obturation in comparison to 68% when there was a positive culture (17). They also showed that the addition of calcium hydroxide for one week significantly increased bacterial reduction in comparison to instrumentation and irrigation alone (18). Several studies have shown that removal of the smear layer resulted in greater bacterial reduction and decreased the time necessary to achieve the disinfecting effect of antimicrobial agents (4,19,20). In addition, removal of the smear layer also facilitated the diffusion of calcium hydroxide to the exterior surface of the root, which could further aid in canal disinfection (21).

The final obturation of the root canal system and the quality of the seal is an important consideration in endodontic therapy. SEM studies have shown that removal of the smear layer enhanced the adaption of thermoplasticized gutta percha and increased the penetration depth of root canal sealers into dentinal tubules (22,23). In vitro bacterial leakage studies have also shown that removal of the smear layer reduced the microleakage through the root canal system (5). In 2007, a systematic review and meta-analysis was conducted on the effect of the smear layer on

sealing ability of canal obturation. These results indicated that 53.8% of comparisons reported no significant difference, 41.5% reported a difference in favor of removing the smear layer, and 4.7% reported a difference in favor of keeping the smear layer. Despite variations in methodology, type of leakage tests and sample size, it was concluded that smear layer removal improved the fluid-tight seal of the root canal system (6).

The ability of sodium hypochlorite (NaOCl) to dissolve organic tissues is well known. However, it does not have the capacity to remove the smear layer from instrumented root canal walls (9,24). McComb and Smith were the first to show that ethylenediaminetetraacetic acid (EDTA) could be used to remove the smear layer (2). SEM studies using several irrigants found that the smear layer was not completely removed when either NaOCl or EDTA was used alone. When NaOCl was used as the only irrigant, pulpal remnants and predentin were removed but a smear layer remained. When EDTA was used as the only irrigant, demineralization of the smear layer occurred, however, neither pulpal remnants nor predentin were removed. The combination of a final irrigation sequence with EDTA followed by NaOCl resulted in removal of the pulpal remnants and smear layer, which resulted in a smoothly planed surface with patent dentinal tubules (9,24,25).

Chelators (i.e. EDTA) dissolve calcified structures due to their ability to remove and bind calcium ions. Thus, EDTA can cause decalcification and dissolution of inorganic components of the smear layer. Because EDTA has a strong demineralizing effect, it can also cause enlargement of dentinal tubules and softening of dentin (26). For efficient removal of the smear layer without causing excessive dentin erosion, studies have suggested a 1min application with 1mL of 17% EDTA per canal (27–29). However, the presence of dentin debris and the smear layer, which would be generated throughout the root canal therapy procedure, significantly decreased the

properties of NaOCl (4,30). One way to simplify this protocol, rather than alternating NaOCl and EDTA, would be to use a mixture of a chelator and NaOCl to concomitantly disinfect the root canal system and remove the smear layer and hard tissue debris. In 2005, this concept of continuous chelation was introduced by Zehnder et al (31). When mixed together, NaOCl has little effect on the chelating ability of EDTA but the antimicrobial efficacy and tissue dissolution properties of NaOCl were significantly reduced (32). Etidronic acid (1-hydroxyethane 1,1-diphosphonic acid; HEDP) has been identified as a weak chelator that is compatible with NaOCl for 60 minutes when mixed together. (31). When comparing EDTA and HEDP, it was found that the smear layer was removed after 60sec and 5min, respectively. However, when the contact time was extended to 10min, dentin erosion was significantly increased with EDTA but not with HEDP (33). The slower rate of chelation with HEDP could be better suited for longer periods of use without causing excessive dentin erosion and has been suggested for use with NaOCl throughout the entire root canal therapy procedure.

Some studies have found that the use of NaOCl as a final irrigant following removal of the smear layer results in marked erosion of the root canal dentin (34,35). A possible alternative would be to use a chelator with strong antimicrobial properties as the final irrigant. QMix 2in1 irrigating solution is a product on the market that is designed to fulfill this purpose. QMix contains EDTA, chlorhexidine and surfactant. It is a clear solution that does not require chairside mixing prior to its use. QMix has been shown to have comparable antimicrobial properties to NaOCl and smear layer dissolution properties comparable to EDTA (36,37). Peracetic acid (PAA) is another alternative that has been proposed to dissolve the smear layer and concomitantly continue to disinfect the root canal system (38). PAA solutions are amongst the strongest disinfectants known, with antibacterial, sporicidal, antifungal and antiviral properties

(39). Currently, these solutions are used in veterinary medicine, water treatment, the food industry and for sterilization of medical equipment (40,41). In an aqueous solution, PAA is in equilibrium with hydrogen peroxide, acetic acid and acetylhydroperoxide. It is the acetic acid content that is probably responsible for the smear layer dissolution. Acetic acid forms complexes with calcium, which are easily soluble in water (42). In vitro studies have found that 1% PAA, when used as a root canal irrigant, has comparable antibacterial action to 2.5% NaOCl (40,43). In regard to smear layer removal, Lottanti et al. studied the effects of EDTA, etidronic acid and PAA irrigation. They found that a 2.25% PAA solution is comparable with 17% EDTA in removing the smear layer (38). However, 2.25% PAA is caustic when in contact with oral mucosa and lower concentrations have been recommended for future research. De-Deus et al. studied the effect of a 0.5% PAA solution in comparison to 2.25% PAA and 17% EDTA, and found comparable smear layer removal after 60 sec of contact time (42). It is not clear if even lower concentration than 0.5% PAA exhibit comparable smear dissolution properties.

Currently, there is a lack of comparative studies on multiple chelators as an alternative to EDTA. The goal of this study was to evaluate in vitro smear layer dissolution using alternative chelating solutions (HEDP, PAA, QMix) in comparison to EDTA.

Methods

Specimen Preparation

Nine extracted and intact human anterior teeth were stored in 10% neutral formalin. The crown of each tooth was removed prior to each sample being embedded in an epoxy resin cylinder to facilitate manipulation and improve the metallographic preparation. Dentine discs approximately 3 ± 0.3 mm thick were cut from the cervical third of the root using a low-speed saw (Isomet, Buhler, Ltd; Lake Bluff, NY, USA) with a diamond disc and continuous water irrigation to prevent overheating. A standard metallographic procedure was employed on the axial cross sections, involving grinding and polishing, to prepare the surfaces for the experimental process and to produce a standardized smear layer (44).

Experimental Procedure

The irrigation solutions tested were PAA (AAA Wholesale, San Francisco, CA), 17% EDTA (Kerr, Orange, CA), 18% HEDP (Cublen K8514P, Zschimmer & Schwarz, Burgstadt, Germany) and QMix (Dentsply Tulsa Dental Specialties, Johnson City, TN). According to the manufacturer, the PAA solution contained 4.5% (wt/vol) peracetic acid, 3.5% acetic acid and 7.3% hydrogen peroxide. PAA solutions were prepared by diluting 4.5% PAA with bi-distilled water resulting in concentrations of 2%, 1%, 0.3%, 0.2% and 0.1% solutions of PAA. HEDP solution was prepared using HEDP powder mixed with bi-distilled water to wt/vol concentration of 18%. QMix is a pre-mixed solution containing chlorhexidine, EDTA and surfactant.

The dentin samples were randomly divided into the following 9 irrigation groups listed below. Each analysis area on the disc was exposed to 1mL of the test solutions for 60sec, except for HEDP which was exposed for 5min. After each experimental time, the demineralizing process was interrupted with 5mL of bi-distilled water.

Group 1:

3% NaOCl for 60sec followed by 0.1% PAA for 60sec

Group 2:

3% NaOCl for 60sec followed by 0.2% PAA for 60sec

Group 3:

3% NaOCl for 60sec followed by 0.3% PAA for 60sec

Group 4:

3% NaOCl for 60sec followed by 1% PAA for 60sec

Group 5:

3% NaOCl for 60sec followed by 2% PAA for 60sec

Group 6:

3% NaOCl for 60sec followed by 17% EDTA for 60sec

Group 7:

Mixture of 18% HEDP + 5.25% NaOCl for 5min

Group 8:

3% NaOCl for 60sec followed by 18% HEDP for5 min

Group 9:

3% NaOCl for 60sec followed by QMix for 60sec

Scanning Electron Microscopy and Scoring

Following the irrigation protocol, the dentin samples were dehydrated with alcohol, mounted on stubs, then sputter coated with gold and observed using a scanning electron microscope (Hitachi SU-70 FE-SEM; Hitachi Ltd, Tokyo, Japan). Nine operative fields were scanned per block, and SEM images were captured at 2000x magnification. Two calibrated evaluators examined and scored the images. Each image was scored according to the parameters below.

Score 1 - no smear layer, all dentinal tubules open;

Score 2 - small amount of smear layer, more than half of the dentinal tubules open;

Score 3 - homogenous smear layer covering the root canal wall, less than half of the dentinal tubules open;

Score 4 - complete root canal wall covered by a homogeneous smear layer, no open dentinal tubules.

Statistics

Agreement between two blinded reviewers was assessed using Kappa Statistic. For disagreements, the higher of the two scores was selected for analysis. Differences in smear layer

ratings were compared using nonparametric Kruskal-Wallis test. Post hoc pairwise comparisons were performed using Dwass, Steel, Critchlow-Fligner (DSCF) Method for multiple comparisons. Significance level was set at 0.05.

Results

Agreement between the two raters was excellent as defined by Cicchetti (45) (k=0.77; 95% CI: 0.64-0.90). There were 11 (14%) instances of disagreement for which Rater 1 scored the image as a "3" and Rater 2 scored as a "4." These cases were scored as a "4" for further analysis.

The image montages in Figure 1 show the degree of smear layer removal for the nine experimental groups. There was evidence of a significant difference in the median score based on the irrigant used (p-value<0.0001). Table 1 includes the median and range of values that were scored for the images in each of the 9 groups. Post hoc pairwise comparisons found that the scores were significantly lower for 17% EDTA at 60sec than all of the other test groups. HEDP alone at 5min and HEDP+NaOCl at 5min were not significantly different from each other (p-value>0.05) but were significantly lower compared to all tested concentrations of PAA at 60sec and QMix at 60sec. There was no significant difference between all concentrations of PAA at 60sec and Qmix at 60sec (p-value<0.05). Figure 2 displays all scores for each of the groups.

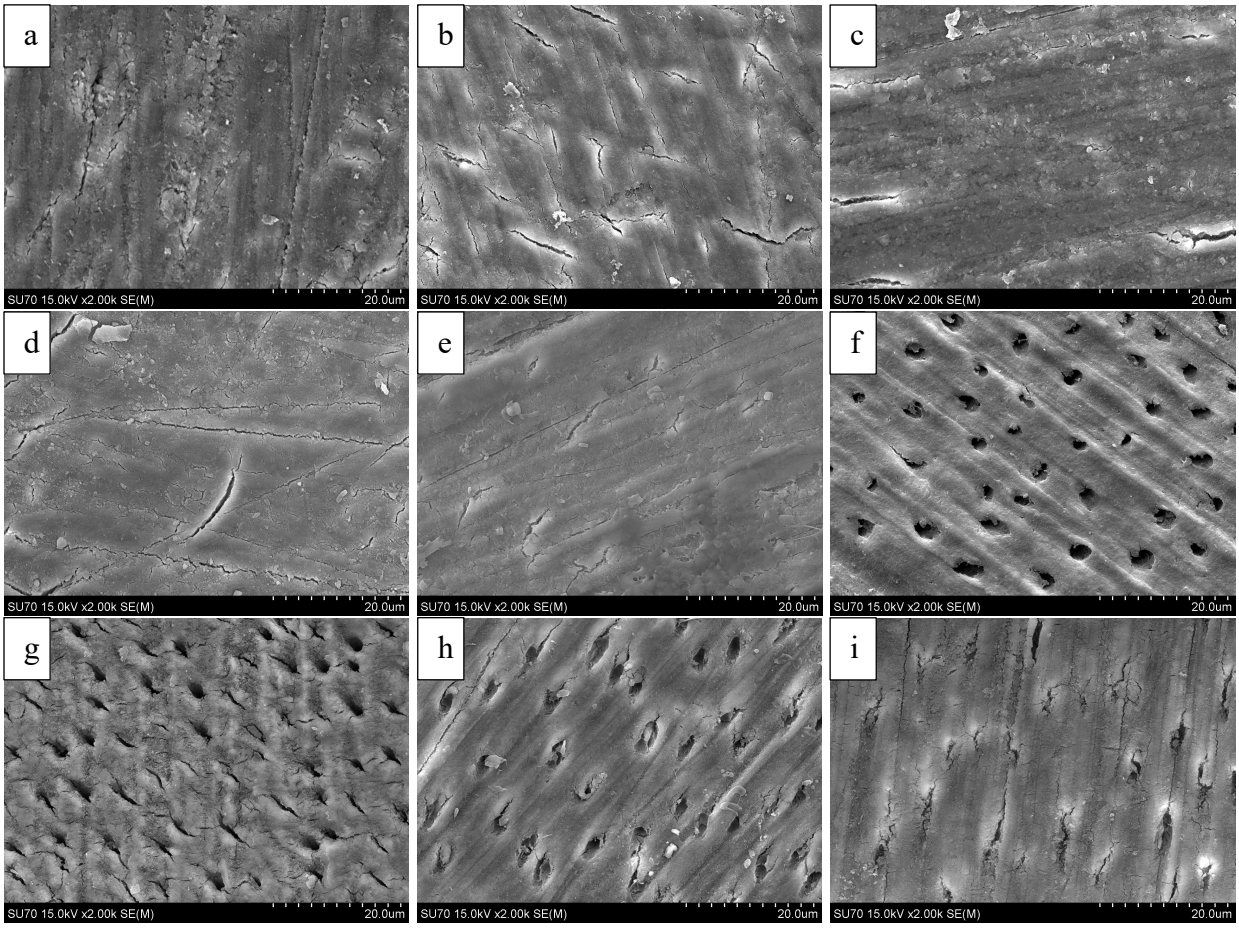

Figure 1. SEM smear layer evaluation after demineralization with each test solution. 2000x mag.

a) 0.1% PAA, b) 0.2% PAA, c) 0.3% PAA, d) 1% PAA, e) 2% PAA, f) 17% EDTA,
g) 18% HEDP+NaOCl, h) 18% HEDP, i) QMix

Table 1: Summary of Smear Layer Scores by Irrigant

Irrigant	n	Median	Minimum	Maximum	P-value*	
0.1% PAA	5	4	4	4	0.0367	a
0.2% PAA	9	4	4	4	0.0030	a
0.3% PAA	9	4	3	4	0.0050	a
1% PAA	9	4	4	4	0.0030	a
2% PAA	9	4	3	4	0.0050	a
17% EDTA	9	1	1	3	REF	b
HEDP	9	3	1	4	0.2123	a,b
HEDP+NaOCl	9	3	2	4	0.0550	a,b
QMix	9	3	3	4	0.0143	a

*P-value from Dwass, Steel, Critchlow-Fligner post hoc comparisons to 17% EDTA
Irrigants labeled with the same letter were not significantly different (last column)

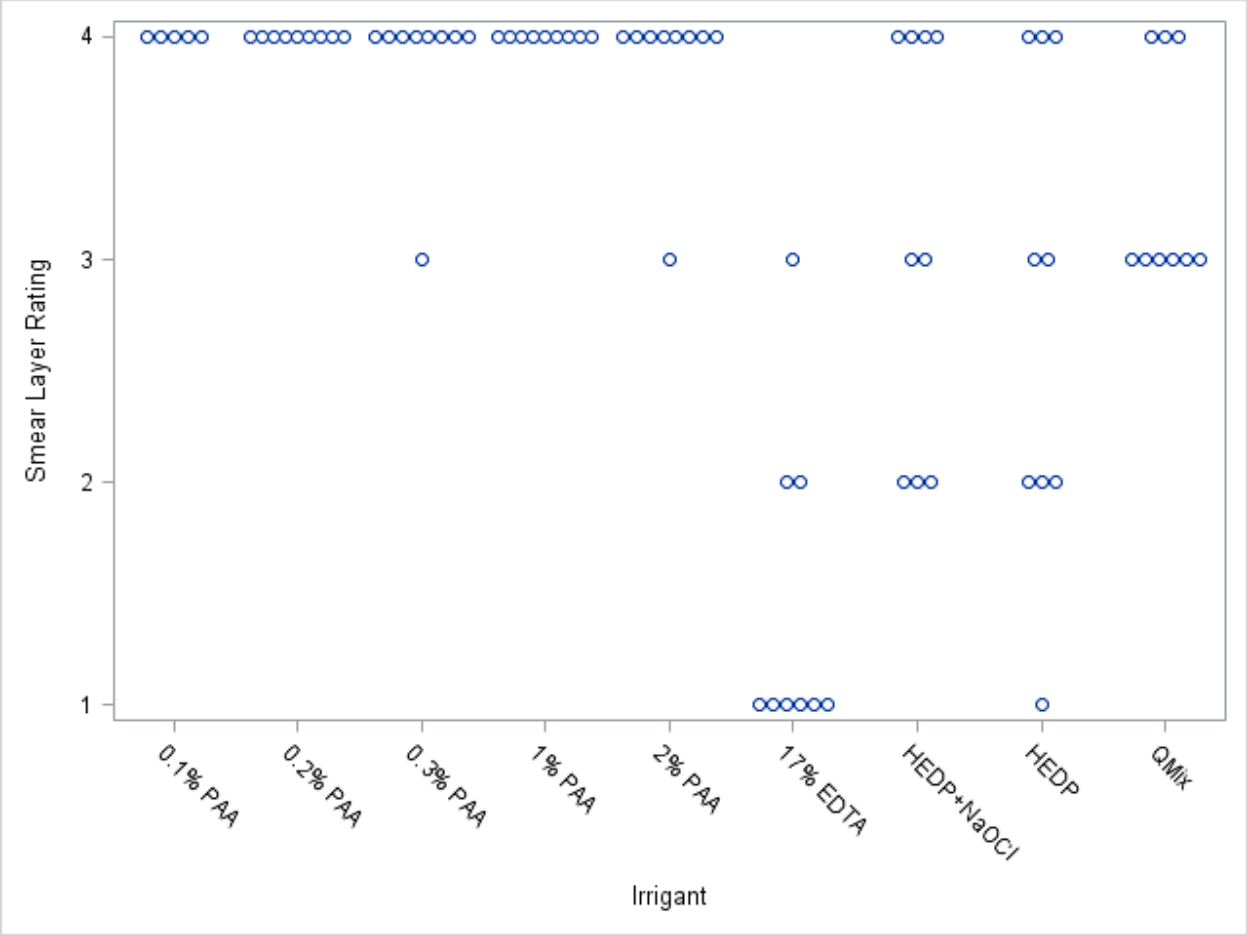

Figure 2: Plot of Image Scores by Irrigant

Discussion

This study aimed to evaluate smear layer dissolution using alternative chelating solutions when compared to EDTA. HEDP used alone or in combination with NaOCl and used for 5 min did exhibit moderate smear layer dissolution capability, which is consistent with previous studies (31,33). PAA used for 1 min, at 0.1-2% concentrations, and QMix used for 1 min were not effective in dissolution of the smear layer. This finding is in contrast to other studies, which indicated that the smear layer dissolution ability of 2.25% PAA, 0.5% PAA and QMix are comparable to EDTA (36,38,42). EDTA used for 1 min was the most effective in dissolution of the smear layer in comparison to all other solutions tested.

The use of a combined HEDP/NaOCl solution as a single irrigant is attractive because of its potential for concurrent disinfection and smear layer removal during the root canal instrumentation phase. Using EDTA for this purpose is not ideal. EDTA inactivates NaOCl when mixed together, can cause excessive erosion when used for longer periods of time, provides no antibacterial effect on its own, and has also been shown to cause greater canal deviation when used during root canal instrumentation (27,31,46). Unlike EDTA, HEDP can be mixed with NaOCl and maintain the antibacterial and organic tissue dissolution properties of NaOCl for at least 60 min (47–49). The results in this study do not support a complete replacement of EDTA with HEDP solutions as it took five times longer to produce an inferior result. However, there is still potential for use of HEDP as an adjunct during instrumentation followed with EDTA in the final irrigation step. In vitro studies have indicated that HEDP+NaOCl has the ability to reduce

hard tissue debris accumulation during instrumentation, and that its antimicrobial properties are not reduced in the presence of dentin debris and the smear layer (48–50). Perhaps HEDP mixtures would allow for use of lower NaOCl concentrations and still maintain strong tissue dissolution and antibacterial properties. Full strength NaOCl has been found to cause a greater decrease in dentin flexural strength in comparison to lower concentrations, which suggests NaOCl concentration may be a contributing factor in fracture of endodontically treated teeth (51). The effect of NaOCl+HEDP on dentin flexural strength warrants further study.

The use of a chelator with strong antimicrobial properties would be advantageous as a final irrigant to dissolve the smear layer and disinfect the root canal system. Previous studies have indicated that PAA and QMix exhibit antimicrobial properties (36,43). However, the results in this study indicate that 0.1%-2% PAA at 60sec and regular strength QMix at 60sec are not effective in dissolving the smear layer as a sole irrigant. NaOCl does have erosive effects on dentin when used as a final irrigant after EDTA (34,35). This has been shown to decrease the microhardness of the root canal dentin and possibly contribute to the risk of vertical root fracture in endodontically treated teeth (52). PAA and QMix could potentially be used as an alternative to NaOCl as a final irrigant for disinfection after smear layer dissolution with a strong chelator if they are less erosive to the dentin. However, further research would be necessary.

The vast research efforts on smear layer dissolution have been predominantly in vitro studies and are, unfortunately, difficult to compare due to the lack of standardization of methodology (53). A limitation of this study was that the method used to generate the smear layer did not replicate what occurs in a clinical situation. The flat surface of dentin discs was polished using a fine grit sanding paper to produce a standardized smear layer. However, this surface was the cross-section of the root dentin and not the wall of the root canal system, where

the ends of the dentinal tubules are generally oriented. Previous studies generated a smear layer through mechanical instrumentation of the root canal system using hand and rotary instruments, which more closely replicates the clinical situation. The dentin disc method was used in this study because conventional rotary instrumentation leaves 35% or more of the root canal surface areas unchanged and it has been shown that untouched areas generate little to no smear layer (8,54). SEM observation of areas where no smear layer was generated could have had an influence on the scores of the tested solutions.

The method of application for each test solution was another limitation in this study. Dentin discs were placed into small cups containing solution without any agitation. The flat surface of the dentin discs allowed for better control of surface contact with each solution by eliminating anatomical variability that may be present within a root canal system. In a clinical situation, irrigants are introduced into the canal via a needle syringe and this can even be agitated with adjunctive devices to enhance smear layer removal (55). In addition, there is variability between studies in the amount of exposure time to irrigating solutions and this is significant because it has been shown that smear layer dissolution can be affected by exposure time (29).

A point of improvement for future study would be to calibrate the 2 reviewers before having them review the images. There was no calibration initially and thus the initial agreement between the 2 reviewers was poor (data not shown). The agreement between the 2 reviewers improved to strong agreement after calibration ($k=0.77$; 95% CI: 0.64-0.90). One reviewer also did have some limited involvement in the imaging of the samples, and this may have introduced bias into that reviewer's scoring. To eliminate this risk of bias, a different reviewer without involvement in the study could have been used.

This study was performed under in vitro conditions so it should not be concluded that direct clinical application would produce the same results. Further research on the use of alternative chelating solutions is recommended. Future studies may examine the effect of varying solution contact times, the effect of added agitation, the effect on dentin microhardness and flexural strength, antimicrobial efficacy, toxicity to oral tissues, and potential interactions with other endodontic irrigants.

Conclusion

Within the limitations of this study, it can be concluded that EDTA at 60 sec was more effective at dissolving the smear layer than all other test solutions. HEDP at 5min and HEDP+NaOCl at 5 min were more effective than PAA at 60 sec (for all tested concentrations) and QMix for 60 sec. HEDP solutions have the potential to be an adjunct to EDTA.

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