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Name of Candidate: Grant R. Rutherford
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Glen M. Imamura
RESEARCH DEPARTMENT, NAVAL POSTGRADUATE DENTAL SCHOOL
Committee Chairperson

HINMAN.SUSAN.ELIZABETH.1261278240 Digitally signed by HINMAN.SUSAN.ELIZABETH.1261278240 Date: 2022.06.06 09:08:15 -04'00' 6/6/22

Susan E. Hinman
PROGRAM DIRECTOR, ENDODONTICS DEPARTMENT, NAVAL POSTGRADUATE DENTAL SCHOOL
Committee Member

WATSON.NORA.LYNN.1567954338 Digitally signed by WATSON.NORA.LYNN.1567954338 Date: 2022.06.05 14:20:02 -04'00' 6/5/22

Nora E. Watson
DEPARTMENT OF RESEARCH PROGRAMS, WALTER REED NATIONAL MILITARY MEDICAL CENTER
Committee Member

THE EFFECT OF SMEAR LAYER REMOVAL ON ENDODONTIC OUTCOMES

by

Grant Russell Rutherford
Commander, Dental Corps
United States Navy

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DEDICATION

To my amazing family - Lindsay, Hanna, Daphne & Will

DISCLAIMER

The views presented here are those of the author and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences, the Department of Defense or the U.S. Government.

ABSTRACT

The Effect of Smear Layer Removal on Endodontic Outcomes

Grant Russell Rutherford, DDS, 2022

Thesis directed by: CAPT Susan E Hinman
Director of Advanced Specialty Program in Endodontics
Endodontics Department
Naval Postgraduate Dental School

Introduction: During the instrumentation phases of endodontic treatment, a "smear layer" of organic and inorganic debris is created. To date, no *in vivo* studies have evaluated the effect of smear layer removal on the healing of periapical pathosis in permanent teeth. **Objectives**: The purpose of this double-blind randomized controlled clinic trial is to evaluate whether removal of the smear layer, via irrigation with ethylenediaminetetraacetic acid (EDTA), has an effect on endodontic outcomes, and to determine the effects, if any, of other covariables on healing outcomes. **Methods**: Consented human subjects were randomly assigned to one of two treatment groups; one group had the smear layer removed via irrigation with 17% ETDA while the other group had the smear layer left intact via irrigation with 0.9% saline. A clinical and radiographic follow-up examination was conducted no sooner than 12 months following treatment. Data from the treatment and follow-up exam were utilized to determine the endodontic outcome. **Results**: Interim analysis of 238 subjects using Fisher's exact test revealed a healed rate of 70% for saline irrigation and 64% using EDTA, for an overall healed rate of 66.8%, and no significant difference between the two irrigation protocols ($p=0.41$). Increased patient age ($p<0.05$), presence of a pre-operative sinus tract ($p=0.03$), pulpal necrosis ($p=0.01$), and pre-operative radiolucency ($p<0.001$) were all covariate factors associated with lower healed rates. **Conclusion**: Interim results of this study indicate no benefit to smear layer removal during initial endodontic treatment of permanent teeth. It was additionally determined that increased patient age is a significant factor, as well as the presence of pre-operative sinus tract and/or periapical radiolucency, and/or a diagnosis of pulpal necrosis, all of which were associated with lower rates of healing.

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LIST OF ABBREVIATONS

EDTA	ethylenediaminetetraacetic acid
mm	millimeters
NPDS	Naval Postgraduate Dental School
NSRCT	non-surgical root canal treatment
PAI	periapical index
SPSS	Statistical Package for the Social Sciences (IBM, inc.)

CHAPTER 1: Introduction

The goals of endodontic therapy include elimination of infection and inflammation, and the successful resolution of patients' signs and symptoms. Modern non-surgical root canal therapy incorporates numerous techniques and procedural steps to achieve these goals. Mechanical shaping, chemical disinfection, and quality obturation of the root canal system are the cornerstones of endodontic treatment that are universally agreed upon as requirements for healing and successful outcomes. Although current success rates for endodontic treatment range from 80% to 94% [1, 2] the endodontic community is constantly striving to further improve outcomes.

As in the cavity preparation step of operative dentistry, during the instrumentation phases of endodontic treatment, a "smear layer" is created. First described in root canals by McComb et al. in 1975, this smear layer becomes adherent to the dentin walls and consists of organic and inorganic debris containing dentin, necrotic and vital pulp tissues, and bacteria[3]. When restoring a cavity preparation with a bonded resin restoration, removal of the smear layer is a critical step for enabling micromechanical retention of the bonding agent by engaging the exposed collagen fibrils and dentinal tubules[4]. However, in endodontics, removal of the smear layer is somewhat controversial since, to date, there has been no demonstrable benefit of this additional step.

Ethylenediaminetetraacetic acid (EDTA) is one of the most common irrigants utilized for removal of the smear layer. A chelating agent, EDTA works by removing the inorganic component of the smear layer and may partially demineralize the underlying dentin surface thus exposing a portion of its organic matrix[5]. It was demonstrated to effectively remove the smear layer by McComb in 1975 in the same paper in which she described the smear layer in endodontics[3].

A 2021 survey conducted by Tsotsis et al. concluded that 93% of endodontists remove the smear layer[6], which is notably higher than the 77% identified by Dutner et al. in 2012[7], and nearly double the 51% identified by Moss et al. in 2001[8], demonstrating that the practice has increased over the last two decades and is now nearly universally embraced. Various authors have noted the supposed benefits of smear layer removal, citing that elimination of this debris layer opens the dentinal tubules, exposing the more deeply penetrated bacteria to chemical disinfection and enabling penetration and sealing of the tubules during obturation[9-13]. However, others argue that leaving the smear layer intact offers greater benefit than removal. Drake et al. demonstrated that leaving the smear layer intact resulted in fewer bacteria within dentin tubules[14]. Additionally, others have highlighted the dentinal erosion that occurs with the combined use of sodium hypochlorite and EDTA[15, 16], as well as reduced dentin microhardness[17].

Numerous *in vitro* studies have evaluated the efficacy of various techniques, instruments, and irrigants on removing the smear layer in endodontics. Additionally, the effect of smear layer removal on endodontic outcomes has been evaluated for primary teeth with varying results[18-20]. However, to date, no *in vivo* studies have been published regarding the effect of smear layer removal on the healing of periapical pathosis in permanent teeth. Therefore, the purpose of this double-blind randomized controlled clinic trial is twofold: 1) to evaluate whether removal of the smear layer, via irrigation with ethylenediaminetetraacetic acid (EDTA), has an effect on endodontic outcomes, and 2) to determine the effects, if any, of other covariables on healing outcomes.

CHAPTER 2: Materials and Methods

Materials and Methods adapted from Fleming, A. 2020 USUHS Thesis Manuscript

The Institutional Review Board (IRB) at the Walter Reed National Military Medical Center (WRNMMC), Bethesda, Maryland, approved this study, WRNMMC IRB #352491, “The Effect of Smear Layer Removal on Endodontic Outcomes.” WRNMMC also provided funding. Since the inception of this study, the Material and Methods section of this manuscript has been modified from the initial approved protocol.

The Endodontics Department at the Naval Postgraduate Dental School (NPDS) serves all active duty military, their dependents, retired military, as well as foreign military members and other categories of eligible beneficiaries. Inclusion criteria for patients to be enrolled were as follows: patients must be 18 years or older, in good health (American Society of Anesthesiology health status classification I or II), require initial, one-appointment non-surgical root canal therapy, and must agree to return for a 1-year follow-up examination.

Patients with a history of periodontal disease, previously initiated or previously treated root canal therapy, active antibiotic use or presenting with an acute apical abscess, were ineligible to participate in this study. Those patients who were allergic to any medication or dental material used in the study, including latex or gutta percha, and subjects who reported being pregnant, were also excluded from participation.

Once consented and enrolled, subjects were randomly assigned to one of two treatment groups. Orthograde and angled pre-operative periapical radiographs were exposed. Medical conditions, clinical symptoms, diagnostic and treatment data (see Table 1) were collected using standardized data collection forms.

All treatment was provided by NPDS endodontic residents using dental operating microscopes and overseen by an Endodontic Department faculty member. With the exception of the irrigation variable, either 17% EDTA or 0.9% sterile saline, a standardized treatment protocol was utilized regardless of group assignment. Subjects were anesthetized and the tooth to be treated was isolated with rubber dam and Oraseal® caulking adhesive (Ultradent Products, South Jordan, UT). Straight-line access was established using #2 round or #557 carbide burs (Henry Schein, Melville, NY) and EndoZ burs (Dentsply Sirona, Tulsa, OK). Coronal flaring was created using #2, #3, and #4 Gates Glidden drills (SybronEndo Corporation, Orange, CA). Canal working lengths were established using a Root ZX® (J Morita, Irvine, CA) and confirmed radiographically. A glide path was created using 0.02 taper #10, #15, #20 FlexoFile® (Dentsply Sirona, Tulsa, OK) stainless steel files to working length. The canals were cleaned and shaped with 0.04 Vortex Blue™ (Dentsply Sirona, Tulsa, OK) rotary files using a crown down technique to at least a master apical file size #35 with .04 taper. Recapitulation was performed with 0.02 taper #10 Flex-O-Files to working length and irrigated with 8.25% NaOCl, delivered from a 30-gauge side vented irrigation tip between all file sizes for a total intraoperative irrigation volume not exceeding 2 ml. The canals were dried with sterile paper points (Henry Schein, Melville, NY).

To blind providers to the final irrigation solutions, each was given a syringe containing either 17% EDTA or 0.9% saline, labeled “irrigant A” or “irrigant B.” The clinician delivered 1 ml of the test irrigant 1 mm short of working length over 1 minute per canal, after which identical treatment for all subjects resumed.

A final rinse of 3 ml of 8.25% NaOCl per canal was performed and the canals were dried with sterile paper points (Henry Schein, Melville, NY). A System B® (Kerr Corporation, Brea, CA) plugger that bound within the canal 5-7 mm short of working length was selected. Working length was confirmed using a 0.04 taper master gutta percha cone (Diadent, Burnaby, BC, Canada). Roth 801 sealer (Roth International LTD, Chicago, IL) was delivered into the canal and the walls coated. The master cone was seated to working length and the canal was obturated with gutta percha using a continuous wave technique. The canal was backfilled using a thermoplasticized backfill technique. Alcohol-soaked cotton pellets were used to clean the chamber prior to temporizing the access with a sterile cotton pellet and Fuji Triage® (GC America Inc., Alsip, IL) or Cavit™ Temporary Filling Material (3M ESPE Dental, St Paul, MN). A post-operative radiograph was exposed using an XCP® (Dentsply Rinn, York, PA) device with Blu-Mousse® (Parkell Inc., Edgewood, NY) bite registration material in order to reproduce the vertical and horizontal angles of the original radiograph at the one-year follow-up appointment. Subjects were instructed to return to their referring dentist for the permanent restoration.

A follow-up examination was conducted no sooner than 12 months following completion of treatment. Providers reviewed health histories and recorded clinical data including results from diagnostic testing on standardized follow-up data collection forms. A follow-up periapical radiograph was taken using the positioning device previously created at the treatment appointment.

All radiographs were assessed using a modified PAI scoring technique, described by Ørstavik[21]. PAI scoring was conducted by three calibrated, board-certified endodontists. Coronal restorations of the immediate post-operative radiograph and the 1-year follow-up radiograph were masked to eliminate reviewer bias. Radiographs were coded, randomized, and individually projected onto a screen in a dark room. Radiographs were scored individually; when there was disagreement, a forced consensus was initiated. A PAI score of 1 or 2 was considered healed while a PAI score of 3, 4 or 5 was considered non-healed. All data were entered into SPSS Statistics (IBM, Armonk, NY). R software was used for statistical analysis (Core Team 2021).

Separate pulpal and apical diagnoses were made based on diagnostic testing conducted during the follow-up exam.

Outcome assessment. Data from the treatment and follow-up exam were utilized to determine the endodontic outcome. Subjects classified as "healed" were asymptomatic with an absence of a radiographic lesion at the time of follow-up, while "non-healed" subjects were symptomatic with or without a radiographic lesion.

Sample size was established for the desired precision for estimation of an anticipated 80% healed rate at 12-months, assuming a 40% projected drop-out rate. In order to assess the true healed rates to within 5 percentage points, a power analysis recommended a total 440 subjects, with 220 per test group. Chi-square and Fisher's exact tests ($\alpha < 0.05$) were used to perform all statistical comparisons of patient, treatment and clinical characteristics by healed status at 12-months follow up.

CHAPTER 3: Results

For this interim analysis, 362 total subjects were enrolled, 342 subjects were eligible for follow-up and 279 subjects were recalled, for an overall recall rate of 83%. Data from 40 subjects was excluded due to either the enrolled tooth having been extracted or due to a protocol deviation (most commonly multiple appointments required to complete treatment). Overall, data from 238 subjects was analyzed.

The healed rate at 12 months follow up was 70% for cases irrigated with 0.9% saline, and 64% for cases irrigated with EDTA (Figure 1), for an overall healed rate of 66.8% and no significant difference between the two protocols (Chi-square $p=0.41$).

Additional analysis of covariables using Chi-square or Fisher's exact tests (see Table 2) revealed four factors that were significantly associated with healing: patient age, pulpal status, presence of pre-operative radiolucency, and presence of pre-operative sinus tract. As seen in Figure 2, teeth with pulpal necrosis had a significantly lower healed rate of 57% versus teeth with vital pulps at 75% ($p=0.01$). Figure 3 demonstrates that teeth with a pre-operative radiolucency had a significantly lower healed rate of 54% compared to teeth without pre-operative radiolucency at 79% ($p<0.001$). Furthermore, 68% of teeth without a pre-operative sinus tract healed, whereas only 38% of teeth with a pre-operative sinus tract healed ($p=0.03$). Lastly, healing rates tended to be higher among younger versus older age groups ($p<0.05$). Additionally, while not statistically significant, a trend toward poorer outcomes was noted for patients with diabetes ($p<0.09$) and/or hypertension ($p<0.08$).

Table 1. List of all covariables considered.

Gender	Pre-op/Post-op swelling	Presence of pre-op radiolucency
Age	History of ortho treatment	Pre-op pulpal diagnosis
Tooth position	History of external resorption	Pre-op apical diagnosis
Tooth type	History of bleaching	Patency
Pre-op/Post-op diabetes	History of internal resorption	Procedural complications
Pre-op/Post-op hypertension	Pre-op/Post-op caries	Intra-orifice barrier
Pre-op/Post-op smoker	Pre-op/Post-op cold sensitivity	Obturation fill length
Pre-op/Post-op coronary heart disease	Pre-op/Post-op mobility	Post-treatment apical diagnosis
Pre-op/Post-op pain	Pre-op/Post-op bleeding on probing	Post-treatment pulpal diagnosis
Pre-op/Post-op EPT response	Pre-op/Post-op restoration	Time elapsed between initial treatment and permanent restoration
Pre-op/Post-op palpation	Pre-op/Post-op probing depths	Presence of post-op intracanal post
Pre-op/Post-op percussion	Pre-op/Post-op open margin	Follow-up apical diagnosis
Pre-op/Post-op sinus tract	Pre-op/Post-op lamina dura	

Table 2. List of specific covariables further analyzed for significance. Data from the 17% EDTA and 0.9% saline groups were pooled to assess the influence of covariate factors on healing. p-values obtained from Chi-square or Fisher's exact tests.

Covariate Factors	Healed (N=159)	Non-healed (N=79)	p-value
PATIENT AGE:			0.05
18-35 Years Old	51 (78.5%)	14 (21.5%)	
36-65 Years Old	90 (63.8%)	51 (36.2%)	
>65 Years Old	18 (56.2%)	14 (43.8%)	
GENDER:			0.31
Male	120 (69.0%)	54 (31.0%)	
Female	39 (60.9%)	25 (39.1%)	
TOOTH TYPE:			0.22
Single root	71 (71.7%)	28 (28.3%)	
Multiple root	88 (63.3%)	51 (36.7%)	
HYPERTENSION:			0.11
No	127 (69.8%)	55 (30.2%)	
Yes	32 (57.1%)	24 (42.9%)	
DIABETES:			0.09
No	149 (68.7%)	68 (31.3%)	
Yes	10 (47.6%)	11 (52.4%)	
PRE-OP SINUS TRACT:			0.03
No	154 (68.4%)	71 (31.6%)	
Yes	5 (38.5%)	8 (61.5%)	
PRE-OP COLD SENSITIVITY:			0.01
NR	64 (57.1%)	48 (42.9%)	
R/NL	44 (73.3%)	16 (26.7%)	
R/L	51 (77.3%)	15 (22.7%)	
PRE-OP RADIOLUCENCY:			<0.001
No	95 (79.2%)	25 (20.8%)	
Yes	64 (54.2%)	54 (45.8%)	
***TEST IRRIGANT:**			0.41
0.9% Saline	81 (69.8%)	35 (30.2%)	
17% EDTA	78 (63.9%)	44 (36.1%)	
OVEREXTENSION OF ROOT FILLING:			0.05
No	156 (68.1%)	73 (31.9%)	
Yes	3 (37.5%)	5 (62.5%)	

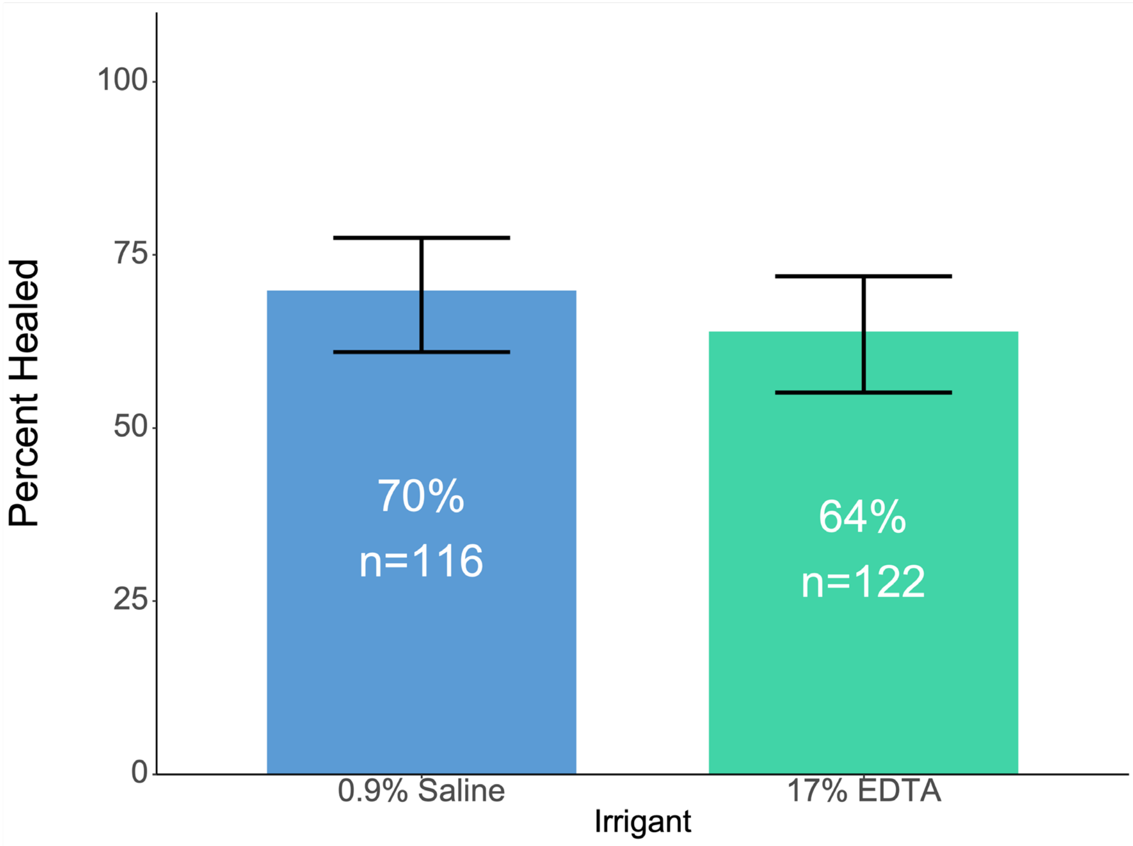

Figure 1. Comparison of the Irrigation Protocols on Healed Rates. Bar graph showing the number of subjects, the healed rate, and 95% confidence intervals for the two irrigation protocols. The protocol using 0.9% saline did not remove the smear layer, while the protocol using 17% EDTA did remove the smear layer. The healed rate did not statistically differ between the two groups (Chi-square $p = 0.41$).

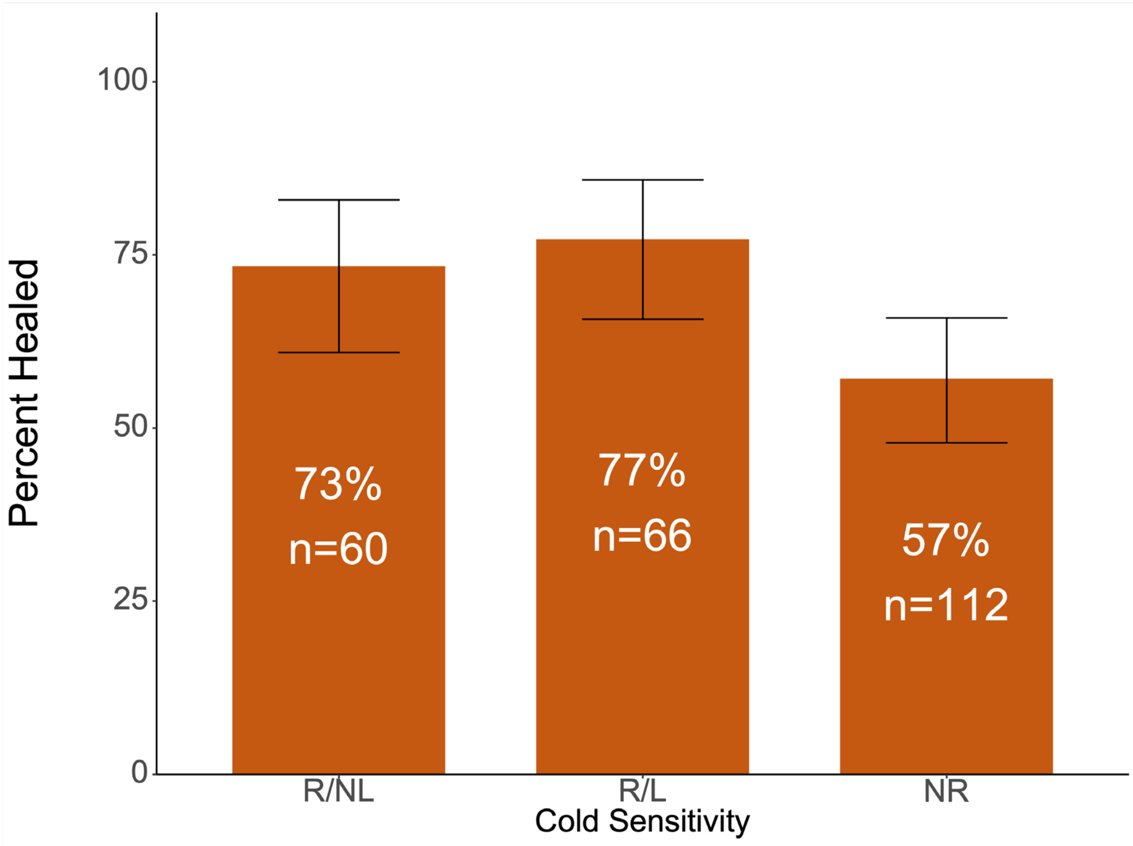

Figure 2. Comparison of Pre-Operative Response to Cold on Healed Rates. Bar graph showing the number of subjects, the healed rate, and 95% confidence intervals of subjects with a pre-operative response to cold. Teeth with a non-lingering response (R/NL) or lingering response (R/L), were considered vital. Teeth with no response to cold (NR), were considered necrotic. Fisher's Exact Test revealed a significant difference between pooled healed rates of vital teeth compared to necrotic teeth ($p = 0.01$).

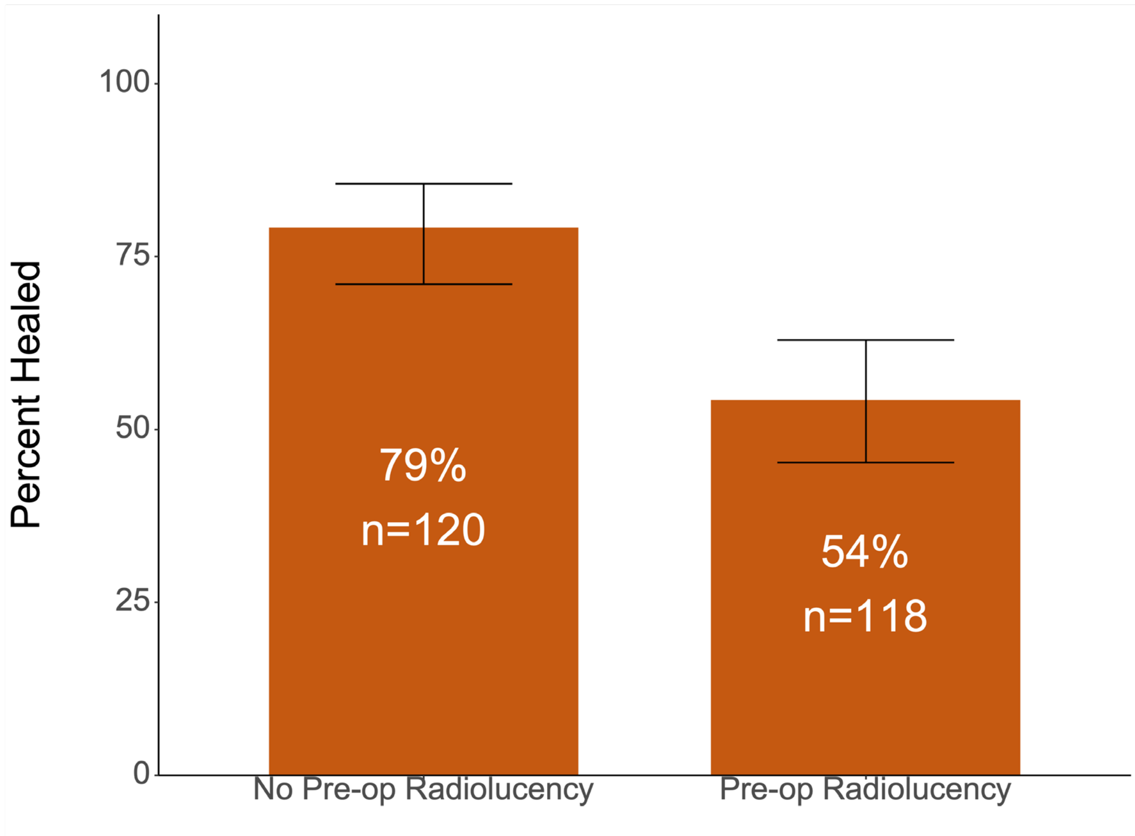

Figure 3. Comparison of Pre-Operative Radiolucency on Healed Rates. Bar graph showing the number of subjects, the healed rate, and 95% confidence intervals of teeth presenting with and without a pre-operative radiolucency. Fisher's Exact Test revealed a significant difference in healed rates between teeth with no pre-operative radiolucency when compared to teeth presenting with a pre-operative radiolucency ($p < 0.001$).

CHAPTER 4: Discussion

The signs and symptoms of endodontic disease are the direct result of microbial infection of the root canal system[22]. Endodontic infections are often complex in nature and therefore can be difficult to eradicate. Complex anatomy, namely canal curvatures, accessory canals and foramina, fins, and isthmuses, make it impossible to completely instrument and disinfect all aspects of a root canal system. Additionally, the structure of the pulp-dentin complex, namely the dentin tubules, results in natural micro-porosities that can harbor debris and microorganisms. Dentin tubules vary in density and amount of branching and interconnectivity throughout the length of the tooth[23]. Furthermore, endodontic infections consist of numerous species of bacteria[24, 25] and possibly fungi and viruses as well[26, 27]. These microbes create intricate communities called "biofilms" which have physical and chemical protective factors for their members that contribute to their tenacity and antimicrobial resistance[25]. These polymicrobial biofilms have implications for root canal therapy as well as for regenerative endodontic procedures[24, 28]. Failure to eliminate or significantly reduce the quantity of pathogenic microorganisms may result in unsuccessful outcomes.

McComb et al. first described the smear layer in endodontics in 1975, as an adherent layer of debris on dentin walls consisting of organic and inorganic debris containing dentin, necrotic and vital pulp tissues, and bacteria[3]. Others have further elaborated on the smear layer's complexity, citing its approximate thickness of 1-5µm, describing the smear "plugs" that are created when this debris is packed into the dentin tubules via mechanical means and/or capillary-action and reach depths of 40-110µm, and emphasizing its tenacity and difficulty of removal[29-31]. Different instrumentation techniques may produce varying qualities and quantities of smear layer[9]. Interestingly, a smear layer may even be formed when only activated irrigation is used without mechanical instrumentation[15, 32].

There are numerous materials that have been investigated for their ability to remove the smear layer in endodontics. Sterile saline, citric acid, phytic acid, etidronic acid, peracetic acid, acetic acid, malic acid, apple vinegar, sodium citrate, sodium hypochlorite, chlorhexidine, MTAD (mixture of tetracycline, an acid, and a detergent), EDTA (ethylenediaminetetraacetic acid), Q-mix (chlorhexidine in combination with EDTA and a detergent), and a plethora of other proposed irrigants have all been studied in attempt to disinfect and/or remove the smear layer[33-36].

Although the efficacy of EDTA in removing the smear layer was identified early on, it took years of research to determine the safest and most effective concentration, volume, and contact time adequate for smear layer removal without detrimental effects on the dentin. The current protocol utilized by most practitioners for removal of the smear layer involves using a 17% EDTA concentration at a volume of 1 milliliter (mL) per canal delivered over the course of 1 minute, followed by a final rinse with sodium hypochlorite. This was the protocol followed for this research study. In 1983, Yamada et al. demonstrated the safety and efficacy of the 17% EDTA concentration[37]. Calt et al. established in 2002 that 1 minute contact time of 17% EDTA is effective in removing the smear layer with minimal negative effect on the peritubular and intertubular dentin[38]. Lastly, in 2005, Crumpton et al. showed that 1 mL of 17% EDTA was a sufficiently effective volume and comparable to 10 mL[39]. In addition to removing the smear layer, EDTA has also been shown to have antifungal activity[26], the ability to dissolve some root canal sealers during endodontic retreatment[40], and to promote angioneogenesis due to its effect on dentin and the resultant release of growth factors[41].

The literature has repeatedly and conclusively demonstrated the efficacy of sodium hypochlorite in disinfecting root canal systems via dissolution of organic tissue, including vital tissue[42], necrotic tissue[43], and elimination of bacterial biofilms[25]. However, the efficacy of sodium hypochlorite is dependent upon its ability to directly contact the microorganisms and/or organic tissue targeted for removal. The smear layer may pose a barrier to allowing adequate penetration and contact of sodium hypochlorite to bacteria embedded within the smear layer itself and/or in the occluded dentin tubules[44]. The combination of sodium hypochlorite and EDTA irrigation has been shown to be more effective in reducing bacteria than sodium hypochlorite alone[10, 45]. Similarly, a final rinse with sodium hypochlorite after smear layer removal with EDTA has been shown to successfully remove the smear layer, superficial debris and biofilm, and intratubular bacteria by numerous studies5[, 37, 46, 47]. These results seem logical given that sodium hypochlorite dissolves organic tissue while EDTA removes inorganic tissue, both of which are present in the instrumented root canal[48]. Notably, Ng et al. identified the use of EDTA followed by a final rinse with sodium hypochlorite as a factor contributing to improved prognoses for endodontic retreatment, but not for initial treatment[1].

Typically, irrigants are introduced into the root canal system via an irrigation syringe and gentle manual pressure. However, additional techniques and adjunctive equipment have been introduced over the years in attempt to increase the efficacy of various irrigants. Notably the use of sonic or ultrasonic activation of EDTA has been shown to enhance debris removal[49-53]. Additionally, the use of lasers, alone or in conjunction with irrigant(s) has also been explored[54-56]. None of the techniques studied to date are universally accepted since none have shown complete efficacy at removal of the smear layer throughout the complete length of all canals[57], with the apical portion of canals being the most difficult to clean.

Various authors have cited the potential benefits of smear layer removal, stating that opening of the dentinal tubules allows access to the more deeply penetrated bacteria by chemical disinfectants, as well as enables penetration and sealing of the tubules during canal obturation[9-12]. In addition to use during routine endodontic treatment in hopes of enhancing disinfection, removal of the smear layer has also been demonstrated to enhance diffusion of calcium ions through dentinal tubules for treatment of resorption with calcium hydroxide intracanal medicament[58]. Furthermore, removal of the smear layer has been demonstrated to reduce leakage and enhance the apical seal[17, 59-61].

In contrast, other authors have stated that leaving the smear layer intact is in fact more beneficial than removal. A study by Drake et al. found that leaving the smear layer intact actually resulted in fewer bacteria within dentin tubules[14]. Additionally, others have highlighted the potentially detrimental erosion of dentin that occurs with the combined use of EDTA and sodium hypochlorite[15, 16], as well as potential reduced dentin microhardness[62]. It has also been argued that leaving the smear layer intact may actually result in less apical microleakage[63]. Moreover, adding an additional procedural step that requires extra treatment time, supplies, and expense without significant benefit yet demonstrated, seems unnecessary to some clinicians.

Despite the nearly universal practice of smear layer removal by endodontists, no *in vivo* clinical study has been completed demonstrating the benefit of this additional step. This is the first double-blind randomized controlled clinical trial assessing the effect of smear layer removal on endodontic outcomes. The findings of this interim analysis show there may not be any benefit to removing the smear layer using a 17% EDTA and 8.25% NaOCl irrigation protocol.

Four factors were identified as associated with reduced healing rates: teeth with pulpal necrosis, presence of pre-operative apical radiolucency and/or sinus tract, and increased patient age. Similar findings were previously demonstrated in research by Sjögren et al. who found that pulp status was the best predictor of success after endodontic treatment and that teeth with necrotic pulps and periapical lesions had significantly lower success rates than teeth with vital pulps[64]. Additionally, the presence of pre-operative radiolucency was demonstrated to be a prognostic factor for reduced healing by Ng et al.[1] as well as Chugal et al.[65].

Interestingly, our study found that healing rates tended to be higher among younger versus older age groups ($p<0.05$). This is in contrast to the findings of previous studies which concluded that patient age was not a significant factor when evaluating outcomes[66, 67], with the exception of Imura et al. who determined age was a significant factor for healing of non-surgical retreatment cases[2].

Furthermore, our finding of lower healed rates in diabetic patients and in patients with hypertension approached significance and is consistent with the findings of previous research. Saleh et al. demonstrated that diabetic patients are nearly three-times more likely to exhibit periapical lesions than nondiabetic patients[68]. Additionally, An et al. as well as Segura-Egea et al. both found higher a prevalence of apical lesions in patients with hypertension, although their results were also not statistically significant [69, 70].

There are several limitations to this study. The sample size of 238 subjects at time of this interim analysis must be taken into consideration since initial power analysis determined a sample size of 440 to be adequate for determining significance. Furthermore, our study utilized a single follow-up period starting at one-year post-treatment. Multiple studies have shown that while a majority of healed cases will heal by one year with certain cases taking up to four years or longer to demonstrate complete healing[71]. Lastly, and possibly most significantly for outcomes assessment is the fact that our study used a strict assessment, allowing only for "healed" or "non-healed" categorization with no "healing" category. Using the periapical index (PAI) scoring system, three board-certified endodontic faculty assessed all radiographs. Twenty-six follow-up radiographs were given a PAI score of "3" which is indicative of an uncertain periapical status, but for the purposes of our study these cases were categorized as "non-healed". Additionally, per the PAI protocol, only a single immediate post-operative radiograph and single follow-up radiograph were eligible for assessment. We know from research by Brynolf et al. that viewing multiple radiographs from different angles increases diagnostic accuracy, especially for multi-rooted teeth[72]. This combination of factors may have resulted in certain healed cases being categorized as non-healed.

There are several reasons possible reasons to explain our finding of no difference in irrigation protocols. Clegg et al. demonstrated that 6% sodium hypochlorite is effective in eradicating bacteria and biofilms[25]. Several years later, Cullen et al. confirmed that 8.25% sodium hypochlorite was even more effective at quickly dissolving pulpal tissue while still being safe to dentin[73]. Ferrer-Luque et al. found that rotary instrumentation alone significantly reduces bacterial load independent of either distilled water or sodium hypochlorite irrigation[74]. Therefore, the use of both 8.25% sodium hypochlorite and rotary instrumentation in all cases in the current study may overshadow any effect resulting from the removal of the smear layer itself. Another reason smear layer removal may not affect outcomes relates to the finding of Zhao et al. that 35-56% of the canal surfaces may remain untouched during mechanical instrumentation, therefore, a smear layer would not be created on these surfaces[75]. These conclusions lend support to the current study's findings that removing the smear layer may not be significant.

CHAPTER 5: Conclusions

Interim results of this prospective double-blind randomized controlled clinical trial indicate no benefit to smear layer removal during initial endodontic treatment of permanent teeth. Therefore, the null hypothesis cannot be rejected at this time. Additionally, it was noted that increased patient age, necrotic teeth, and teeth with a pre-operative radiolucency and/or sinus tract, all negative impacted healed rates.

REFERENCES

1. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int Endod J 2011;44(7):583-609.
2. Imura N, Pinheiro ET, Gomes BP, et al. The outcome of endodontic treatment: a retrospective study of 2000 cases performed by a specialist. J Endod 2007;33(11):1278-82.
3. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod 1975;1(7):238-42.
4. Wieczowski G, Yu XY, Davis EL, Joynt RB. Microleakage in Various Dentin Bonding Agent/Composite Resin Systems. Operative Dent 1992(5):62-67.
5. Baumgartner JC, Mader CL. A Scanning Electron Microscope Evaluation of Four Root Canal Irrigation Regimens. J Endod 1987;13(4):147-57.
6. Tsotsis P, Dunlap C, Scott R, Arias A, Peters OA. A survey of current trends in root canal treatment: access cavity design and cleaning and shaping practices. Aust Endod J 2021;47(1):27-33.
7. Dutner J, Mines P, Anderson A. Irrigation trends among American Association of Endodontists members: a web-based survey. J Endod 2012;38(1):37-40.
8. Moss HD, Allemang JD, Johnson JD. Philosophies and practices regarding the management of the endodontic smear layer: results from two surveys. J Endod 2001;27(8):537-9.
9. Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. Dent Clin North Am 1990;34(1):13-25.
10. Soares JA, Roque de Carvalho MA, Cunha Santos SM, et al. Effectiveness of chemomechanical preparation with alternating use of sodium hypochlorite and EDTA in eliminating intracanal Enterococcus faecalis biofilm. J Endod 2010;36(5):894-8.
11. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Dent Traumatol 1990;6:142-49.
12. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94(6):658-66.
13. Zancan RF, Di Maio A, Tomson PL, Duarte MAH, Camilleri J. The presence of smear layer affects the antimicrobial action of root canal sealers. Int Endod J 2021;54(8):1369-82.
14. Drake DR, Wiemann AH, Rivera EM, Walton RE. Bacterial Retention in Canal Walls In Vitro: Effect of Smear Layer. J Endod 1994;20(2):78-82.
15. Simezo AP, da Silveira Bueno CE, Cunha RS, et al. Comparative Analysis of Dentinal Erosion after Passive Ultrasonic Irrigation versus Irrigation with Reciprocating Activation: An Environmental Scanning Electron Study. J Endod 2017;43(1):141-46.
16. Qian W, Shen Y, Haapasalo M. Quantitative analysis of the effect of irrigant solution sequences on dentin erosion. J Endod 2011;37(10):1437-41.

17. Likhitkar MS, Kulkarni SV, Burande A, et al. To evaluate the influence of smear layer with different instruments and obturation methods on microleakage of root canal filled teeth: In vitro study. J Int Soc Prev Community Dent 2016;6(3):240-4.
18. Pintor AVB, dos Santos MRM, Ferreira DM, et al. Does Smear Layer Removal Influence Root Canal Therapy Outcome? A Systematic Review. J Clin Pediatr Dent 2016;40(1):1-7.
19. Tannure PN, Azevedo CP, Barcelos R, Gleiser R, Primo LG. Long-term Outcomes of Primary Tooth Pulpectomy With and Without Smear Layer Removal: A Randomized Split-mouth Clinical Trial. Pediatr Dent 2011;33:316-20.
20. Barcelos R, Tannure PN, Gleiser R, Luiz RR, Primo LG. The influence of smear layer removal on primary tooth pulpectomy outcome: a 24-month, double-blind, randomized, and controlled clinical trial evaluation. Int J Paediatr Dent 2012;22(5):369-81.
21. Orstavik D, Kerekes K, Eriksen HM. The periapical index: A scoring system for radiographic assessment of apical periodontitis. Endod Dent Traumatol 1986;2:20-34.
22. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20(3):340-49.
23. Mjor IA, Nordahl I. The density and branching of dentinal tubules in human teeth. Arch Oral Biol 1996;41(5):401-12.
24. Cameron R, Claudia E, Ping W, Erin S, Ruparel NB. Effect of a Residual Biofilm on Release of Transforming Growth Factor beta1 from Dentin. J Endod 2019;45(9):1119-25.
25. Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod 2006;32(5):434-7.
26. Sen BH, Akdeniz BG, Denizci AA. The effect of ethylenediamine-tetraacetic acid on Candida albicans. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90(5):651-5.
27. Sabeti M, Slots J. Herpesviral-bacterial coinfection in periapical pathosis. J Endod 2004;30(2):69-72.
28. Aksel H, Albanyan H, Bosaid F, Azim AA. Dentin Conditioning Protocol for Regenerative Endodontic Procedures. J Endod 2020;46(8):1099-104.
29. Pashley DH. Smear layer: overview of structure and function. Proc Finn Dent Soc 1992;88:215-24.
30. Aktener BO, Cengiz T, Piskin B. The Penetration of Smear Material into Dentinal Tubules during Instrumentation with Surface-Active Reagents: A Scanning Electron Microscopic Study. J Endod 1989;15(12):588-90.
31. Mader CL, Baumgartner JC, Peters DD. Scanning Electon Microscopic Investigation of the Smeared Layer on Root Canal Walls. J Endod 1984;10(10):477-83.
32. Kanaan CG, Pelegrine RA, da Silveira Bueno CE, et al. Can Irrigant Agitation Lead to the Formation of a Smear Layer? J Endod 2020;46(8):1120-24.
33. Jagzap JB, Patil SS, Gade VJ, et al. Effectiveness of Three Different Irrigants - 17% Ethylenediaminetetraacetic Acid, Q-MIX, and Phytic Acid in Smear Layer

Removal: A Comparative Scanning Electron Microscope Study. Contemp Clin Dent 2017;8(3):459-63.
34. Karunakaran JV, Kumar SS, Kumar M, Chandrasekhar S, Namitha D. The effects of various irrigating solutions on intra-radicular dentinal surface: An SEM analysis. J Pharm Bioallied Sci 2012;4(Suppl 2):S125-30.
35. Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. Int Endod J 2009;42(4):335-43.
36. Spano JC, Silva RG, Guedes DF, et al. Atomic absorption spectrometry and scanning electron microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators. J Endod 2009;35(5):727-30.
37. Yamada RS, Armas A, Goldman M, Sun Lin P. A Scanning Electron Microscopic Comparison of a High Volume Final Flush with Several Irrigating Solutions: Part 3. J Endod 1983;9(4):137-42.
38. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod 2002;28(1):17-9.
39. Crumpton BJ, Goodell GG, McClanahan SB. Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. J Endod 2005;31(7):536-8.
40. Keles A, Koseoglu M. Dissolution of root canal sealers in EDTA and NaOCl solutions. J Am Dent Assoc 2009;140:74-79.
41. Saghiri MA, Asatourian A, Morgano SM, Wang S, Sheibani N. Moderately Acidic pH Promotes Angiogenesis: An In Vitro and In Vivo Study. J Endod 2020;46(8):1113-19.
42. Rosenfeld EF, James GA, Burch BS, Lincoln N. Vital Pulp Tissue Response to Sodium Hypochlorite. J Endod 1978;4(5):140-46.
43. Hand RE, Smith ML, Harrison JW. Analysis of the Effect of Dilution on the Necrotic Tissue Dissolution Property of Sodium Hypochlorite. J Endod 1978;4(2):60-64.
44. Yang S, Bae K. Scanning Electron Microscopy Study of the Adhesion of Prevotella nigrescens to the Dentin of Prepared Root Canals. J Endod 2002;28(6):433-37.
45. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.
46. Goldman M, Goldman LB, Cavaleri R, Bogis J, Sun Lin P. The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study: part 2. J Endod 1982;8(11):487-92.
47. Guerreiro MYR, Belladonna FG, Monteiro LPB, et al. The influence of the addition of surfactants to sodium hypochlorite on the removal of hard tissue debris. Int Endod J 2020;53(8):1131-39.
48. Bosaid F, Aksel H, Makowka S, Azim AA. Surface and structural changes in root dentine by various chelating solutions used in regenerative endodontics. Int Endod J 2020;53(10):1438-45.
49. Virdee SS, Seymour DW, Farnell D, Bhamra G, Bhakta S. Efficacy of irrigant activation techniques in removing intracanal smear layer and debris from mature

permanent teeth: a systematic review and meta-analysis. Int Endod J 2018;51(6):605-21.
50. Schmidt TF, Teixeira CS, Felippe MC, et al. Effect of Ultrasonic Activation of Irrigants on Smear Layer Removal. J Endod 2015;41(8):1359-63.
51. Orlowski NB, Schimdt TF, Teixeira CDS, et al. Smear Layer Removal Using Passive Ultrasonic Irrigation and Different Concentrations of Sodium Hypochlorite. J Endod 2020.
52. Kuah HG, Lui JN, Tseng PS, Chen NN. The effect of EDTA with and without ultrasonics on removal of the smear layer. J Endod 2009;35(3):393-6.
53. Rodig T, Dollmann S, Konietschke F, Drebenstedt S, Hulsmann M. Effectiveness of different irrigant agitation techniques on debris and smear layer removal in curved root canals: a scanning electron microscopy study. J Endod 2010;36(12):1983-7.
54. Arslan H, Ayranci LB, Karatas E, et al. Effect of agitation of EDTA with 808-nanometer diode laser on removal of smear layer. J Endod 2013;39(12):1589-92.
55. DiVito E, Peters OA, Olivi G. Effectiveness of the erbium:YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation. Lasers Med Sci 2012;27(2):273-80.
56. Takeda FH, Harashima T, Kimura Y, Matsumoto K. Efficacy of Er:YAG Laser Irradiation in Removing Debris and Smear Layer on Root Canal Walls. J Endod 1998;24(8):548-51.
57. Violich DR, Chandler NP. The smear layer in endodontics - a review. Int Endod J 2010;43(1):2-15.
58. Foster KH, Kulild JC, Weller RN. Effect of Smear Layer Removal on the Diffusion of Calcium Hydroxide through Radicular Dentin. J Endod 1993;19(3):136-40.
59. Clark-Holke D, Drake D, Walton R, Rivera E, Guthmiller JM. Bacterial penetration through canals of endodontically treated teeth in the presence or abscence of the smear layer. J Dent 2003;31:275-81.
60. Nischith KG, Srikumar GP, Razvi S, Chandra RV. Effect of smear layer on the apical seal of endodontically treated teeth: an ex vivo study. J Contemp Dent Pract 2012;13(1):23-6.
61. Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F. Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. J Endod 2007;33(2):96-105.
62. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. J Dent 1999;27:43-46.
63. Timpawat S, Vongsavan N, Messer HH. Effect of removal of the smear layer on apical microleakage. J Endod 2001;27(5):351-3.
64. Sjogren UH, B.; Sundqvist, G.; Wing, K. Factors Affecting the Long-term Results of Endodontic Treatment. J Endod 1990;16:298-504.
65. Chugal NM, Clive JM, Spångberg LSW. Endodontic infection: some biologic and treatment factors associated with outcome. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2003;96(1):81-90.
66. Storms JL. Factors that influence the success of endodontic treatment. J Can Dent Assoc 1969;35:83-97.

67. Kojima KI, K.; Nagamatsu, K.; Hara, A.; Nakata, K.; Morita, I.; Nakagaki, H.; Nakamura, H. Success rate of endodontic treatment of teeth with vital and nonvital pulps. A meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:95-99.
68. Saleh W, Xue W, Katz J. Diabetes Mellitus and Periapical Abscess: A Cross-sectional Study. J Endod 2020;46(11):1605-09.
69. An GK, Morse DE, Kunin M, Goldberger RS, Psoter WJ. Association of Radiographically Diagnosed Apical Periodontitis and Cardiovascular Disease: A Hospital Records-based Study. J Endod 2016;42(6):916-20.
70. Segura-Egea JJ, Jimenez-Moreno E, Calvo-Monroy C, et al. Hypertension and dental periapical condition. J Endod 2010;36(11):1800-4.
71. Orstavik D. Time-course and risk analyses of the development and healing of chronic apical periodontitis in man. Int Endod J 1996;29:150-55.
72. Brynolf I. Roentgenologic periapical diagnosis IV. When is one roentgenogram not sufficient? Swed Dent J 1970;63:415-23.
73. Cullen JK, Wealleans JA, Kirkpatrick TC, Yaccino JM. The effect of 8.25% sodium hypochlorite on dental pulp dissolution and dentin flexural strength and modulus. J Endod 2015;41(6):920-4.
74. Ferrer-Luque CM, Bejarano I, Ruiz-Linares M, Baca P. Reduction in Enteroccocus faecalis counts - a comparison between rotary and reciprocating systems. Int Endod J 2014;47(4):380-6.
75. Zhao D, Shen Y, Peng B, Haapasalo M. Root canal preparation of mandibular molars with 3 nickel-titanium rotary instruments: a micro-computed tomographic study. J Endod 2014;40(11):1860-4.