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THE EFFECT OF SMEAR LAYER REMOVAL ON ENDODONTIC OUTCOMES

by

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

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ABSTRACT

THE EFFECT OF SMEAR LAYER REMOVAL ON ENDODONTIC OUTCOMES
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Introduction: A layer of organic and inorganic debris referred to as the smear layer is produced during mechanical instrumentation of the root canal system. The combination of ethylene-diamine-tetraacetic-acid (EDTA) and sodium hypochlorite (NaOCl) has been shown to effectively remove the smear layer. To date, no prospective controlled in vivo studies have been published regarding the effect of smear layer removal on endodontic outcomes in permanent teeth. The purpose of this randomized, prospective, double-blinded clinical trial was to compare the endodontic outcomes of teeth where the smear layer was either removed or left intact. A secondary analysis assessed the influence of covariate factors on healing. **Methods:** Subject's meeting study inclusion criteria were randomly assigned to one of two irrigation groups. Root canals were instrumented in a standardized manner followed by a final irrigation of either 1ml of 17% EDTA or 1ml of 0.9% saline. Clinical and radiographic evaluations were completed no earlier than 12-months post-treatment to assess outcome. A modified periapical index (PAI) score (1-5) was used for radiographic analysis. Data were analyzed using Fisher's exact test ($\alpha<0.05$). **Results:** For this interim analysis of 205 subjects, no significant difference in outcome was found between groups ($p=0.37$). Pre-operative necrosis ($p=0.01$), pre-operative apical lesion ($p<0.0001$) and pre-operative diabetes ($p=0.02$) were the only covariates found to affect healing rates (why talk about healing rates? It was never

defined in the abstract). **Conclusion:** Within the limitations of this in-vivo clinical study, removal of the smear layer did not affect endodontic outcomes.

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REVIEW OF THE LITERATURE

Endodontic therapy continues to evolve while maintaining its goal to reduce the presence of bacteria and diseased tissue within the root canal system. In the absence of bacteria, pulpal and periapical disease is not found [1]. Mechanical instrumentation of the root canal to facilitate removal of diseased pulp tissue and bacteria creates what is known as the smear layer on and within the dentin wall [ref]. Studies assessing the effectivenesss of chemomechanical debridement have led to further description and analysis of this layer. It is composed of tooth derived inorganic and organic material derived from pulp remnants, dentinal debris and bacteria [2, 3]. Particulate matter found within the layer range in size from less than 0.5 to 15µm. Its thickness ranges from 2-5µm with extensions of up to 110µm into the dentinal tubules [4, 5]. The smear layer has been shown to encase bacteria and tissue remnants within the dentinal tubules [ref]. Understanding the properties and effect of the presence or absence of the smear layer on the coronal and apical seal have been studied and reported [6, 7]. However, the presence or absences of smear layer on endodontic outcomes of initial root canal therapy in the permanent dentition has yet to be determined (established?) [8].

Rinsing with saline or sodium hypochlorite (NaOCl) solutions alone do not remove the smear layer, it remains firmly embedded along the canal wall and dentinal tubules [9]. Seventeen percent ethylene-diamine-tetra-acetic acid (EDTA), a chelator, has been shown to remove the smear layer when used in combination with a final rinse of 6% NaOCl [9]. Studies have defined the parameters for most efficient removal. A concentration, volume and time of 1ml 17% EDTA over 1-minute with a final rinse of 3ml 6% NaOCl, produces the desired result while minimizing deleterious effects and dentin erosion [9-11]. Additional research using newer products and techniques have also successfully removed the smear layer. These reports incorporate hydrogen

peroxide, mild acids such as citric acid, acetic acid, BioPure® MTAD®. Qmix®, ultrasonics and lasers [2, 9, 12-17]. However, none of these studies have demonstrated increased efficacy over the combination of 17% EDTA and 6%NaOCl.

To date, no *in-vivo* outcome study has been published evaluating the removal of the smear layer as part of initial nonsurgical endodontic therapy in permanent teeth. Recommendations in the literature on whether the smear layer should be removed remain controversial. Proponents for its' removal empirically cite that "cleaner is better" and advocate the following reasons: elimination of trapped bacteria [2], decrease bacterial leakage [18], and increase the quality of the seal [19, 20]. Conversely, not removing the smear layer may prevent bacterial penetration into dentinal tubules [21]. Those opposed to removing the smear layer cite the following: increased dentin permeability, decreased apical seal [22, 23] or bacterial leakage [3]. Although these authors provided evidence supporting their positions, they did not address possible effects on healing. Therefore, the purpose of this randomized, prospective, double-blinded clinical trial was to compare the endodontic outcomes of teeth where the smear layer was either removed or left intact. A secondary analysis assessed the influence of covariate factors on healing.

MATERIALS AND METHODS

The Institutional Review Board at the Walter Reed National Military Medical Center (WRNMMC), Bethesda, MD approved this study, WRNMMC IRB #352491, "The Effect of Smear Layer Removal on Endodontic Outcomes". WRNMMC also provided funding. The Material and Methods section of this manuscript was modified from the approved protocol.

The Endodontics Department at the Naval Postgraduate Dental School (NPDS) serves US Navy active duty, their family members, retired military and a few other eligible beneficiaries.

Patients meeting the following inclusion criteria; 18 years or older, in good health (American Society of Anesthesiology health status classification I or II), require initial, single appointment NSRCT and agreed to return for a 1-year follow-up examination, were asked to participate. Patients with a history of periodontal disease, previously initiated or treated root canal therapy, on antibiotic therapy or presenting with an acute apical abscess were ineligible to participate. Those patients allergic to any medication or dental material used in the study, including latex or gutta percha, and subjects who reported being pregnant were also not asked to participate.

Once consented and enrolled, subjects were randomly assigned to one of two treatment groups. Straight and angled pre-operative periapical radiographs were taken. Medical conditions, clinical symptoms and diagnostic and treatment information were collected using standardized data collection forms. (Was a pre-operative pulpal and periapical diagnosis established?)

All treatment was provided by NPDS endodontic residents using dental operating microscopes and verified (supervised or mentored?)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 being 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 Maillefer, 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 Maillefer, Tulsa, OK) stainless steel files to working length. The canals were cleaned and shaped with 0.04 Profile (Dentsply Maillefer, 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 FlexoFiles to working length and irrigated with 6% NaOCl, delivered from a 30-gauge side vented irrigation tip between all file sizes for a total intraoperative irrigation volume not exceeding 2ml. 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 1ml of the test irrigant 1mm short of working length over 1 minute per canal, after which identical treatment for all subjects resumed.

A final rinse of 3ml of 6% NaOCl per canal was performed and the canals 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 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 thermaplasticized 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 taken 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 radiograph at the follow-up appointment. Subjects were instructed to return to their referring dentist for the permanent restoration.

A follow-up examination, was conducted no less 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 post-operative periapical radiograph was taken using the positioning device previously created at the treatment appointment.

All radiographs were assessed using a (modified?) PAI scoring, described by Ørstavik [24]. PAI scoring was conducted by 3 calibrated, board certified endodontists. Coronal restorations of the immediate post-operative (pre-operative?) and 1-year follow-up radiographs were masked to eliminate reviewer bias. Radiographs were coded, randomized and individually projected onto a screen in a dark room. After conventional film were converted into digitized images, they were randomized and individually viewed on a single laptop with the ability to adjust the density and contrast. Radiographs were scored individually, and when there was disagreement, forced consensus was used. 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).

A pulpal and apical diagnosis was made based on diagnostic testing conducted during the follow-up exam.

Outcomes 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 by estimating an 80% healed rate at 12-months. (Wasn't a drop-out rate part of this calculation?) In order to assess the true healed rate to within 5 percentage points, 440 subjects, 220 per group, were required. Fisher's exact test ($\alpha<0.05$) was used to perform all statistical comparisons using R software (manufacturer).

RESULTS

This interim analysis reports that a total of 311 subjects enrolled in the study, 24 subjects enrolled since the last interim analysis. 11 subjects did not complete the NSRCT at NPDS, resulting in 235 subjects who were eligible for follow-up. 205 subjects completed the follow-up examination for a follow-up rate of 87%. A total of 36 subjects with a completed follow-up were unable to be analyzed due to extraction (n = 15) or deviation from protocol (n = 21). The most common protocol deviation was due to multiple appointment treatments but also included surgical root canal treatment, missed canal, use of sealer not per protocol and known use of EDTA. The remaining 166 subjects were analyzed. As shown in Figure 1, 72% of subjects assigned to the 0.9% Saline group healed while 64% of subjects assigned to the 17% EDTA group healed. Comparison of the two irrigation protocols demonstrated no significant difference between groups (p=0.37). Covariate factors evaluated that possibly could affect are listed in Table 1. Because of the limited number of subjects evaluated, healed rates were derived by combining data from the two groups for all covariate analyses. A pre-operative pulpal diagnosis of necrotic, the presence of a pre-operative radiolucency, and pre-operative diabetes were the only covariates significantly influencing healed rates (Figures 2-4). As shown in Figure 2, teeth with a pre-operative response to cold, 78% with a response non-lingering (R/NL) and 77% with a response with lingering (R/L), were considered vital. Fifty-six percent of teeth exhibited no pre-operative response to cold (NR) and were considered necrotic. Pooling healed rates of teeth testing vital compared to healed rated of those testing necrotic revealed a significant difference in response to cold (p=0.01). As shown in Figure 3, 81% of those subjects without a pre-operative radiolucency healed, whereas 48% of those subjects with a pre-operative radiolucency healed. Comparison of the groups using Fisher's exact revealed a significant difference (p<0.0001). As

shown in Figure 4, Diabetes was the only medical condition that appeared to play a role in healing. Medical history data collected prior to initiation of treatment revealed 71% of subjects without history of diabetes healed, while 40% of those subjects who were diabetic healed. Comparison of healed rates using Fisher's exact revealed a significant difference between the groups on this self-reported measure ($p=0.02$).

Table 1. Covariate Factors

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

Table 1. A list of covariate factors analyzed. For this interim analysis data from the 17% EDTA and 0.9% saline groups were pooled to assess the influence of covariate factors on healing.

Figure 1. *Comparison of Healed Rates between the Irrigation Protocols on Healed Rates*

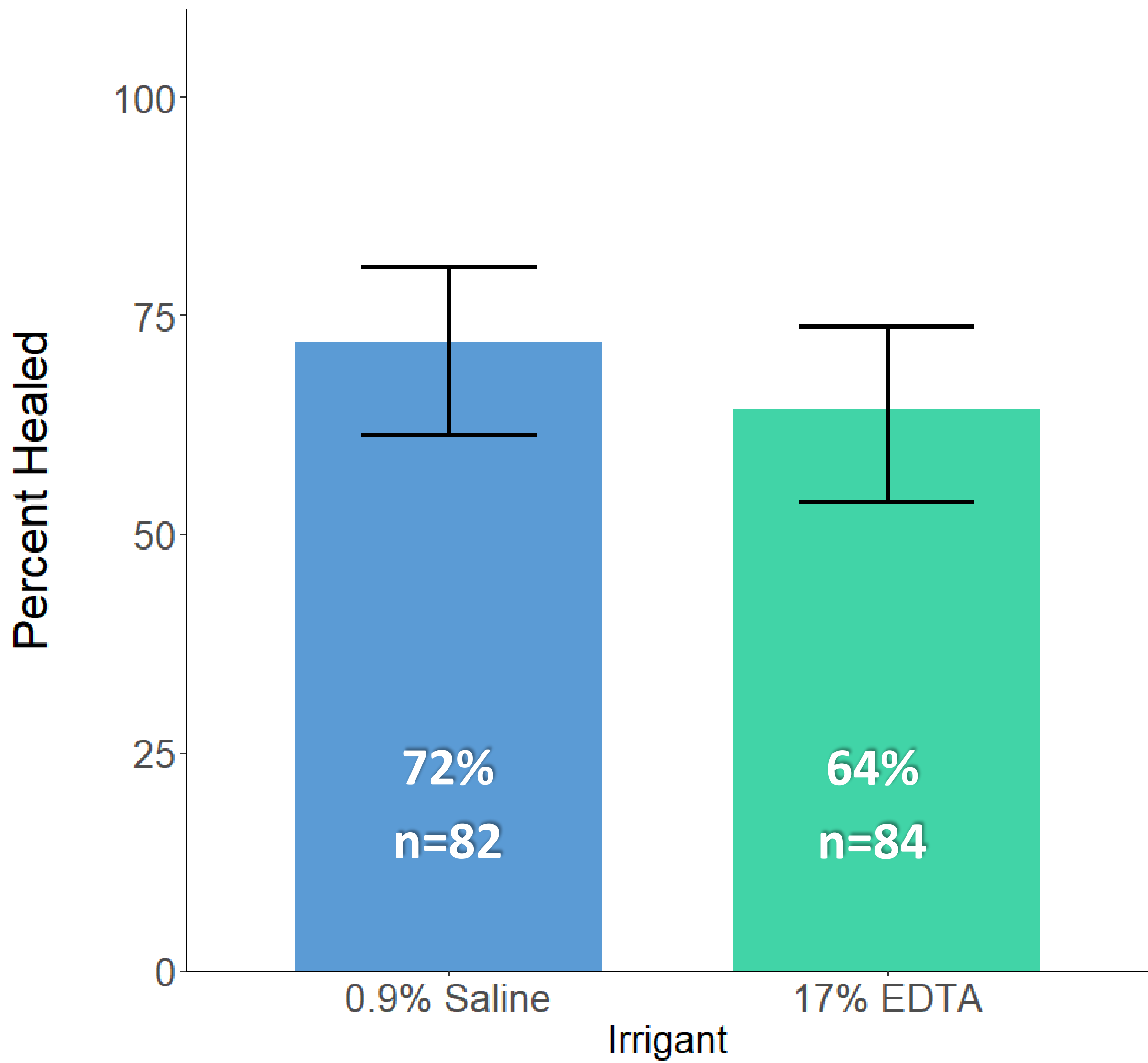

Figure 1. The graph shows the number, healed rate and 95% confidence intervals of subjects in the two irrigation protocols for this interim analysis. Fisher's exact revealed no significant difference in healed rate between groups ($p = 0.37$).

Figure 2. *Comparison of Pre-Operative Response to Cold on Healed Rates*

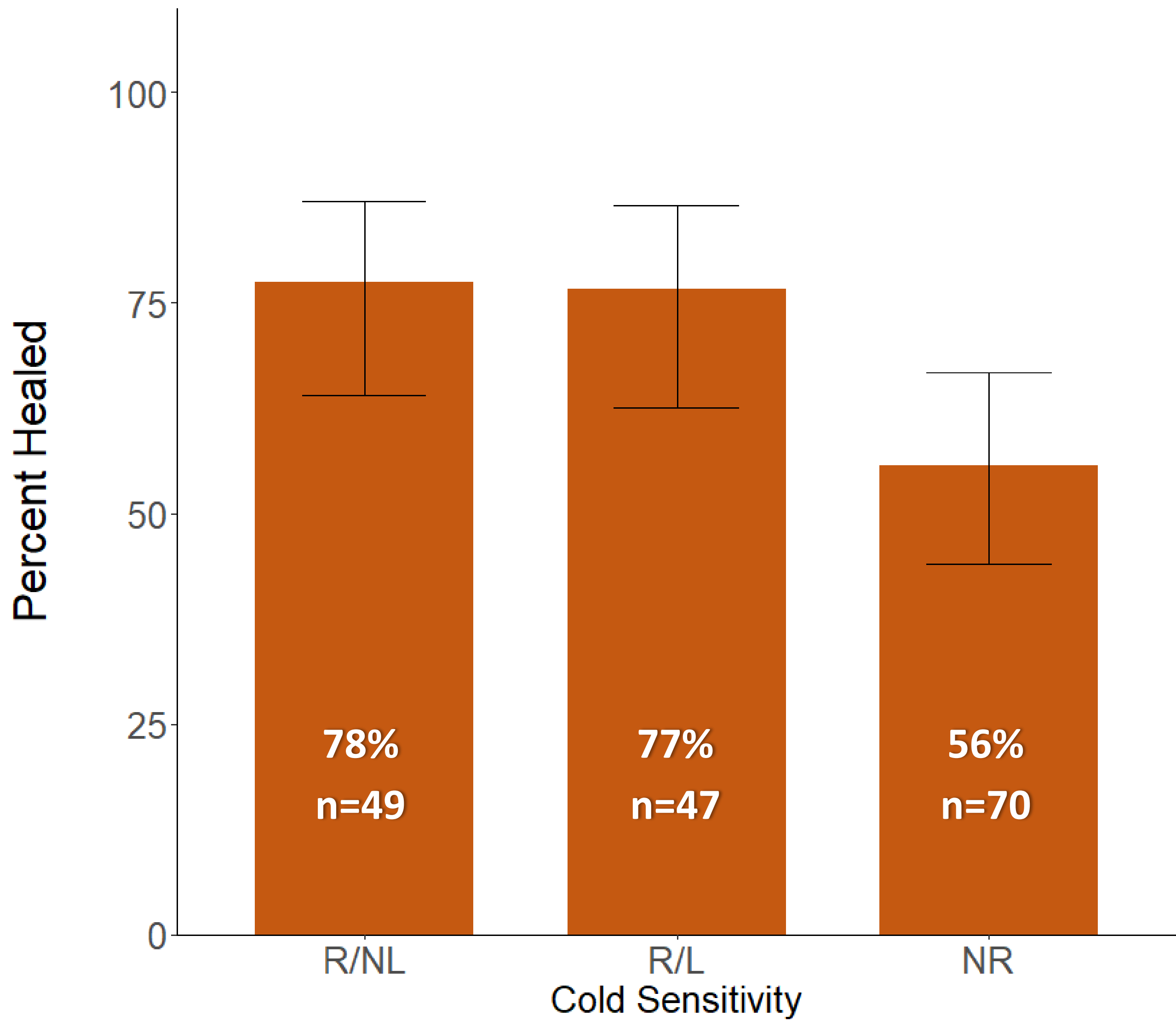

Figure 2. The graph shows the number, healed rate and 95% confidence intervals of subjects with a pre-operative response to cold. Teeth with a response non-lingering (R/NL) or response with lingering (R/L), were considered vital. Teeth with no response to cold (NR), were considered necrotic. Fisher's exact revealed a significant difference between pooled healed rates of vital teeth that responded to cold compared to necrotic teeth with no response to cold (p-0.01).

Figure 3. *Comparison of Pre-Operative Radiolucency on Healed Rates*

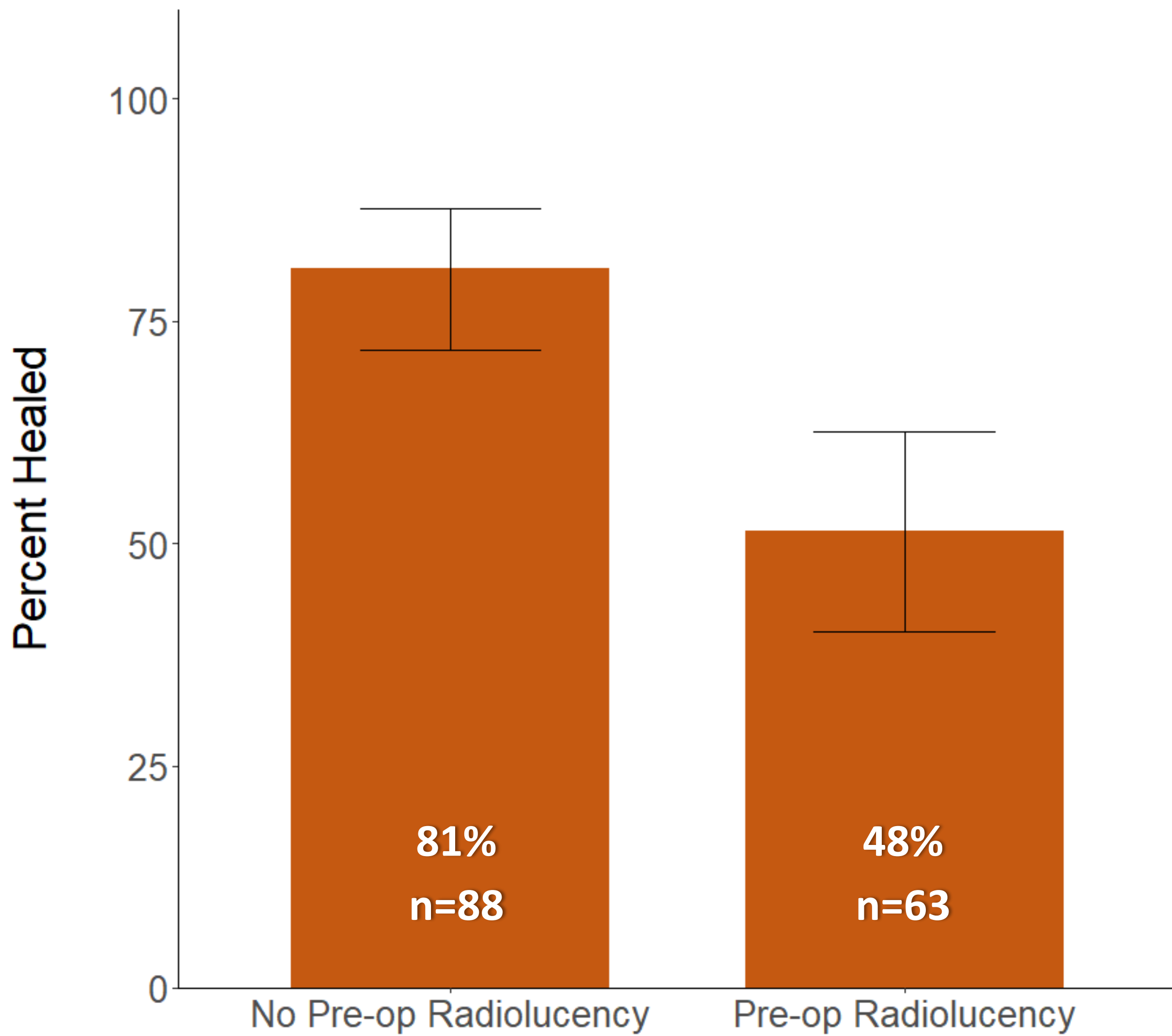

Figure 3. The graph shows the number, healed rate and 95% confidence intervals of teeth presenting with a pre-operative radiolucency. Fisher's exact revealed a significant difference in healed rates between teeth with no pre-operative radiolucent lesion when compared to teeth presenting with a pre-operative radiolucent lesion ($p<0.0001$).

Figure 4. *Comparison of Subjects who Pre-Operatively Reported to be Diabetic on Healed Rates*

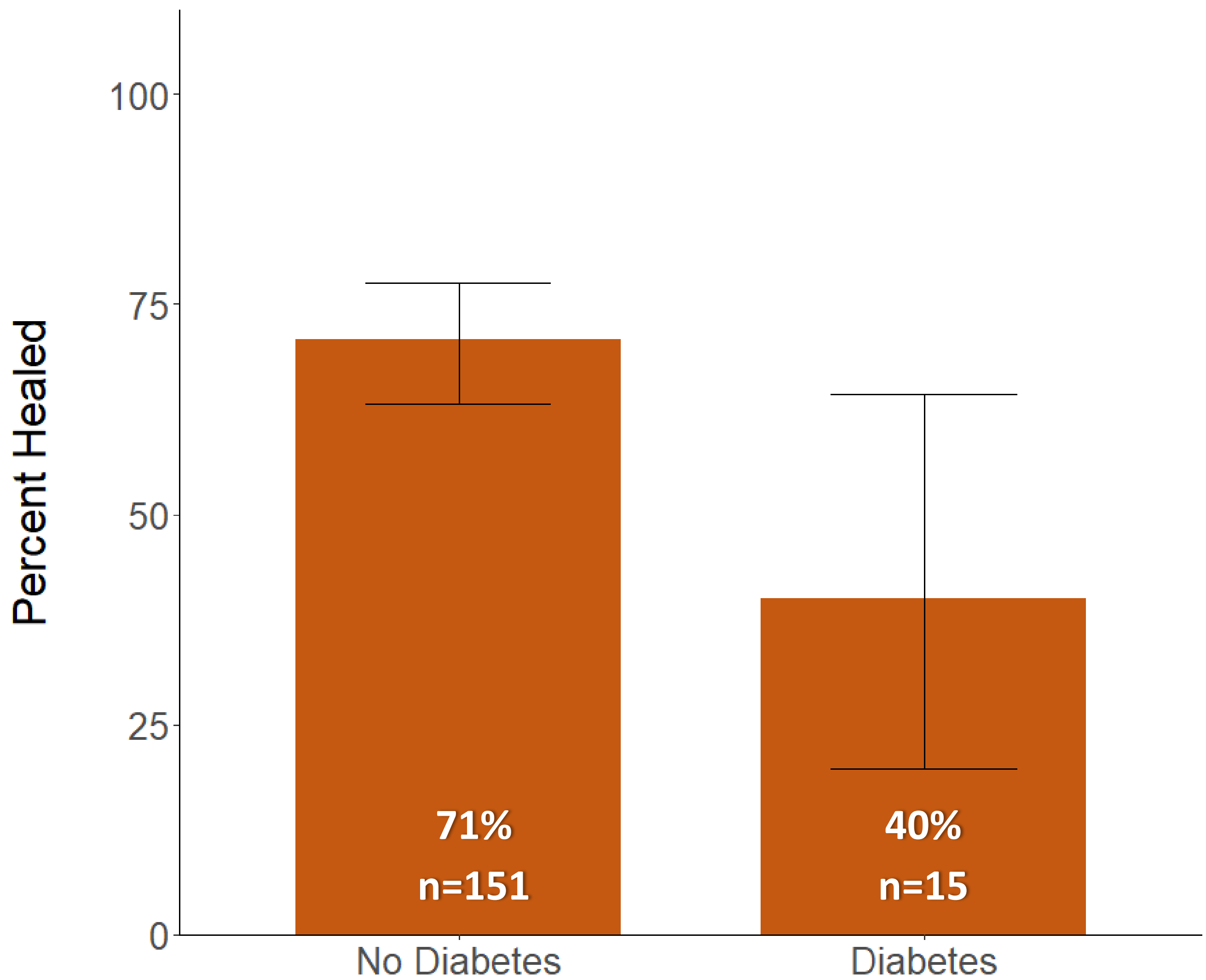

Figure 4. The graph shows the number, healed rated, and 95% confidence intervals of subjects with a history of diabetes. Seventy-0ne percent of subjects without pre-operative diabetes healed, whereas 40% with pre-operative diabetes healed. Comparison of healed rates revealed a significant difference between the 2 groups on healing ($p = 0.02$).

DISCUSSION

Currently the literature is lacking with regards to randomized, double blinded, prospective clinical trials assessing the single variable of smear layer removal on endodontic outcomes in permanent teeth. Studies assessing the effects of smear layer removal have focused on singular effects within the limitations of in vitro studies. Studies in favor of removal report the release of trapped bacteria within and under the smear layer [2], increased ability to improve the seal between obturation material and tooth structure [19, 20], decreased microleakage [18] and enhanced ability of intracanal medicaments to diffuse through the dentin [25]. *In vitro* studies in favor of not removing smear layer cite an increase in apical microleakage [22, 23] or increased dentinal erosion resulting from the combined use of EDTA and NaOCl [26]. Bacterial leakage has been shown to increase [3, 22, 23] and bacterial count within dentinal tubules was found to be lower when the smear layer was left intact [21].

The purpose of this prospective randomized double-blinded clinical study was to investigate the influence of smear layer removal on endodontic outcomes during single-visit initial NSRCT. In an effort to minimize variability a standardized protocol was established and followed by all providers treating enrolled subjects. Exact materials and techniques were provided as part of the protocol to maintain consistency between treated subjects. Calibration of endodontic providers was completed prior to participation in the study via PowerPoint presentation and written instructions were made available to practitioners during treatment for reference and guidance.

This interim analysis determined that removing the smear layer using a combination of 17% EDTA and 6% NaOCl did not lead to improved healed rates. The results of this study are in agreement with a prospective study, though methodology differed and comparison may not be

accurate [27]. Smear layer removal has been evaluated on a primary tooth model with findings advocating for the removal of the smear layer [28], and a reported no significant difference [29]. Differences noted between the studies and the current include: primary vs. permanent teeth, citric acid vs. EDTA as the smear layer removal irrigant, multi-visit vs. single visit treatment, use of an intra canal medicament vs. no medicament, obturation with zinc-oxide eugenol vs gutta percha, and multi-year vs. one-year follow-up. Due to the number of additional variables and methodologies a comparison is very difficult to make.

The presence or absence of the smear layer was not found to be a statistically significant variable. Based on previous studies that have shown that apical pathosis is caused by intracanal bacteria [1] and the reduction of intracanal bacteria when utilizing rotary instrumentation [30] and 6% NaOCl [31] may overshadow the effects of the smear layer. Reduction of bacterial load from the canal both in the planktonic state and as part of a biofilm without a way to quantify how much of a smear layer is produced may indeed overshadow what potential minimal smear layer is being produced and its effect on healing. It has been reported that 35-56% of a canals surface remains untouched during mechanical instrumentation [32] which could result in a low production of smear layer covered surfaces within the canal. Mechanical instrumentation alone is not effective at removing pulp tissue but when combined with chemical irrigation it was found to significantly reduce canal debris and the bacterial load [33]. Various irrigation systems are available to aid in removing debris from the canal system and in this study syringe irrigation via a 30-gauge Max-i-probe syringe (Dentsply Maillefer, Tulsa, OK) was placed 1mm short of the working length [34, 35]. Positive pressure irrigation techniques are able to facilitate large volumes of solutions into the canal system, however there are limitations such as vapor lock [36]. When compared to negative pressure systems, neither method completely eliminated accumulated hard tissue debris [37]. These factors

may lend credit to this study's findings that removing the smear layer may not be significant when evaluating outcomes.

In addition to the primary objective, covariate factors were analyzed to determine statistical influence on endodontic outcomes. Of those evaluated, the presence of pre-operative necrotic pulpal diagnosis and a pre-operative radiolucency were found to be statistically significant in effecting healing rates. This is in agreeance with such studies by Ng et al [27], Marquis et al [38], and Imura et al [39]. These studies found decreases in healed rates when a pre-operative lesion was present and an increased healed rate when the pre-operative lesion was not present. In addition, pre-operative diabetes was found to significantly decrease outcomes in this study. Although there were only 15 subjects enrolled with pre-operative diabetes, this finding is a trend consistent with previously published studies where diabetes is a prognostic factor associated with endodontic outcomes [40, 41]. Pre-operative diabetes has also been associated with decreased endodontic success when a pre-operative lesion is present [42]. Based on this interim analysis, these covariates were the only factors associated with a decreased endodontic outcome.

The limitations of this interim analysis include the reported sample size at this time, length of follow-up and the use of strict criteria during outcomes assessment, where only asymptomatic patients with a PAI score of 1 or 2 on follow-up were considered healed. A power analysis was completed prior to protocol approval in order to determine sample size. This analysis was completed assuming an 80% healed rate based on a previously published outcome study [27]. For this interim analysis, the sample size (205 evaluated subjects) is well below the sample size needed to have a sufficient power (440) and therefore the results of this study could potentially change as more subjects are enrolled and analyzed.

Orstavik reported that at 12-months following completion of NSRCT approximately 90% of teeth that will heal, will show signs of healing [24]. Healing was noted to continue past the 12-month mark and noted within the study it could take four years for complete healing to be recorded. Due to the transient military population and the relatively high capture rate based on that study the recall examination was set at no earlier than 12-months from completion of initial NSRCT though a longer recall time should result in an increased healed rate [43]. Loose healed rate was not considered for this study which would reduce the overall healed rate and places it lower than other published studies that did not use strict criteria during PAI scoring [27]. This study's classification of "healed" or "non-healed" without a "healing" category is a limitation. Additional scoring systems have been compared against the PAI scoring system with higher intra- and inter-observer agreement values with other indexing assessments for periapical health assessed radiographically [44].

CONCLUSION

The interim analysis of this prospective randomized double-blinded clinical trial reveals that the healed rate of single-visit initial non-surgical root canal treatment was not significantly altered by the presence or absence of the smear layer in permanent teeth at this time. Additionally, it was noted that necrotic teeth, teeth with a pre-operative radiolucency or subjects with diabetes; negatively impacted healed rates. Based on these results, we cannot reject the null-hypothesis at this time.

REFERENCE LIST

1. Kakehashi, S., H.R. Stanley, and R.J. Fitzgerald, *The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats.* Oral Surg Oral Med Oral Pathol, 1965. **20**: p. 340-9.
2. McComb, D. and D.C. Smith, *A preliminary scanning electron microscopic study of root canals after endodontic procedures.* J Endod, 1975. **1**(7): p. 238-42.
3. Pashley, D.H., *Smear layer: overview of structure and function.* Proc Finn Dent Soc, 1992. **88 Suppl 1**: p. 215-24.
4. Aktener, B.O., T. Cengiz, and B. Piskin, *The penetration of smear material into dentinal tubules during instrumentation with surface-active reagents: a scanning electron microscopic study.* J Endod, 1989. **15**(12): p. 588-90.
5. Mader, C.L., J.C. Baumgartner, and D.D. Peters, *Scanning electron microscopic investigation of the smeared layer on root canal walls.* J Endod, 1984. **10**(10): p. 477-83.
6. Goldberg, F., L.P. Artaza, and A. De Silvio, *Apical sealing ability of a new glass ionomer root canal sealer.* J Endod, 1995. **21**(10): p. 498-500.
7. Madison, S. and K.V. Krell, *Comparison of ethylenediamine tetraacetic acid and sodium hypochlorite on the apical seal of endodontically treated teeth.* J Endod, 1984. **10**(10): p. 499-503.
8. Yang, S.E. and K.S. Bae, *Scanning electron microscopy study of the adhesion of Prevotella nigrescens to the dentin of prepared root canals.* J Endod, 2002. **28**(6): p. 433-7.
9. Yamada, R.S., et al., *A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: Part 3.* J Endod, 1983. **9**(4): p. 137-42.
10. Calt, S. and A. Serper, *Time-dependent effects of EDTA on dentin structures.* J Endod, 2002. **28**(1): p. 17-9.
11. Crumpton, B.J., G.G. Goodell, and S.B. McClanahan, *Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation.* J Endod, 2005. **31**(7): p. 536-8.
12. Arslan, H., et al., *Effect of agitation of EDTA with 808-nanometer diode laser on removal of smear layer.* J Endod, 2013. **39**(12): p. 1589-92.
13. Patil, P.H., et al., *Efficacy of new irrigating solution on smear layer removal in apical third of root canal: A scanning electron microscope study.* J Conserv Dent, 2018. **21**(2): p. 190-193.
14. Schmidt, T.F., et al., *Effect of Ultrasonic Activation of Irrigants on Smear Layer Removal.* J Endod, 2015. **41**(8): p. 1359-63.
15. Silva, P.V., et al., *Chitosan: a new solution for removal of smear layer after root canal instrumentation.* Int Endod J, 2013. **46**(4): p. 332-8.
16. Spano, J.C., 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): p. 727-30.
17. Torabinejad, M., et al., *A new solution for the removal of the smear layer.* J Endod, 2003. **29**(3): p. 170-5.
18. Clark-Holke, D., et al., *Bacterial penetration through canals of endodontically treated teeth in the presence or absence of the smear layer.* J Dent, 2003. **31**(4): p. 275-81.
19. Shahravan, A., et al., *Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis.* J Endod, 2007. **33**(2): p. 96-105.
20. Andriukaitiene, L., et al., *The effect of smear layer removal on E. faecalis leakage and bond strength of four resin-based root canal sealers.* BMC Oral Health, 2018. **18**(1): p. 213.
21. Drake, D.R., et al., *Bacterial retention in canal walls in vitro: effect of smear layer.* J Endod, 1994. **20**(2): p. 78-82.
22. Timpawat, S. and S. Sripanaratanakul, *Apical sealing ability of glass ionomer sealer with and without smear layer.* J Endod, 1998. **24**(5): p. 343-5.

23. Shamsy, E., et al., *Evaluation of the Apical Seal of Mineral Trioxide Aggregate in the Absence and Presence of Smear Layer.* J Contemp Dent Pract, 2019. **20**(3): p. 298-303.
24. Orstavik, D., *Time-course and risk analyses of the development and healing of chronic apical periodontitis in man.* Int Endod J, 1996. **29**(3): p. 150-5.
25. Torabinejad, M., et al., *Clinical implications of the smear layer in endodontics: a review.* Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2002. **94**(6): p. 658-66.
26. Qian, W., Y. Shen, and M. Haapasalo, *Quantitative analysis of the effect of irrigant solution sequences on dentin erosion.* J Endod, 2011. **37**(10): p. 1437-41.
27. Ng, Y.L., V. Mann, and K. Gulabivala, *A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health.* Int Endod J, 2011. **44**(7): p. 583-609.
28. Barcelos, R., et al., *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): p. 369-81.
29. Tannure, P.N., et al., *Long-term outcomes of primary tooth pulpectomy with and without smear layer removal: a randomized split-mouth clinical trial.* Pediatr Dent, 2011. **33**(4): p. 316-20.
30. Ferrer-Luque, C.M., et al., *Reduction in Enteroccocus faecalis counts - a comparison between rotary and reciprocating systems.* Int Endod J, 2014. **47**(4): p. 380-6.
31. Clegg, M.S., et al., *The effect of exposure to irrigant solutions on apical dentin biofilms in vitro.* J Endod, 2006. **32**(5): p. 434-7.
32. Zhao, D., et al., *Root canal preparation of mandibular molars with 3 nickel-titanium rotary instruments: a micro-computed tomographic study.* J Endod, 2014. **40**(11): p. 1860-4.
33. Bystrom, A. and G. Sundqvist, *Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy.* Scand J Dent Res, 1981. **89**(4): p. 321-8.
34. Sedgley, C.M., et al., *Influence of irrigant needle depth in removing bioluminescent bacteria inoculated into instrumented root canals using real-time imaging in vitro.* Int Endod J, 2005. **38**(2): p. 97-104.
35. Kahn, F.H., P.A. Rosenberg, and J. Gliksberg, *An in vitro evaluation of the irrigating characteristics of ultrasonic and subsonic handpieces and irrigating needles and probes.* J Endod, 1995. **21**(5): p. 277-80.
36. Tay, F.R., et al., *Effect of vapor lock on root canal debridement by using a side-vented needle for positive-pressure irrigant delivery.* J Endod, 2010. **36**(4): p. 745-50.
37. Versiani, M.A., et al., *Micro-CT evaluation of the efficacy of hard-tissue removal from the root canal and isthmus area by positive and negative pressure irrigation systems.* Int Endod J, 2016. **49**(11): p. 1079-1087.
38. Marquis, V.L., et al., *Treatment outcome in endodontics: the Toronto Study. Phase III: initial treatment.* J Endod, 2006. **32**(4): p. 299-306.
39. Imura, N., et al., *The outcome of endodontic treatment: a retrospective study of 2000 cases performed by a specialist.* J Endod, 2007. **33**(11): p. 1278-82.
40. Cabanillas-Balsera, D., et al., *Association between diabetes and nonretention of root filled teeth: a systematic review and meta-analysis.* Int Endod J, 2019. **52**(3): p. 297-306.
41. Nagendrababu, V., et al., *Association between diabetes and the outcome of root canal treatment in adults: an umbrella review.* Int Endod J, 2020. **53**(4): p. 455-466.
42. Fouad, A.F. and J. Burleson, *The effect of diabetes mellitus on endodontic treatment outcome: data from an electronic patient record.* J Am Dent Assoc, 2003. **134**(1): p. 43-51; quiz 117-8.
43. Friedman, S., *Prognosis of initial endodontic therapy.* Endod Topics, 2002. **2**: p. 59-88.
44. Tarcin, B., et al., *Radiologic Assessment of Periapical Health: Comparison of 3 Different Index Systems.* J Endod, 2015. **41**(11): p. 1834-8.