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Impact on Bacterial Micro-leakage in Exposed Root Canal Obturation Material in Teeth Irrigated  
with Different Solutions

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

By

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May, 2019

## **Acknowledgements**

I would like to thank everyone on my thesis committee for all their help and guidance.

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## Abstract

### IMPACT ON BACTERIAL MICRO-LEAKAGE IN EXPOSED ROOT CANAL OBTURATION MATERIAL IN TEETH IRRIGATED WITH DIFFERENT SOLUTIONS

By: Khalifa W. Alhaddad, D.D.S

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

Virginia Commonwealth University, 2019

Thesis Advisor: Garry L. Myers, D.D.S

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**Purpose:** Determine the timeframe of bacterial penetration that occurs to the apex when obturation material (gutta percha) is exposed to bacteria for a set period of time (45 days) and to determine if bacterial penetration of the obturated root is influenced by the type of irrigant used during the final rinse (17% EDTA vs 2% Chlorhexidine vs full strength 5.25% NaOCl).

**Methods:** Thirty-six extracted teeth, including six controls, were instrumented and irrigated with 5.25% NaOCl followed by a final rinse of either: 17% EDTA, 2% Chlorhexidine, or 5.25% NaOCl, and then obturated. Each root was suspended between two chambers: the coronal chamber inoculated with brain heart infusion broth and  $10^8$  colony-forming units of *Enterococcus faecalis*, the apical chamber with brain heart infusion broth. The latter was checked daily for turbidity, indicating bacterial leakage.

**Results:** After excluding teeth with clear indications of experimental failure, 21 teeth were included in the analysis. Leakage rates were not significantly difference across the three groups (Chlorhexidine: 14%, EDTA: 67%, NaOCl: 50%; p-value=0.1581). Time to leakage was not significantly difference across the three groups (p-value=0.2470).

**Conclusion:** Within the limitations of this study it was shown that leakage occurs between 4-42 days and that there was no significant difference between the different solutions in preventing leakage.



## **Introduction**

The goals of endodontic therapy have classically been described as the “Endodontic Triad”, which are: thorough debridement of the root canal, sterilization of the root canal, and complete obturation of the root canal.(1) Ideally, if all three objectives are met then root canal therapy should be successful. However, endodontic treatment sometimes results in failure even when all the objectives are seemingly met. Reasons for failure include: poor debridement of the root canal system, missed anatomy, voids in the obturation, and systemic factors. Another reason of failure in endodontic therapy is contamination of the obturation material or gutta percha . (2)

Contamination of gutta percha from the oral environment occurs when either the temporary or permanent restoration is missing or leaking or when the tooth is fractured. Contamination can also occur when the endodontically treated tooth is restored without a rubber dam, thus exposing the obturated canals to saliva from the oral environment. The oral cavity is home to many bacterial species that have been linked to apical periodontitis.(3)

Studies investigating the effects of gutta percha contamination date back as early as the 1950s, when Dow and Ingle examined gutta percha contamination using radioisotopes.(4) Later studies on gutta percha contamination began using different methods to evaluate the contamination of gutta percha. One method that became very popular was dye leakage study.

One of the most influential dye leakage studies on gutta percha contamination was a three-part study done Madison and Swanson. In part one of the study, they examined

contamination of gutta percha in extracted teeth at different time periods. The teeth were instrumented and obturated with gutta percha and then the temporary restorations were removed to expose the gutta percha. Experimental groups were next exposed to artificial saliva for varying time periods: 3, 7, 14, 28, and 56 days. All the teeth were then submerged in Pelican ink for 48 hours and then rendered transparent by using methyl salicylate after decalcification and dehydration procedures. The results of the study showed that gutta percha contamination occurred as early as three days, and all experimental teeth showed leakage ranging from 79-85% of the root.(3)

In Part two of this study, Madison and Swanson examined the impact of sealer types on gutta percha contamination. The results of this study again showed that leakage occurs into the gutta percha and that the AH26 group demonstrated the greatest dye penetration between sealer groups.(5) Part three of the study was an in vivo study performed on monkeys. The experimental set up was identical to part one and two of the study except this time monkey teeth were used rather than extracted teeth. Dye leakage occurred in all the experimental teeth with most cases showing penetration 1-4 mm into the canals after 1 week.(6)

While the results of dye leakage studies performed by Swanson and Madison and many others were enlightening, many clinicians were skeptical about the results. The main argument against dye leakage studies were that they failed to include the main culprit and cause of endodontic failures: the bacterial micro-organisms. Many of the dye leakage models used artificial saliva and did not include any bacteria in the experiments. Another argument against dye leakage studies were that the dye or ink used in these experiments was not clinically relevant because it behaves differently than bacteria in terms of penetration and mobility. This was shown in a study done by De-Deus. In his extracted teeth experiment, samples that did not leak in the

bacterial leakage model leaked when they were exposed to the blue methylene dye leakage model. This study and other similar studies led to a shift in gutta percha contamination studies from dye leakage studies to bacterial microleakage studies.(7)

The earliest of these bacterial microleakage studies was done by Torabinejad in 1990. In his experiment, forty-five extracted teeth were instrumented and then obturated with laterally condensed gutta percha and Roth's sealer. The coronal portion of gutta percha was removed to create space for the artificial saliva and bacteria and they made sure to leave at least 10mm of Gutta percha in the canal. The bacteria used in this study were *Staphylococcus epidermidis*, which are nonmotile, and *Proteus vulgaris*, which are highly motile. The results of the study showed that over 50% of the teeth were completely contaminated after 19 days exposure to *Staphylococcus epidermidis* and after 42 days exposure to *Proteus vulgaris*. These results were quite different to those shown in the dye leakage studies.(8)

Magura's study on gutta percha contamination as related to time involved using both a dye leakage model and a histological exam. The time periods used in this study were 2, 7, 14, 28, and 90 days. The results of this study showed that salivary penetration assessed histologically was significantly less than when visualized with dye analysis. Another important point was that salivary penetration at 90 days was significantly greater than at the four other time periods. The 90 day time period was the only time period that Magura thought was clinically significant and warranted complete retreatment of the root canal system.(9)

Following Torabinejad's study in 1990 Khayat performed another bacterial microleakage study to examine gutta percha contamination. The results showed that teeth obturated with lateral condensation showed leakage as early as 8 days with a of range 8–48 days while teeth obturated with the warm vertical technique showed leakage as early as 4 days with a range of 4–46 days.

This study showed that complete bacterial contamination occurred within an average of less than thirty days. (10)

The results obtained from bacterial leakage studies were different from the dye leakage studies, specifically in how long it took the contamination to occur as well as the depth of the contamination. Nevertheless, both study models showed that contamination and leakage of Gutta percha can occur and that it can penetrate all the way to the apex. However, there were studies done later that concluded that gutta percha contamination from the oral environment did not lead to contamination of the entire root canal system. Ricucci's study in 2003 was a histobacteriological study that was done on root canal treated teeth that were exposed to the oral environment for a minimum of three months. The results were that 96% of the roots examined had bacterial contamination that was limited to the coronal third and none of the roots showed contamination in the apical third. (11)

Even with there being conflicting studies on whether bacterial contamination occurs, there were many studies looking into ways to prevent bacterial contamination of gutta percha. Examples included studies looking at different obturation techniques, temporary materials, and the use of orifice barriers. One area of research that has not been studied extensively is if the different irrigants used during endodontic procedures have an effect on preventing or reducing bacterial contamination. One study that investigated this was done by Marley et al. in 2001.

The goal of Marley et al.'s research was to determine if the use of chlorhexidine gluconate, a.k.a Peridex, as a final irrigant would have an effect on the apical seal of the gutta percha. Their study not only compared chlorhexidine gluconate with NaOCl and saline but also compared the different irrigants with multiple endodontic sealers such as Roth's sealer, AH26, and Sealapex. Different combinations of irrigants and sealers were used, and the fluid filtration

method was used to evaluate leakage at 90 and 180 days. The results of their research showed that there was no significant difference between the groups in preventing leakage. (12)

The aim of this study is similar to Marley's study but with a few modifications. Three irrigants will be investigated: 17% EDTA, 2% Chlorhexidine and 5.25% NaOCl. All three irrigants will be used as a final rinse before obturation with gutta percha and Roth's sealer. The objective of this study will be to determine the timeframe of bacterial penetration that occurs to the apex when obturation material (gutta percha) is exposed to bacteria for a set period of time (45 days) and to determine if bacterial penetration of the obturated root is influenced by the type of irrigant used during the final rinse.

## Methods

All extracted teeth used in this study were sterilized in formaldehyde solution for over twenty-four hours. Instrumentation was completed in a disinfected clinical environment. After instrumentation was complete the teeth were then sterilized in an autoclave prior to the final obturation. All obturation, mounting, and inoculating of teeth was performed in a biological safety cabinet with the use of sterile surgical gloves.

The 36 teeth selected for the experiment consisted of incisors, canines, and premolars with one canal. All the teeth were decoronated and had 3mm of the root resected to achieve a final tooth length of 14-16mm. The teeth were accessed and instrumented to an apical ISO size of 80 using hand and rotary files to a working length of 1mm short of the apex. During instrumentation all the teeth were irrigated with 5.25% NaOCl and 17% EDTA. After instrumentation all the teeth were autoclaved.

After completion of the final rinse with the specific irrigant the teeth were then obturated using a warm vertical condensation technique with Roth's sealer and a master cone of size 80/02. The teeth were mounted in a modified disposable polypropylene centrifuge tube at the midroot level on both the coronal side and root side using a layer of each of the following materials: cyanoacrylate (KRAZY GLUE), nail polish (ESSIE) and rope wax.

The upper chamber was filled with 2 mL of a 1:10 dilution of *Enterococcus faecalis* at  $\sim 5 \times 10^8$  CFU/mL in brain heart infusion broth (BHI). The lower chamber consisted of 2 ml BHI in

a flat-bottomed, 20-mL, transparent scintillation vial. The tip of the centrifuge tube with the tooth attached was introduced into and sealed to the neck of the vial with sticky wax so that the tip of the root would reach approximately 2 mm into a reservoir of 2 mL of broth in the lower chamber. (Figure 1).

After inoculation and tightening of the lid onto its respective upper chamber, the mounted teeth were placed in an incubator and were evaluated daily. Leakage was determined by visualizing turbidity in the lower chamber. When turbidity was seen, the teeth were determined to have leaked. (Figure 2).



Figure 1: Set-Up

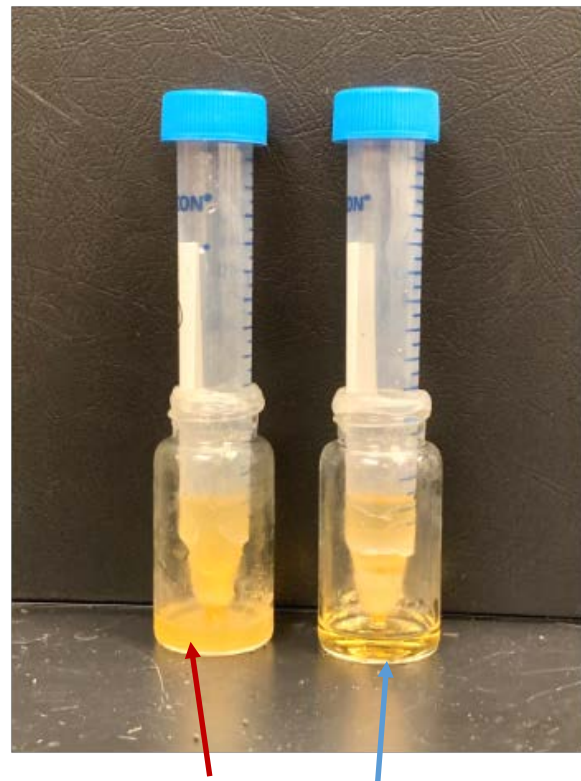


Figure 2: Turbidity vs. Lack of turbidity

Verification of bacterial leakage was also done by testing for salt tolerance, which is suggestive of *Enterococcus faecalis* leakage rather than the presence of a contaminant. A 3  $\mu$ l sample of the turbid lower broth was introduced into a tube containing BHI + 6.5% NaCl and left for 24-48

hours in an incubator. Evidence of turbidity would indicate the presence of *Enterococcus faecalis*. (Figure 3).



Figure 3: Appearance of BHI + 6% NaCl prior to and after inoculation with *Enterococcus faecalis*.

A. Uninoculated control. B. Inoculated with *E. faecalis* and incubated for 24 h at 37°C.



Experimental and Control groups:

Group A/B/C: (10 teeth each)

Teeth were accessed and instrumented to an apical size of 80. During instrumentation all the teeth were irrigated with 5.25% NaOCl and 17% EDTA. After instrumentation all the teeth were autoclaved. Group A had a final rinse of only 5.25% NaOCl for 1 min and were dried with paper points. Group B had a final rinse of only 17% EDTA for 1 min and were also dried with paper points. Group C had a final rinse of only 2% Chlorhexidine for 1 min and were dried with paper points. After completion of the final rinse with the specific irrigant, the teeth were then obturated using a warm vertical condensation technique with Roth's sealer and a master cone of size 80/02. After the teeth were obturated they were placed in the bacterial leakage model described previously. The time/day when turbidity was noted in the lower chamber was recorded.

Group D: Positive control (3 teeth)

Teeth were accessed and instrumented to an apical size of 80. During instrumentation all the teeth were irrigated with 5.25% NaOCl and 17% EDTA. After instrumentation all the teeth were autoclaved. These teeth were not obturated. The teeth were then placed in the bacterial leakage model described previously. The time/day when turbidity was noted in the lower chamber was recorded.

Group E: Negative control (3 teeth)

Teeth were accessed and instrumented to an apical size of 80. During instrumentation all the teeth were irrigated with 5.25% NaOCl and 17% EDTA. After instrumentation all the teeth were autoclaved. No final rinse was done. These teeth were obturated. The teeth were then placed in the bacterial leakage model described previously with the exception that the upper chamber did not contain any *Enterococcus faecalis*. The time/day when turbidity was noted in the lower chamber was recorded.

**Statistical Methods:** Percent with leakage was compared using Fisher's exact test and time to leakage was compared using Kaplan-Meier survival with log-rank test. For log-rank test, post hoc pairwise comparisons were adjusted for using Tukey's adjustment to maintain an overall significance level of 0.05. All analyses were repeated with Intention to Treat (ITT) and Per Protocol (PP) analysis methods. The PP analysis excluded cases where it was apparent the leakage had occurred due to a failed experimental design which resulted in leakage around the tooth and not through the tooth. Significance level was set at 0.05. SAS EG v.6.1 (SAS Institute, Cary, NC) was used for all analyses.

## Results

The experiment was conducted for 45 days for all groups. There were nine instances where it was apparent that leakage had occurred around the tooth. Cases of contamination due to experimental setup did not differ significantly among the three treatment groups (Chlorhexidine: 30%, EDTA: 40%, NaOCl: 20%; p-value=0.8792). These cases were included in the ITT analysis but were subsequently excluded and analysis was repeated for the PP analysis. Summary results are given in Table 1.

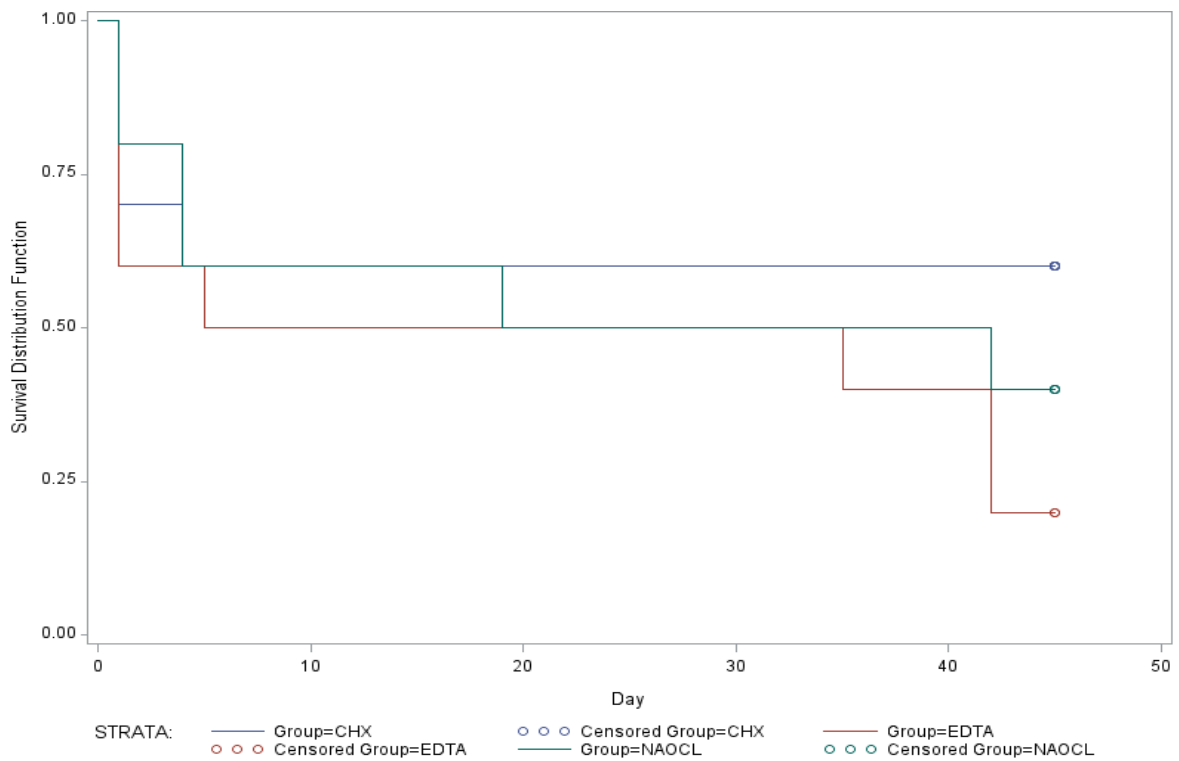
Table 1: Summary of Leakage Results by Group

	<b>Leaked</b>	<b>No Leakage</b>	<b>Experimental Failure</b>
Chlorhexidine	1 (10%)	6 (60%)	3 (30%)
EDTA	4 (40%)	2 (20%)	4 (40%)
NaOCl	4 (40%)	4 (40%)	2 (20%)
Positive Control	3 (100%)	0 (0%)	0 (0%)
Negative Control	0 (0%)	3 (100%)	0 (0%)

## Intention to Treat Analysis

ITT analysis included all 30 teeth. Leakage rates were not significantly different across the three groups (Chlorhexidine: 40%, EDTA: 80%, NaOCl: 60%;  $p$ -value=0.2484). Kaplan-Meier survival curves are given in Figure 4. Time to leakage was not significantly different across the three groups ( $p$ -value=0.3220).

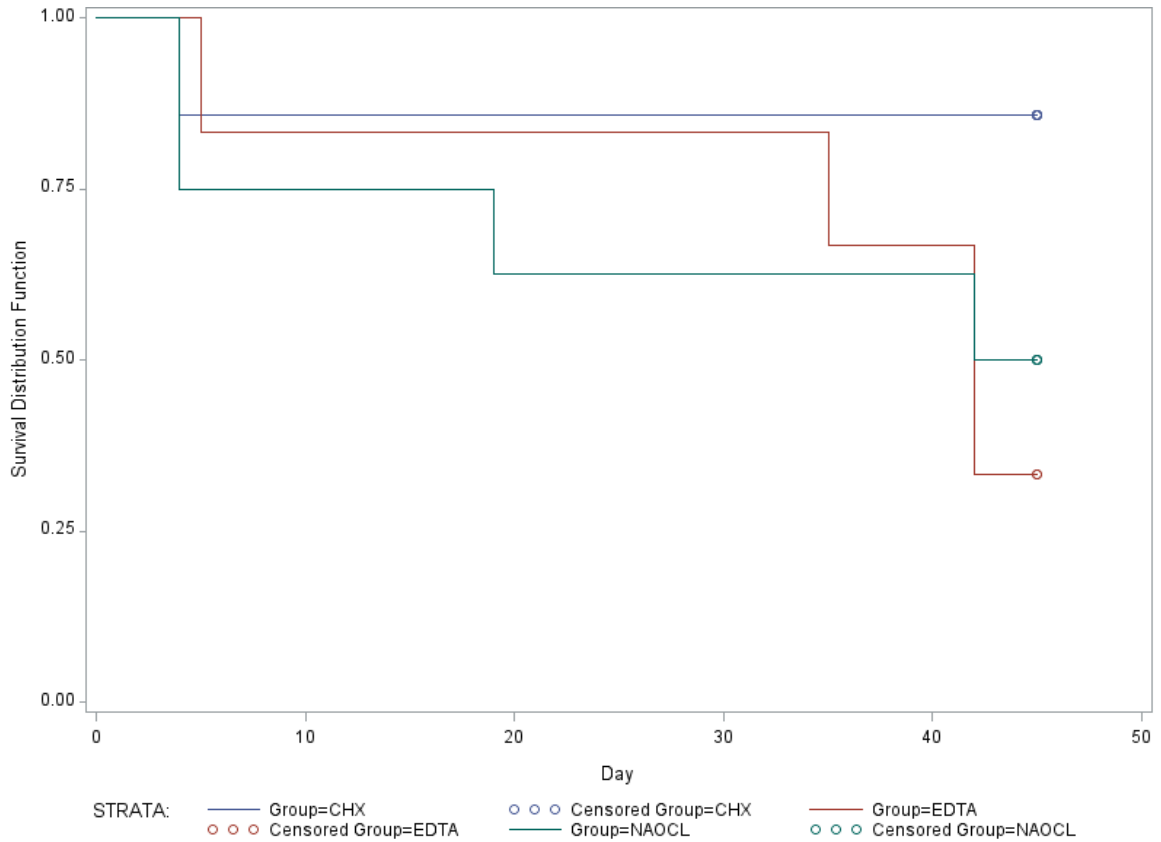
Figure 4: Kaplan-Meier Curves Based on Treatment Group (ITT)



## Per Protocol Analysis

After excluding teeth with clear indications of experimental failure, 21 teeth were included in the analysis. Leakage rates were not significantly different across the three groups (Chlorhexidine: 14%, EDTA: 67%, NaOCl: 50%;  $p$ -value=0.1581). Kaplan-Meier survival curves are given in Figure 5. Time to leakage was not significantly different across the three groups ( $p$ -value=0.2470).

Figure 5: Kaplan-Meier Curves Based on Treatment Group



In the Chlorhexidine group one out of the seven samples leaked, (Chlorhexidine = 14% leakage). In the EDTA group four out of the six samples leaked, (EDTA = 67% leakage). In the NaOCl group, four out of the eight samples, (NaOCl = 50% leakage). The earliest day that leakage occurred was on the fourth day, in which two of the NaOCl samples leaked and one of the Chlorhexidine samples leaked. One sample of the EDTA group leaked on the fifth day as well. The remaining samples leaked across different days with the last day of leakage recorded being day forty-two, in which one of the NaOCl samples leaked and two of the EDTA samples leaked.

## **Discussion**

Contamination of gutta percha from the oral environment occurs when either the temporary or permanent restoration is missing or leaking, when the tooth is fractured, or if treatment is done without a rubber dam. Contamination of gutta percha by the oral environment has been a topic that has been studied for a long time. Different techniques and modalities were used for the different studies and unsurprisingly the results are quite diverse. The classic dye leakage studies done by Madison and Swanson showed that leakage of the entire root occurred as early as three days. However, dye leakage studies have been shown to be not as accurate as bacterial-leakage studies due to the fact that artificial saliva is used instead of bacteria and that the dye or ink used in these experiments is not clinically relevant because it behaves differently from bacteria in terms penetration and mobility.

Bacterial-leakage model studies such as those done by Torabinejad, showed that leakage occurred after or within 19-42 days in about 50% of the samples. Other bacterial-leakage studies include those done by Magura, in which leakage occurred in 100% of the samples at 90 days and Khayat, in which leakage occurred in 100% of the samples between 4-48 days. However, Ricucci's study showed that no apical leakage occurred in teeth that were exposed to the oral environment for three months. Even with there being conflicting studies on whether bacterial contamination occurs, there were many techniques and materials used to prevent bacterial

contamination gutta percha. This study focused on the irrigants used during final rinse before obturation.

The different irrigants that were examined were 5.25% NaOCl, 17% EDTA, and 2% Chlorhexidine. The use of 5.25% NaOCl in Endodontics has been long studied and established. 5.25% NaOCl has been shown to have favorable properties as an irrigant such as its antimicrobial efficacy, tissue dissolving properties, and safe clinical use. Harrison et al.'s study in 1981 showed that full strength or 5.25% NaOCl had the best antimicrobial properties when compared with the diluted forms of NaOCl such as the 0.5%, 1%, and 2.5%. This was done by taking absorbent points that were contaminated with *S. faecalis* and exposing them to varying time intervals with different dilutions of NaOCl. After exposure to one of the test solutions, the points were placed in culture medium, incubated and evaluated for growth. 5.25% NaOCl killed all bacteria after 45 sec, 2.5% NaOCl after 2 min, 1% NaOCl after 5 min, and 0.5% NaOCl and 3% H<sub>2</sub>O<sub>2</sub> after 15 min. This study showed that NaOCl was not only an effective antibacterial agent but also that the 5.25% form was more effective than the diluted. (11)

A similar study was conducted by Hand et al. in 1978 but in this study the focus was on NaOCl's ability to dissolve necrotic tissue. Weighed rat connective tissues were placed in contact with test solutions of either 5.25%, 2.5%, 1.0%, or 0.5% NaOCl, normal saline, distilled water, or 3% hydrogen peroxide in the ratio of 1 ml of solution per 10 mg of tissue. After seven minutes, the tissues were removed from the solutions, and reweighed to determine the percent of weight loss or gain. The results were that 5.25% NaOCl was significantly more effective as a necrotic tissue solvent than any of the other test solutions.(12)

Another property of NaOCl that makes it an effective irrigant is its ability to physically disrupt bacterial biofilm. This was demonstrated in a study by Clegg in 2006 where he compared



6% NaOCl and 2% Chlorhexidine in their ability to disrupt and eliminate biofilm. Polymicrobial biofilm was created on root dentin hemisections and the samples were immersed into test solutions of 6% NaOCl and 2% Chlorhexidine. The results were that 6% NaOCl was the only irrigant that was able to render bacteria non-viable and disrupt and eliminate biofilm, meanwhile Chlorhexidine was able to disrupt the biofilm but not eliminate it. (13)

EDTA has also been studied extensively as an irrigant for use during endodontic treatment. The main advantage that EDTA has over the other irrigants is its ability to dissolve inorganic material, such as debris created during treatment and the smear layer. This was first recognized by McComb and Smith, who described the smear layer as a surface layer completely obscuring the dentinal tubules composed of superficial debris and embedded erythrocytes. Their study also looked what solution or irrigant would be able to remove the smear layer. The different irrigants they evaluated were distilled water, 6% NaOCl, 20% Polyacrylic acid and 17% EDTA. The results of their study showed that %17 EDTA was the only irrigant that removed the smear layer.(14)

The discovery of the smear layer led to other avenues of research related to it, the main one being the question of whether it was actually beneficial to remove the smear layer during endodontic treatment. This concern was studied by Drake, who conducted a study on the outcome of removing the smear layer. In his study, twenty-six extracted canines were instrumented and irrigated with 2.5% NaOCl and divided into 2 groups. One group was irrigated with saline which resulted in the smear layer not being removed, and the other group was irrigated with 17% EDTA followed by 2.5% NaOCl. After the teeth were sterilized with ethylene oxide, a bacterial solution of *Streptococcus anginosus* was placed inside the canals for 2 hours. A single coarse paper point was then used to remove the fluid from the canal and the teeth

were split, placed in sterile test tubes, incubated and the bacteria counted. The results showed that teeth with the smear layer intact had fewer bacterial counts and the bacteria was only on the surface of the smear layer. The teeth where the smear layer was removed had a higher bacterial count and it was evident that there were bacteria inside the dentinal tubes. (15)

Following Drake's study other endodontists began looking into this phenomenon. In a similar study done by Calas et al they compared bacterial contamination in teeth where the smear layer was removed and in teeth where it wasn't removed. In their study, a smear layer was formed on blocks of bovine incisor dentin. The experimental blocks were rinsed with 6% citric acid for 5 minutes then with 6.25% NaOCl for 10 minutes to remove the smear layer, whereas the control blocks were rinsed only with distilled water. The blocks were then rinsed and sterilized prior to inoculation with two strains of *P. nigrescens*. After an incubation time of three hours, the bacteria adhering to the dentin surface were counted by direct examination with SEM. The results of this study were the opposite of Drake's study, the teeth where the smear layer was removed had a lower bacterial count in comparison to the teeth where the smear layer was not removed.(16)

Besides being able to remove the smear layer to allow for better disinfection of the dentinal tubules, there was another theorized advantage to removing the smear layer. It was thought that removing the smear layer would allow sealer to penetrate into the tubules and enhance the seal of the gutta percha. Kokkas et al.'s study in 2004 showed sealers were able to penetrate further into the dentinal tubes of teeth where the smear layer was removed in comparison to teeth where the smear layer was not removed. In their study the teeth were divided into two groups: Group A had the smear layer left intact and in Group B the smear layer was removed with a 3 ml rinse with 17% EDTA. Each group was obturated with gutta percha and one

of three sealers: AH Plus, Apexit or Roth's 811. After 15 days the teeth were longitudinally split and the penetration of the sealers into dentin was measured using SEM at the coronal, middle and apical levels. The results of this study showed that sealer penetrated deeper in the group in which the smear layer was removed. This and many other studies showed the benefits of using 17% EDTA during endodontic treatment to aid in disinfection of the dentinal tubules and increased sealer penetration. (17)

Chlorhexidine has been used as an irrigant during endodontic treatment for several reasons. One of the reasons is that it has similar properties to 5.25% NaOCl while at the same time not being too harmful to the periapical tissues. This was shown in a study done by Jeansonne et al. in which they compared the antimicrobial properties of chlorhexidine and NaOCl. In their study, 62 extracted human teeth were instrumented using chlorhexidine, NaOCl, or saline as irrigants. Microbiological samples were then taken from the teeth immediately after accessing the canal, after instrumentation and irrigation, and after standing in an anaerobic atmosphere for 24 hours. The results of their study showed that both chlorhexidine and NaOCl significantly reduced the numbers of post-irrigant positive cultures and colony-forming units in comparison with the saline irrigated teeth. One important thing that was noted in the chlorhexidine group was that chlorhexidine was unable to dissolve any tissue remaining in the canals. However, Jeansonne et al. still recommended the use of chlorhexidine as an irrigant, especially in cases where the apex is open, presence of perforations, and/or if the patient is allergic to NaOCl.(18)

Another advantage in using chlorhexidine as an irrigant is in its residual antimicrobial activity, a.k.a substantivity, that has been shown to present for days to weeks after treatment. This was demonstrated by a couple of different studies. One study was done by White et al., in

which they evaluated chlorhexidine's substantivity at different time intervals. This was done by instrumenting the extracted teeth while irrigating with 2% Chlorhexidine, filling the canals with sterile water, and taking samples of the root canal fluid with paper points at 6, 12, 24, 48, and 72 hours after treatment. The results of their study showed that chlorhexidine's antimicrobial properties were evident in all teeth even at the 72 hour mark. (19)

In a similarly designed study to the one done by White et al., Rosenthal et al. evaluated chlorhexidine's substantivity at three, six and twelve weeks. They also checked to see if the residual CHX detected from dentin samples remained antimicrobial by mixing the extracts with cultures of *E. faecalis* for 30 minutes. The results of their study showed that chlorhexidine antimicrobial activity was present even at 12 weeks.(20)

Even with chlorhexidine's many advantages it still has a couple of disadvantages. One disadvantage was already mentioned before in Jeansonne et al and Clegg et al studies and that is that chlorhexidine is not able to dissolve organic/necrotic tissue. Another disadvantage of using chlorhexidine is a that it reacts negatively with other endodontic irrigants such as NaOCl and EDTA. A study done by Basrani et al. showed that mixing chlorhexidine and NaOCl results in the formation of a precipitate. That precipitate is para-chloroaniline, a precipitant with potential carcinogenic effects according to Rasimick et al. Rasimick et al. also showed that mixing chlorhexidine and EDTA results in the formation of a white salt. However, unlike para-chloroaniline, the white salt does not seem to have any negative side effects. They also mention that flushing the canal with saline in between can reduce the chances of any of the precipitants forming, but the fact that these precipitants can occur is enough to deter many Endodontists from routinely using chlorhexidine as an irrigant during endodontic treatment.(21,22)

Roth's sealer was used in the study for several reasons. It is one of the most common root canal sealers used in Endodontics. It has been used for a long period of time due to the fact that it satisfies many of Grossman's ideal properties of a root canal sealer, such as: providing an excellent seal, radio-opaque, bacteriostatic, inexpensive, and ease of mixing and handling. Roth's sealer has also been shown to be effective against *E. faecalis*. Mickel et al.'s study in 1999 showed that when compared to other sealers such as CaOH sealers and AH26, Roth's sealer was the most effective against *E. faecalis*. This was done by inoculating 17 blood agar plates with *E. faecalis* and then placing five discs on each plate containing: Roth's sealer, Kerr sealer, Sealapex, AH-Plus and control ampicillin. The plates were then incubated at 37° C and zones of inhibition were measured at 24 and 48h. The results were that Roth's sealer showed the largest zone of inhibition at both time periods.(23)

Time to leakage in this study ranged from 4-42 days, with the fourth day being the first day leakage was evident and the forty-second day being the last. This is somewhat similar to other bacterial leakage studies. Leakage occurred between 10-73 days with Torabinejad et al.'s study, 4-30 days with Khayat et al.'s study, and 7-90 days with Magura et al.'s study. Eldeniz and Ørstavik's study, whose experimental design was very similar to this study, had leakage occur as early as 2-4 days and as late as 40 days (24). The reason for the early leakage times is probably related to the fact that the apices of the teeth were resected. This would lead to a larger apical size and an overall shorter root length. For example, in Torabinejad et al.'s study the teeth were instrumented to a size 40, while in this study the apical sizes were 80. In the end, resecting 3mm of the apex resulted in a larger apical size and shorter root length, which in turn would mean the bacteria would have a shorter area to traverse and more area to leak through.

In this study only nine of the 21 experimental samples leaked (42%). This is different than most of the bacterial leakage studies. In Torabinejad et al.'s study 50% of the samples leaked, 100% with Khayat et al.'s study, 100% with Magura et al.'s study, and 72% Eldeniz and Ørstavik's study. There are a couple of reasons why the leakage percent in this study is lower than others. It could be related to the use of Roth's sealer, which as previously mentioned, has been shown to be effective against *E. faecalis*. Also, while not statistically significant due to the sample size, leakage occurred less in the chlorhexidine group (14%). This could be related to chlorhexidine's substantivity, where it has been shown that chlorhexidine's antimicrobial ability persists in the canal for up to 12 weeks.

As with many studies there were some limitations and difficulties encountered in this study. One limitation is that fact that experimental set up had some flaws in it. This is evident due to the fact that nine out of the thirty teeth leaked prematurely and were treated as experimental failures. An experimental failure in this study was defined as a tooth that leaked within one day and/or it was evident that bacterial sample leaked around the tooth instead of through the tooth. The possible reason for leakage to occur around the tooth is that seal had gaps in it. Gaps occurred due to the fact that most of the teeth were oval in the buccolingual dimension and the tubes were perfect circles, therefore a gap around the mesiodistal dimension would occur. In addition to applying rope wax around the tooth three layers of super glue were applied between the tooth and test tube to prevent leakage. Unfortunately, leakage still occurred.

Another limitation of this study is the small sample size of the experimental groups: Based on the per protocol analysis, the overall power of the study was 41% for detecting a difference in the proportion of teeth that leaked. To power this study to 80% power, 17 teeth per

group would be required. However, given the average rate of experimental leakage (30%), this would suggest testing at least 25 teeth in each group to have 17 good samples in each group.

## **Conclusion**

Within the limitations of this study it was shown that leakage occurs between 4-42 days and that there was no significant difference between the different solutions in preventing leakage.



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