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Potential Antimicrobial Methods for Provisionalizing Teeth After Endodontic Treatment

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Potential Antimicrobial Methods for Provisionalizing Teeth After Endodontic Treatment

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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Abstract

POTENTIAL ANTIMICROBIAL METHODS FOR PROVISIONALIZING TEETH AFTER ENDODONTIC TREATMENT

By Laura T. Garden, DDS

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Department of Endodontics

Objective: To evaluate the effectiveness of a Chlorhexidine soaked cotton pellet on bacterial leakage.

Methods: Fifty-one extracted teeth, including six controls, were instrumented, obturated, and sealed with either a cotton pellet (CP), 2% Chlorhexidine soaked cotton pellet (CHX), or a Permaflo orifice barrier (OB). 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 and phenol red. The latter was checked daily for turbidity, indicating bacterial leakage.

Results: All open and closed control groups had leaked by day 7. The average CP tooth survived for 13.1 days whereas the CHX and OB teeth leaked by an average of 5.8 days.

Conclusion: There is insufficient evidence to support the use of a Chlorhexidine soaked cotton pellet. The results were not as expected and the study design should be re-evaluated.

Introduction

In the field of endodontics, one of the main goals is to significantly reduce, or completely eliminate, the presence of bacteria in the root canal system. As described by Kakehashi et al in 1965 (1), bacteria in the root canal system causes the development of pulp necrosis and a periapical lesion. Thus, striving to create an aseptic environment will increase the chance of success and also prevent reinfection. Adequate mechanical debridement, irrigation, and obturation are the main endodontic practices applied in order to reduce the microbiota present in the root canal system. These areas are often studied and new products and methods are constantly being developed in order to increase bacterial elimination while maximizing efficiency. However, as discussed by Ray and Trope (2), even the most technically sound root canal will be at an increased risk for failure if an adequate coronal seal is not maintained. Indeed, this is one of the most common reasons for root canal failure (3, 4).

A common treatment after completion of nonsurgical root canal therapy is the placement of a cotton pellet and temporary restoration by the endodontist. A survey completed by Vail et al (5) found that eighty percent of the surveyed Diplomates of the American Board of Endodontics prefer to place a cotton pellet beneath the temporary restoration. This is in alignment with the desires of most general dentists (6). This method is preferred since cotton pellets are readily available, easy to place, and helpful for the restorative dentist to locate the chamber and prevent iatrogenic perforation or over preparation of the tooth. Drawbacks of the cotton pellet include that they become contaminated quickly if the temporary is not well sealed. Parris et al. found bacterial contamination of the cotton pellet as early as one week (7). Further, Newcomb et al

demonstrated entrapment of the cotton fibers within the temporary can cause leakage within minutes (8). There are no studies to date that have been able to prove any sort of antimicrobial or sealing capabilities of a cotton pellet. Again, this material is the endodontic spacer of choice simply due to availability and ease of retrieval. Although endodontists are qualified to place a final restoration, it is often the preference of most general dentists that the placement of the core build up be completed in their own office, often in conjunction with the crown preparation. Further, placement of a provisional restoration by the endodontist is a mainstream technique from an efficiency standpoint and also maintaining the idea that the general dentist is the restorative expert, allowing endodontists to focus on quality root canal treatment.

It is stressed to patients that the tooth can become re-infected if a final, sealed restoration is not placed in a timely fashion. Often, the recommendation is to have the core build up placed within two weeks (9). Studies have found bacterial leakage within five days when using Cavit (10), with an average microbial penetration of 13-18 days (11). Multiple studies have found that the majority of studied provisional materials will leak within the first 12-14 days (9, 10). Even more grave implications are seen when the provisional is lost or the tooth is left unsealed. Swanson and Madison discussed the ability of saliva to penetrate 79-85% of the root length with the absence of a coronal seal (12). These findings were corroborated by Torabinejad et al (13) who detected contamination to the apex by 90% of the *S. epidermidis* samples within 30 days, and anywhere between 10-73 days by *P. vulgaris*. The clinical implications of these unrestored teeth include significantly more inflammation in the periapical tissues after five months versus those with a coronal restoration (14). Further, simply having bacteria contaminate the coronal aspect of an endodontically treated tooth can have inflammatory responses seen around the apex due to the apical movement of smaller endotoxin particles (15).

In an academic setting or emergency based private practice, final restorations are not placed reliably. This is due to increased time between available appointments in a dental school and lack of an established dentist-patient relationship in emergency based care. Often, it is seen that a patient will have root canal therapy completed, be relieved from pain, and never go to a regular dentist to have the provisional restoration replaced with a final.

To combat this challenge, clinicians have started placing what is called an “orifice barrier” that consists of a bonded composite restoration or resin modified glass ionomer into the orifices of the canals to protect the gutta percha from any leakage that may occur if the temporary restoration is inadequate (16). The use of orifice plugs, with 2mm thickness, has been shown to prevent microleakage and decrease the incidence of periapical inflammation (17). Specifically, the use of a resin material or glass ionomer has been shown to provide a better seal than Intermediate Restorative Material (IRM) or another material with zinc oxide eugenol (18, 19). Drawbacks to this method include: additional chairside time, investment of materials, and an inability to place a post in the orifice if desired. Endodontists have also commonly used a flowable composite that is colored to provide an orifice barrier. The use of a flowable composite is more cost effective than a glass ionomer or resin modified glass ionomer and also more efficient. Further, having a contrasting color (for example purple, as seen with Permaflo flowable resin) makes it easy for the general dentist to identify and then remove this material with a Cavitron or ultrasonic instrument if a post space is desired.

Another option that is a compromise between the traditional plain cotton pellet and an orifice barrier is an interim restoration that is inexpensive and quick but has either sealing or antimicrobial ability. This option is a more novel idea and utilizes chlorhexidine. Specifically, it involves soaking a cotton pellet in chlorhexidine and placing it below the temporary restoration.

Chlorhexidine has been a well-researched chemical in endodontics, known for its ability to kill bacteria and even provide substantivity from several days to one week (20-22). It has been studied as an endodontic irrigant and an intracanal medicament, but never as an endodontic spacer below the provisional restoration post obturation (23, 24). This study utilized a cotton pellet soaked in chlorhexidine and evaluated its ability to prevent bacterial leakage in obturated teeth compared to a plain cotton pellet or a bonded orifice barrier. The purpose of this study was to evaluate the effectiveness of a chlorhexidine soaked cotton pellet in an ex vivo model on single rooted teeth. The study design was inspired from a 2010 study in an academic institution that was evaluating the bacterial leakage of provisional restorative materials (9). Days until bacterial contamination of the roots were measured. These findings were compared to the results of extracted teeth temporarily restored via a plain cotton pellet or orifice barrier and evaluated if there were potential clinical applications to this method.

Methods and Materials

A series of five pilot studies were completed in order to refine the most reliable method to conduct this study. The study design was inspired by the article written by Bae et al that described an anaerobic bacterial leakage model(25). All extracted teeth mentioned in this study and the pilot studies were sterilized in formaldehyde solution for over twenty four hours. Instrumentation and obturations were completed in a disinfected clinical environment. After instrumentation, teeth were sterilized in an autoclave prior to the final obturation. Any mounting or inoculating of teeth was performed in a biological safety cabinet with the use of sterile surgical gloves.

In Pilot 1, six extracted single rooted teeth were collected. The coronal aspects of the teeth were sectioned to create a uniform length of 18mm. One tooth served as a negative control and remained closed. After a traditional endodontic access was completed on the remaining five teeth, they were instrumented to an ISO #30/04 file utilizing a crown-down technique. Irrigation with 5.25% NaOCl was utilized throughout instrumentation. One tooth served as a positive control and remained open without obturation. All other teeth were then obturated using a single cone technique with zinc oxide eugenol sealer and a master cone of size 30/04. The roots of all six teeth were then sectioned with a 330 bur to provide a remaining length of 8mm root structure, as measured from the CEJ. As previously mentioned, two teeth served as controls. Tooth #3 had only gutta percha obturation (GPO). Tooth #4 had a cotton pellet (CP) placed. Tooth #5 had a 2% chlorhexidine soaked cotton pellet (CHX) and tooth #6 had a Permaflo (ULTRADENT) orifice barrier. The teeth were mounted in the lid of a disposable polypropylene centrifuge tube at the level of the CEJ via the Dentsply light-cured Temporary Endodontic Restorative Material

(T.E.R.M.). The coronal aspect of the tooth was placed facing inside the test tube with the sectioned root aspect suspended. See Figure 1. Coronal aspect of tooth mountedfor a photograph of the coronal mounting and Figure 2 for the root end mounting.

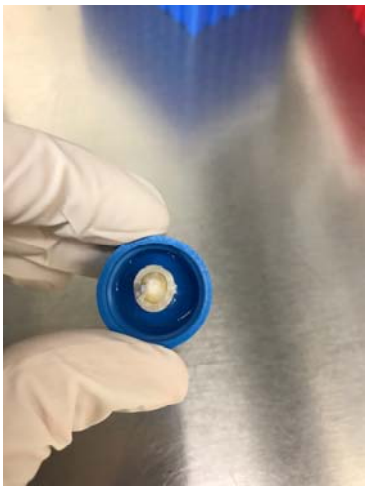


Figure 1. Coronal aspect of tooth mounted

Figure 2. Root aspect of tooth mounted

The upper chamber was filled with 4mL of a 1:100 dilution of *Streptococcus sobrinus (s.s.)* 5 x 10^8 CFU/mL and sterile tryptic soy broth (TSB). This test strain was chosen based off of the article written by Henriques et al (26) describing the most common microbial populations in infections refractory to endodontic treatment. The lower chamber consisted of 1.8mL of 1:40 dilution of filtered phenol red (PR) and sterile tryptic soy broth suspended in one well of a 24-

well microtiter plate.

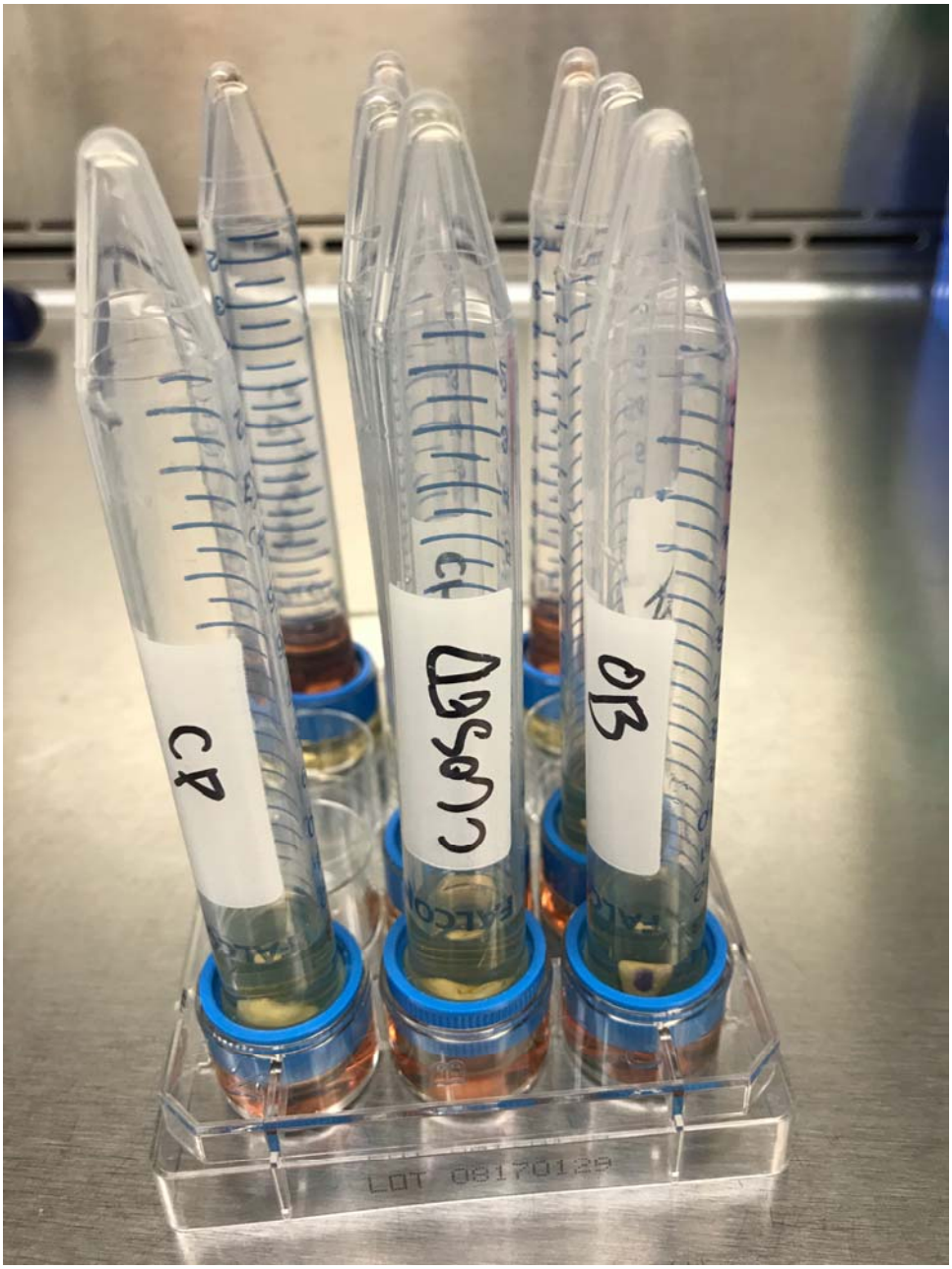


Figure 3. Pilot 1 Set-Up

After inoculation via tightening the lid into its respective upper chamber and seating it firmly into the lower chamber, the mounted teeth were placed in an incubator and were evaluated daily. Figure 3 shows the set-up of the teeth. The yellow color reflects the upper chamber with the *S. sobrinus* and TSB inoculation and the lower chamber has the sterile PR:TSB mixture.

Leakage was determined by visualizing turbidity or a color change in the lower chamber. The top three wells in Figure 4 are an example of the color change from red to yellow that occurs with bacterial leakage. When turbidity or this yellow color was seen, the teeth were determined to have leaked.

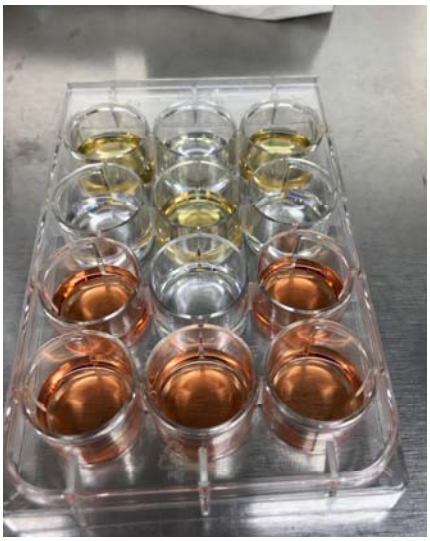


Figure 4. Example of color change in lower chamber

The positive control leaked after three days as expected. The pilot study was run for two weeks and no other leakage occurred. It was postulated that the *Streptococcus sobrinus* cells were no longer viable and the upper chamber was refreshed with additional *s.s.* cells. This initiated the Pilot 2 study. The results of this study showed leakage of the positive control and GPO at 3 days, CHX at 4 days, OB at 5 days, and CP at 6 days. The negative control showed leakage after 1 month. This was thought to indicate a breakdown in the T.E.R.M. and cyanoacrylate mounting since the negative control was an unprepared tooth with no other pathways of leakage. The results of the CP tooth leaking after the longest amount of time was assumed to be due to possible contamination of the other treatment groups. The disinfection protocol was modified to wiping the teeth with 5.25% NaOCl prior to mounting and a subsequent wiping of the polypropylene tube lid after mounting with 5.25% NaOCl-dampened

sterile gauze. Simultaneously, a viability study was completed to compare the viability of $5x10^8$ CFU/mL of *Streptococcus sobrinus* with $5x10^8$ CFU/mL *Enterococcus faecalis*. The purpose of this study was to use two endodontically relevant bacteria and see if there was a difference in their ability to survive without needing to refresh the inoculated chamber. This was measured by taking an overnight culture of each bacterium and adding it to tryptic soy broth to create a 1:100 dilution. The viability of the bacteria was checked daily by transferring a portion of the original culture to a new microfuge tube of tryptic soy broth, placing it in an incubator, and checking for turbidity. The presence of turbidity confirmed cell replication and, thus, that the bacteria were still thriving. The results of this study showed that the *Streptococcus sobrinus* cultures lost their viability after 10 days. On day 22, a CFU count was completed on the *Enterococcus* faecalis strains and resulted in $2.38X10^8$ CFUs, confirming maintained viability. The results of this study incited a change in the chosen inoculation strain to become *Enterococcus faecalis* in order to facilitate more persistent bacteria that could survive longer periods. This would eliminate the need to refresh the samples and potentially introduce contamination and disruption of the experimental set-up.

Pilot 3 was conducted with four new teeth to be assigned to each experimental group and the inoculation was an overnight culture of $5x10^8$ CFU/mL of *Enterococcus faecalis.* There was a positive (open) control group and the GPO group was eliminated. Further, there was no gutta percha obturation in any of the teeth. The set-up was conducted in the same manner as Pilot 1 except the growth media was changed to brain-heart infusion broth (BHI). This media is more typically used for *Enterococcus faecalis.* The results showed leakage of the open group after 2 days, CHX after 3 days, OB after 4 days, and no leakage of the CP group. There was not an explanation for there not being any leakage in the CP group. Pilot 4 was conducted in order to

see if there was a difference in results if the experimental set-ups were inverted. This helped to eliminate gravity as a factor for bacterial leakage and simulated a clinically relevant environment for maxillary teeth. Some of the teeth were mounted with the coronal portion on the lower part of the lid and the roots in the upper portion. For these teeth, the experimental set-up was also flipped with the inoculated chamber being in the well and the PR:BHI solution in the upper chamber. A new sample of eight teeth were prepared in a similar fashion to Pilot 1 and the inoculum was $5x10^8$ CFU/mL of an overnight *Enterococcus faecalis* culture. There was a positive control of a wide open tooth and a negative control of a closed tooth. The GPO group was eliminated. None of the teeth received any gutta percha obturation material. The experimental groups were: CP, CP inverted, CHX, CHX inverted, OB, and OB inverted. The teeth were checked for leakage (turbidity) at the end of 5 days. By day 5, all teeth showed leakage except for the CHX and CHX inverted groups. There did not appear to be any difference or benefit to inverting the set-up.

Lastly, Pilot 5 was conducted with an increased number of samples. There were 51 total teeth. Six teeth served as controls. There was a positive control group that was a wide open tooth and consisted of three teeth. The other three control teeth were assigned to the negative, or closed, control group where the teeth were not accessed or instrumented. The remaining 45 teeth were randomly assigned to one of three treatment groups: CP, CHX, or OB. Each group consisted of 15 teeth. All 51 teeth received a number that allowed for the examiners to be blinded to the assignments. All teeth were prepared with an experimental set-up similar to Pilot 1 except they were instrumented to an ISO size 35/04 and BHI was used as a growth media instead of TSB. In place of T.E.R.M., the teeth were mounted with a permanent composite restoration followed by a layer of cyanoacrylate. An additional layer of nail polish was added to further seal

the outer aspects of the teeth and prevent leakage from the junction where the teeth were mounted to the polypropylene lid, as referenced in previous studies (27, 28). The teeth were checked daily for turbidity every day for 6 days and then results were compiled at the end of 13 days. The results showed the following teeth leaked on day 1: 3 open teeth, 1 OB, 1 CHX. On day 2, 1 closed, 3 OB, 2 CP, and 2 CHX had leaked. By day 6, 3 open controls, 2 closed controls, 6 CP, 4 CHX, and 5 OB teeth had leaked. These results were the same by day 13. Teeth were randomly examined under the microscope to verify absence of any contamination. Single and double diplococci were confirmed via microscopic examination, which is consistent with *Enterococcus faecalis.* It was postulated that there was still leakage occurring along the exterior surface of the tooth allowing communication between the upper chamber and lower chambers. It was determined that a delivery method would be used for the final study to suspend the inoculated solution directly into the access openings and prevent any potential leakage along the external aspect of the tooth.

It should be noted that the teeth used in the experimental study were the same used in Pilot 5. After the Pilot 5 study, all teeth were disinfected by soaking in 5.25% NaOCl for one hour. The additional pilot holes and subsequent mounting of the Monoject Irrigation Syringe Tips in all teeth was an additional step that was performed after Pilot 5.

In the experimental study, 51 teeth were used. The instrumentation, obturation, and mounting of the teeth were completed by one of four researchers: one second year endodontic resident and three fourth year dental students. The teeth were randomly assigned to one of five groups: a positive (open) control group (n=3), a negative (closed) control group (n=3), or one of three treatment groups (n=15 per group). The three treatment groups consisted of a sterilized plain cotton pellet #2 (CP), a 2% chlorhexidine soaked #2 cotton pellet (CHX), and an orifice

barrier (OB) group. The coronal aspects of the teeth were sectioned to create a uniform length of 18mm. For the closed control group (n=3), an initial pilot hole was created to allow suspension of a curved Monoject Irrigation Tip and set aside. There was no communication with the pulp chambers seen. For the remaining 48 teeth, a traditional endodontic access was completed and they were instrumented to an ISO #35/04 file utilizing a crown-down technique. Irrigation with 5.25% NaOCl was utilized throughout instrumentation. After instrumentation, as described in the Pilot 5 set-up, the teeth had been sterilized via autoclave. Next, the teeth were obturated using a single cone technique with zinc oxide eugenol sealer and a master gutta percha cone of size 35/04. The roots of all 51 teeth were then sectioned with a 330 bur to provide a remaining length of 8mm of root structure, as measured from the CEJ. The teeth were suspended at the level of the CEJ in a polypropylene Falcon™ 15ml Conical Centrifuge tube lid (FISHER SCIENTIFIC). This was via a standardized opening that had been created with a 330 bur in the lid. The teeth were secured on both the coronal side and root side with a layer each of light-cured composite resin (Z250), cyanoacrylate (KRAZY GLUE), and nail polish (ESSIE). The coronal aspect of the tooth was placed suspended inside the centrifuge tube with the sectioned root aspect suspended on the outer aspect of the lid.

The test groups were then prepared. The CP and CHX groups had the cotton pellets placed inside the access with light condensing. The OB group was placed via 38% phosphoric acid etch (PULPDENT), OptiBond XTR (KERR), and 2mm of light cured Permaflo. This can be seen in Figure 5.

Figure 5. Example of OB group mounted

All 51 teeth then received the tip of a curved Monoject Irrigation Syringe (MONOJECT) that had been previously separated from the syringe. The curve of the tip was placed within the access of all teeth and the pilot hole of the 3 negative control teeth. It was secured with light-cured flowable composite (KERR). A total of 80 microliters of sterile BHI was added to each Monoject tip in 20 microliter increments with a micropipette. The small increments were utilized to allow delivery to the tip of the syringe and prevent any air bubbles. If air bubbles were detected, the BHI was aspirated and injected again. The 15-ml Falcon ™ Conical Centrifuge Tubes served as the upper chamber and were screwed onto the lids. Figure 6 shows the root end suspension that was inverted and placed into the wells of a microtiter plate. The photograph also shows the Monoject Tips that had been suspended in the coronal accesses.

Figure 6. Root end photograph and Monoject tips

A 1:40 dilution of phenol red(PR): brain-heart infusion(BHI) was created. The phenol red

served as a pH indicator. When the color changed from red to yellow/orange, the change in pH was indicative of bacterial growth/leakage. 1.8mL of the 1:40 PR:BHI was added to each lower chamber. A total of eight Falcon™ Polystyrene Microplates with 24 wells per plate were used as the lower chambers. Alternating wells were filled with the PR:BHI solution and the empty wells were filled with 1.8mL of sterile, distilled water to prevent evaporation of the solutions, as evident in Figure 7.

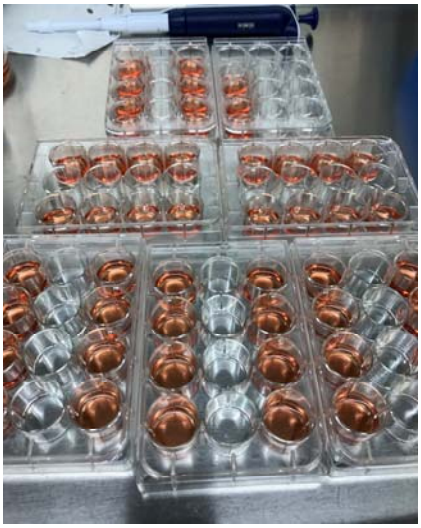


Figure 7. Initial photograph of the lower chamber wells

The experimental set-up was created without any inoculation and placed in Ziploc bags. These were then placed in an incubator for one week to verify absence of contamination. None of the wells showed color change or turbidity, indicating there was no contamination during the initial stages. Next, a 1:100 dilution of an overnight culture of $5x10^8$ CFU/mL *Enterococcus faecalis(e.f.)* in sterile brain-heart infusion (BHI) was created. The 15-ml Falcon™ Conical Centrifuge Tubes were unscrewed and 10 microliters of the *E. faecalis:*BHI solution was added to each Monoject syringe tip via a micropipette. The tubes were screwed back into place and the set-up was placed in large Ziploc bags with moistened paper towels. The bags were placed in an incubator. Day 0 was when the experiment was initiated. The teeth were checked daily during

the week for turbidity and/or color change by the same examiner. If either were seen, the day was documented and the tooth was considered to have “leaked”. The number of the tooth sample was then written on the bottom of the well to confirm it had already leaked when the wells were checked the next day. The experiment was conducted for 20 days. Figure 8 shows an example of a plate with both turbid and non-turbid wells. Cells labeled with a “T” are an example of those that were turbid and cells labeled with an “N” were non-turbid.

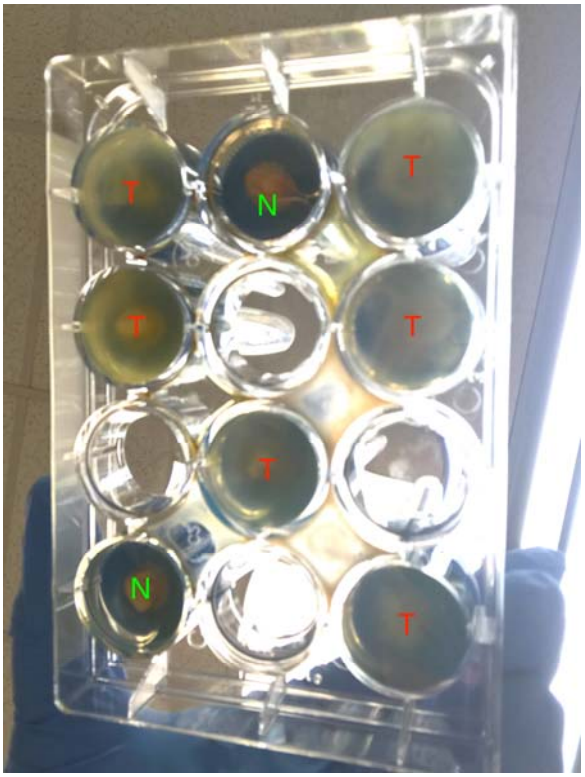


Figure 8. Turbid vs. non-turbid wells

Once the experiment was completed, a post-mortem analysis was performed to determine which wells had contamination versus *Enterococcus faecalis* leakage. *E.f.* is known to grow in media containing high salt concentrations, whereas most other bacteria, including those that might be acquired by accidental contamination would not (29). A spectrophotometric analysis was run where the remaining liquid from all wells was used to inoculate two separate microtiter plates. The first plate contained a BHI-only solution and the second plate contained BHI plus

6.5% NaCl. Figure 9 and Figure 10 show how the samples were taken to be used in the microtiter plates. These plates were analyzed and the wells that produced optical density readings well above those of media alone in the BHI-only plate but not in the 6.5% NaCl:BHI plate were considered to have been inoculated by a contaminating bacterium rather than by *E. faecalis*. Samples of *E.faecalis* and *S.sobrinus* were plated as controls. See Appendix 4 for the complete results of the analysis.

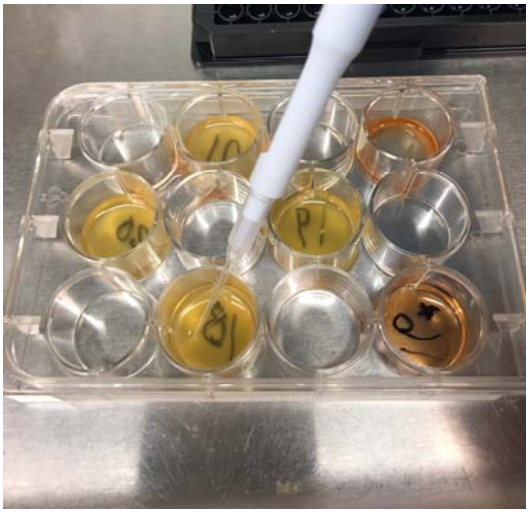


Figure 9. Sampling of well for spectrophotometry

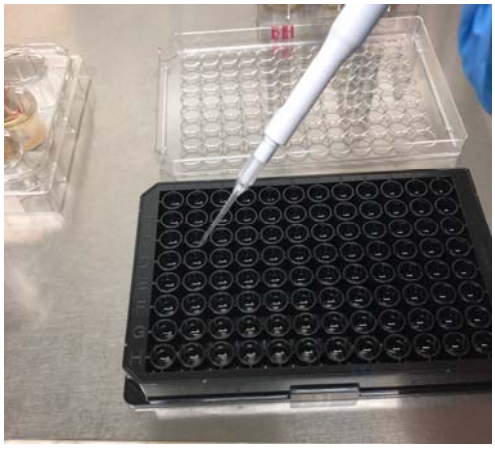


Figure 10. Loading of well for spectrophotometric analysis

Time to leakage was calculated with Kaplan-Meier life table analysis. Comparisons between groups were tested using Cox-Proportional Hazards model. The level of significance was set at $p<0.05$.

Results

See Appendix 3 for the list of all data. The 51 tubes were randomly assigned to groups and randomly ordered. Three tubes were lost (and marked “blank”); One CP tube and 2 OB tubes. As Table 1 indicates, there were an additional 6 tubes marked as contaminated.

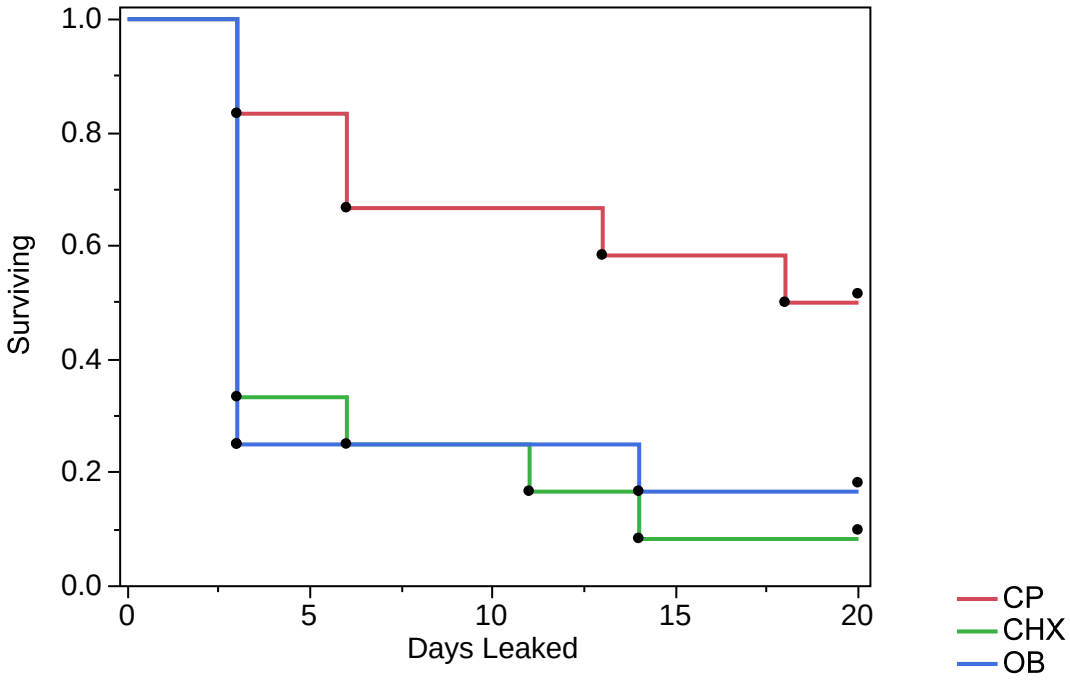
Table 1. General Results

	Unknown		Leakage	
Group	blank	contaminated	NO	YES
Closed	0	1	0	2
Open	0	1	0	2
CP	1	1	6	7
CHX	0	2	1	12
OB	2	1	2	10

Controls: It was anticipated that all of the open tubes would leak and this occurred, all on day 3. It was anticipated that none of the closed tubes should leak. However, they also all leaked (on day 3) or were contaminated on day 7.

Survival analysis: The primary analysis was for those tubes that were not contaminated. As may be seen from Figure 11, the three treatment groups differed by survival time (Wilcoxon chi-square P = 0.0103). The average CP tube survived without leakage for 13.1 days (SE = 2.0 days), whereas both the CHX and OB tubes survived an average of 5.8 days (SE = 1.4 days).

Figure 11. Kaplan-Meier survival analysis – non-contaminated samples



A secondary analysis that included the contaminated samples as failures showed similar results, see Appendix 2.

Discussion

There was a statistically significant difference in the survival of the CP tubes versus the OB and CHX groups. The CP tubes leaked after an average of 13.1 days versus 5.8 days for both the OB and CHX groups. However, this is not in accordance with previous studies. As seen by both Wolcott et al (16) and Yamauchi et al(17), the use of an orifice barrier provides superior sealing versus traditional temporary materials. Although these two studies did not look at the use of a cotton pellet versus an orifice barrier directly, they did look at orifice barriers versus common provisional materials. One can presume that a bonded, permanent restorative material would seal better than a plain cotton pellet. Further, there are no studies to date showing that the use of a plain cotton pellet provides any sort of antimicrobial or sealing properties. Contrarily, Newcomb et al(8) has shown that the use of a cotton pellet will inhibit sealing if the fibers are trapped between the interface of the provisional material and the tooth.

There were six total teeth used for controls in this study. The closed group consisted of teeth that had a pilot hole created in order to mount the Monoject tube but there was no communication with the pulpal spaces. However, dentin was exposed with the potential for interactions through the tubules. The open group was compiled of teeth that were instrumented but did not receive any obturation or temporary material. To confirm an accurate study design, it was expected that the open group would all leak and the closed group would have no leakage by day 20. However, all open and closed control groups leaked by day 3 except for one closed tube that leaked by contamination on day 7. This information confirms that the study design should be re-evaluated before any conclusions are drawn. In Pilot study 1, the closed group did not show any leakage and in pilot study 2, there was no leakage until one month. In pilot studies 4 and 5, the closed control groups showed early leakage. This was potentially attributed to leakage around

the tooth/centrifuge tube lid interface and thought to have been corrected by an additional seal with nail polish. The Monoject tips were applied into the access holes to eliminate any potential leakage at the tooth/lid interface. The thought was this direct delivery of the inoculation would eliminate any concerns about potential spacing between the tooth mounting. Any gaps at this junction could allow communication between the upper and lower chambers other than through the root canal system, rendering the study design inaccurate. In hindsight, a sixth pilot study should have been created in order to confirm accuracy of the new method with the Monoject tips.

Teeth were checked daily during the week. There were no evaluations of the teeth on Saturdays or Sundays. Upon evaluation of the data, only the initial day 3 samples leaked on a Monday. These were documented as leaking on “day 3”. This could have caused the data to be biased high with too long of a survival time since the leakage could have occurred over the weekend on “day 1” or “day 2”. However, no additional samples showed turbidity on a Monday. Thus, the initial failures could have occurred earlier but this does not seem to have greatly affected the overall results.

A postmortem analysis of the closed group was performed with Methylene Blue Dye (VISTA™). The dye was applied to the outer aspect of the tooth to evaluate if leakage occurred from the upper chamber to the lower chamber. Although none of the dye penetrated, it was noted that the dye leaked from the external aspect into the access, presumably via the dentinal tubules. A future study could evaluate the effects of sealing the entire coronal aspect of the teeth instead of only the junction where the teeth were mounted. Further, if the dye leaked through the tubules in the cervical aspect, it is possible that the inoculation leaked through the tubules in the pilot hole, which would explain the leakage seen in the closed control group.

Once the experimental study was mounted, the upper chambers received a sterile brain-

heart infusion broth and were placed in an incubator for one week. None of the teeth showed any turbidity or color change in the lower wells, confirming there was no contamination in the initial set-up. However, once the spectrophotometric analysis was completed, there were indications that six of the tubes contained bacteria other than *E.faecalis* and were considered contaminated. The contaminated tubes were spread out evenly among all treatment and control groups. As seen by the survival analysis (Appendices 1 and 2) including all teeth versus those that were not contaminated, this did not affect the results. Nevertheless, contamination does indicate a flaw in the study design and further confirms a new model should be created. One limitation of this study is the high level of handling necessary that creates a potential source for contamination.

As stated previously, the OB and CHX groups leaked after an average of 5.8 days. According to Barthel et al (11), the use of Cavit allows an average of two weeks before bacterial leakage is evident. It was decided by the authors not to use a provisional material in this study since it could potentially introduce an additional variable. However, future studies could introduce the use of a provisional restoration, which would create a more clinically relevant situation and perhaps help prevent some of the leakage seen that was earlier than previously reported.

Given the information from this study, there is not enough evidence to support the use of a Chlorhexidine soaked cotton pellet as an endodontic spacer. Nevertheless, previous studies have shown the antimicrobial benefits of Chlorhexidine in endodontics (20-24). Once the experimental design is reevaluated, future research is indicated to further pursue any benefits of a 2% Chlorhexidine soaked cotton pellet beneath a temporary restoration.

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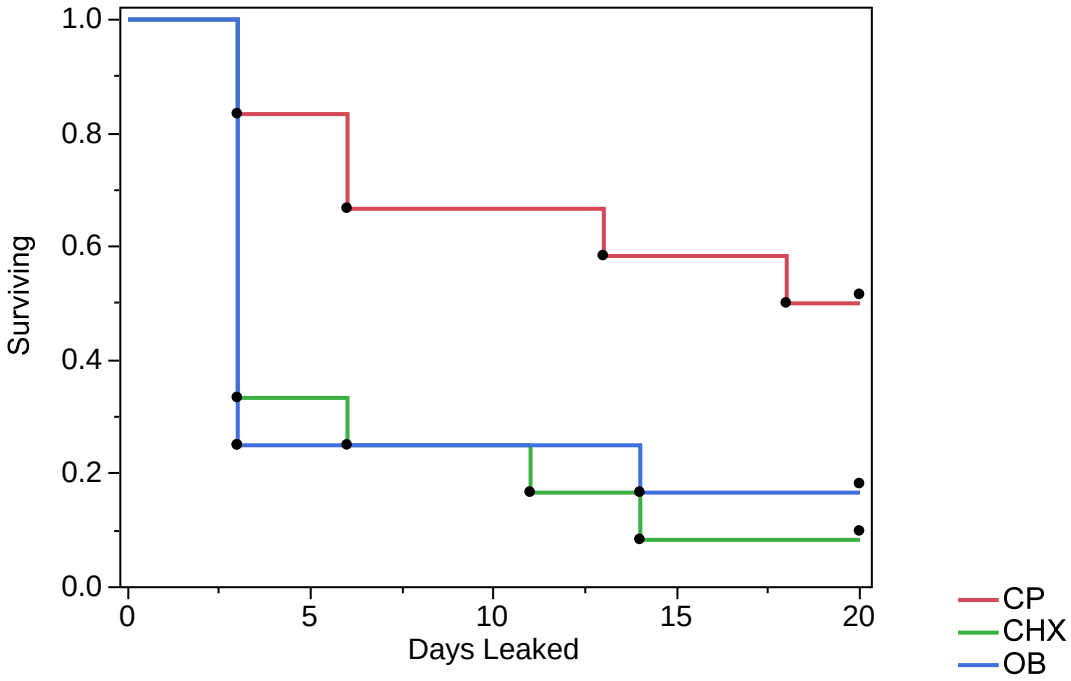
Appendices

Appendix 1 Kaplan-Meier survival analysis of non-contaminated samples

Survival Plot



Summary

Group	Number failed	Number censored	Mean		Std Error
CP	6	6	13.0833	Biased	1.98519
CHX	11	1	5.75	Biased	1.30882
OB	10	2	5.75	Biased	1.44938
Combined	27	9	8.52778	Biased	1.12624

Quantiles

Group	Median Time	Lower 95%	Upper 95%	25% Failures	75% Failures
CP	.	3	.	6	.
CHX	3	3	11	3	8.5
OB	3	3	14	3	8.5
Combined	3	3	13	3	.

Tests Between Groups

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	7.1398	2	0.0282*
Wilcoxon	9.1438	2	0.0103*

CP

Days Leaked	Survival	Failure	SurvStdErr	Number failed	Number censored	At Risk
0.0000	1.0000	0.0000	0.0000	0	0	12
3.0000	0.8333	0.1667	0.1076	2	0	12
6.0000	0.6667	0.3333	0.1361	2	0	10
13.0000	0.5833	0.4167	0.1423	1	0	8
18.0000	0.5000	0.5000	0.1443	1	0	7
20.0000	0.5000	0.5000	0.1443	0	6	6

CHX

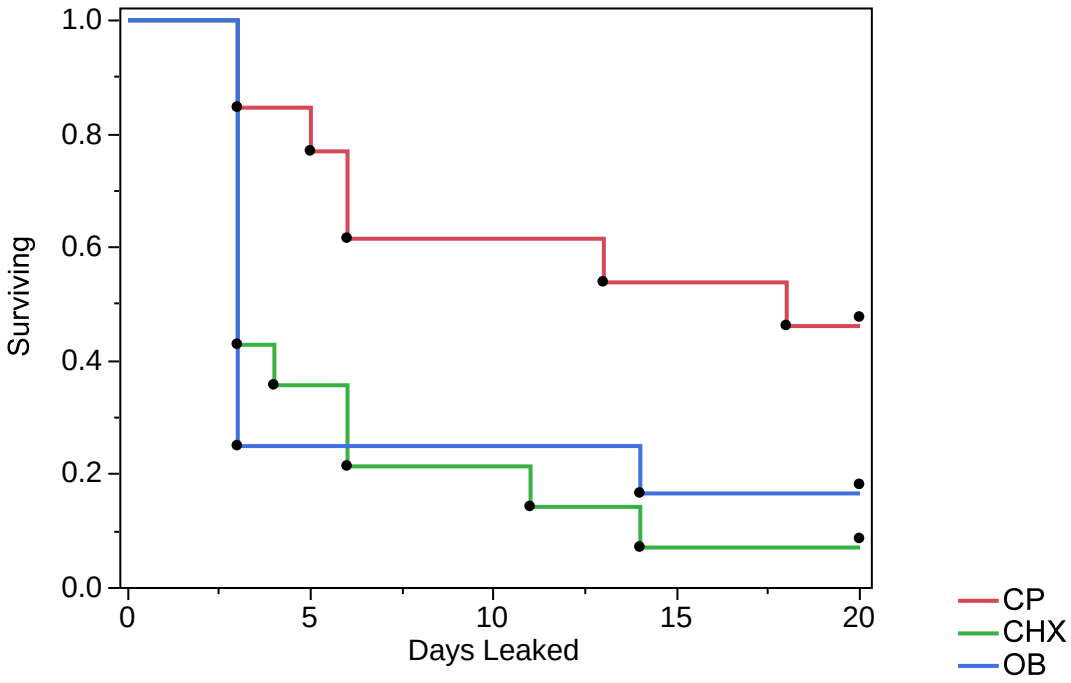
Days Leaked	Survival	Failure	SurvStdErr	Number failed	Number censored	At Risk
0.0000	1.0000	0.0000	0.0000	0	0	12
3.0000	0.3333	0.6667	0.1361	8	0	12
6.0000	0.2500	0.7500	0.1250	1	0	4
11.0000	0.1667	0.8333	0.1076	1	0	3
14.0000	0.0833	0.9167	0.0798	1	0	2
20.0000	0.0833	0.9167	0.0798	0	1	1

OB

Days Leaked	Survival	Failure	SurvStdErr	Number failed	Number censored	At Risk
0.0000	1.0000	0.0000	0.0000	0	0	12
3.0000	0.2500	0.7500	0.1250	9	0	12
14.0000	0.1667	0.8333	0.1076	1	0	3
20.0000	0.1667	0.8333	0.1076	0	2	2

Appendix 2 Kaplan-Meier survival analysis of all samples

Survival Plot



Summary

Group	Number failed	Number censored	Mean	Std Error
CP	7	6	12.4615 Biased	1.91861
CHX	13	1	5.64286 Biased	1.12065
OB	10	2	5.75 Biased	1.44938
Combined	30	9	8.25641 Biased	1.04949

Quantiles

Group	Median Time	Lower 95%	Upper 95%	25% Failures	75% Failures
CP	18	5	.	6	.
CHX	3	3	6	3	6
OB	3	3	14	3	8.5
Combined	4	3	11	3	18

Tests Between Groups

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	7.2189	2	0.0271*
Wilcoxon	9.1143	2	0.0105*

CP

Days Leaked	Survival	Failure	SurvStdErr	Number failed	Number censored	At Risk
0.0000	1.0000	0.0000	0.0000	0	0	13
3.0000	0.8462	0.1538	0.1001	2	0	13
5.0000	0.7692	0.2308	0.1169	1	0	11
6.0000	0.6154	0.3846	0.1349	2	0	10
13.0000	0.5385	0.4615	0.1383	1	0	8
18.0000	0.4615	0.5385	0.1383	1	0	7
20.0000	0.4615	0.5385	0.1383	0	6	6

CHX

Days Leaked	Survival	Failure	SurvStdErr	Number failed	Number censored	At Risk
0.0000	1.0000	0.0000	0.0000	0	0	14
3.0000	0.4286	0.5714	0.1323	8	0	14
4.0000	0.3571	0.6429	0.1281	1	0	6
6.0000	0.2143	0.7857	0.1097	2	0	5
11.0000	0.1429	0.8571	0.0935	1	0	3
14.0000	0.0714	0.9286	0.0688	1	0	2
20.0000	0.0714	0.9286	0.0688	0	1	1

OB

Days Leaked	Survival	Failure	SurvStdErr	Number failed	Number censored	At Risk
0.0000	1.0000	0.0000	0.0000	0	0	12
3.0000	0.2500	0.7500	0.1250	9	0	12
14.0000	0.1667	0.8333	0.1076	1	0	3
20.0000	0.1667	0.8333	0.1076	0	2	2

Appendix 3 Raw data of all samples

Group	Tooth	Days Leaked	Censored1	Censored2	Results
OB	1	3	0	0	Y
CP	2	20	1	1	NO
CHX	3	3	0	0	Y
CHX	4	3	0	0	Y
OB	5	3	0	0	Y
CP	6	20	1	1	NO
CHX	7	3	0	0	Y
CP	8	13	0	0	Y
OB	9	3	0	0	Y
OB	10	3	0	0	Y
Open	11	3	0	0	Y
OB	12	3	0	0	Y
Closed	13	7		0	CONTAMIN
CHX	14	3	0	0	Y
OB	15	3	0	0	Y
OB	16			0	CONTAMIN
Open	17	3	0	0	Y
CHX	18	3	0	0	Y
CP	19	3	0	0	Y
CHX	20	3	0	0	Y
CP	21	6	0	0	Y
CHX	22	6	0	0	Y
Closed	23	3	0	0	Y
CP	24	20	1	1	NO
CP	25	6	0	0	Y
CHX	26	4		0	CONTAMIN
Open	27	3		0	CONTAMIN
CP	28	18	0	0	Y
CHX	29	3	0	0	Y
CP	30	20	1	1	NO
CHX	31	14	0	0	Y
OB	32	3	0	0	Y
CHX	33	11	0	0	Y
OB	34	14	0	0	Y
CHX	35		0	0	Y
CP	36		0	0	Y
OB	37	20	1	1	NO
CHX	38	3	0	0	Y

Group	Tooth	Days Leaked	Censored1	Censored2	Results
OB	39	3	0	0	Y
CP	40	3	0	0	Y
CP	41	20	1	1	NO
OB	42	20	1	1	NO
CP	43	5		0	CONTAMIN
OB	44	3	0	0	Y
CP	45	20	1	1	NO
Closed	46	3	0	0	Y
CP	47				BLANK
OB	48				BLANK
CHX	49	6		0	CONTAMIN
OB	50				BLANK
CHX	51	20	1	1	NO

Notes: The “BLANK” results were not included in any analyses. For the primary analysis, results that were not “CONTAMIN” were included. These observations are marked with 0/1 values in the Censored1 column. A censored=0 value indicated that the tooth leaked on the day specified in “days leaked.” A tooth that did not leak was recorded as censored=1 at the end of the study (after 20 days). For the secondary analysis, results that were “CONTAMIN” were included and these values are marked with 0/1 values in the Censored2 column.

Appendix 4 Spectrophotometric Results

Software Version	2.01.14
Experiment File Path:	C:\Users\Public\Documents\Experiments\Todd\Laura BHI.xpt
Protocol File Path:	
Plate Number	Plate 1
Date	12/21/17
Time	12:08:43 PM
Reader Type:	Synergy H1
Reader Serial Number:	270729
Reading Type	Reader

Procedure Details

Plate Type	96 WELL PLATE
Shake	Linear: 0:01 (MM:SS)
	Frequency: 567 cpm (3 mm)
Read	Absorbance Endpoint
	Full Plate
	Wavelengths: 600
	Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 8

Results

Actual Temperature:	22.4

BHI plate

	1	2	3	4	5	6	7	8
A	0.684	0.05	0.68	0.772	0.615	0.05	0.738	0.668
B	0.772	0.649	0.78	0.679	0.386	0.712	0.578	0.623
C	0.635	0.55	0.487	0.534	0.718	0.656	0.592	0.051
D	0.649	0.247	0.558	0.622	0.518	0.05	1.615	0.485
E	0.688	0.09	0.526	0.779	0.054	0.458	0.549	0.437
F	0.056	0.057	0.338	0.414	0.053	0.642	0.05	0.479
G	0.785	0.057	0.058	0.493	0.514	0.547	0.567	0.277
H	0.463	0.09	0.087	0.086	0.084	0.082	0.081	0.079

Background subtracted (use .048 as background)

	1	2	3	4	5	6	7	8
A	0.636	0.002	0.632	0.724	0.567	0.002	0.69	0.62
B	0.724	0.601	0.732	0.631	0.338	0.664	0.53	0.575
C	0.587	0.502	0.439	0.486	0.67	0.608	0.544	0.003
D	0.601	0.199	0.51	0.574	0.47	0.002	1.567	0.437
E	0.64	0.042	0.478	0.731	0.006	0.41	0.501	0.389
F	0.008	0.009	0.29	0.366	0.005	0.594	0.002	0.431
G	0.737	0.009	0.01	0.445	0.466	0.499	0.519	0.229
H	0.415	0.042	0.039	0.038	0.036	0.034	0.033	0.031

BHI + 6.5% NaCl plate

	1	2	3	4	5	6	7	8
A	0.64	0.053	0.701	0.73	0.548	0.05	0.728	0.663
B	0.527	0.401	0.841	0.333	0.063	0.378	0.575	0.088
C	0.681	0.352	0.341	0.525	0.525	0.496	0.54	0.051
D	0.441	0.061	1.274	0.413	0.335	0.05	0.467	0.422
E	0.497	0.293	0.35	0.553	0.051	0.463	0.513	0.36
F	0.052	0.052	0.203	0.37	0.051	0.468	0.049	0.05
G	0.213	0.05	0.055	0.371	0.353	0.279	0.256	0.078
H	0.102	0.061	0.06	0.059	0.058	0.057	0.057	0.056

Background subtracted (use .048 as background)

	1	2	3	4	5	6	7	8
A	0.591	0.004	0.652	0.681	0.499	0.001	0.679	0.614
B	0.478	0.352	0.792	0.284	0.014	0.329	0.526	0.039
C	0.632	0.303	0.292	0.476	0.476	0.447	0.491	0.002
D	0.392	0.012	1.225	0.364	0.286	0.001	0.418	0.373
E	0.448	0.244	0.301	0.504	0.002	0.414	0.464	0.311
F	0.003	0.003	0.154	0.321	0.002	0.419	0	0.001
G	0.164	0.001	0.006	0.322	0.304	0.23	0.207	0.029
H	0.053	0.012	0.011	0.01	0.009	0.008	0.008	0.007

Readings <0.01 Negative- NO LEAKAGE

>0.01 and <0.299 Contaminated

>0.299 *E.Faecalis* LEAKED

Key – Corresponding tooth number and bacterial control samples

A	1	2	3	4	5	6	7	8
B	9	10	11	12	13	14	15	16
C	17	18	19	20	21	22	23	24
D	25	26	27	28	29	30	31	32
E	33	34	35	36	37	38	39	40
F	41	42	43	44	45	46	47	48
G	49	50	51	Ef1	Ef1	Ef2	Ef2	Ss
H	Ss							

A	Y	N	Y	Y	Y	N	Y	Y
B	Y	Y	Y	Y	C	Y	Y	C
C	Y	Y	Y	Y	Y	Y	Y	N
D	Y	C	C	Y	Y	N	Y	Y
E	Y	Y	Y	Y	N	Y	Y	Y
F	N	N	C	Y	N	Y	BLANK	BLANK
G	C	BLANK	N	Ef1	Ef1	Ef2	Ef2	Ss
H	SS							

INTERPRETATION OF RESULTS
Y=LEAKED
N=NO LEAKAGE
C=CONTAMINATED

Vita

Dr. Laura Garden was born on June 30, 1986 in Roanoke, Virginia. She received a Bachelor of Arts with Distinction from the University of Virginia in 2008 prior to attending Virginia Commonwealth University where she earned a Doctor of Dental Surgery degree in 2012. She completed an Advanced Education in General Dentistry certificate at the University of North Carolina in 2013. Dr. Garden then returned to Virginia where she worked as a general dentist at South River Dentistry in Midlothian, Virginia for three years. She is a member of the American Dental Association, Virginia Dental Association, Richmond Dental Society, and American Association of Endodontists. Dr. Garden will graduate from Virginia Commonwealth University with a Master of Science in Dentistry and a Certificate in Endodontics.