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GUTTA PERCHA CARTRIDGES AND THE RISK OF *E. FAECALIS* CROSS-CONTAMINATION

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DEDICATION

To my mother, Lynne Torigoe

DISCLAIMER

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ABSTRACT

Gutta Percha Cartridges and the Risk of *E. faecalis* Cross-contamination

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Objective: To determine if gutta percha cartridges have to potential to cross-contaminate root canals with *E. faecalis*. Gutta percha cartridge disinfection methods were also evaluated. **Methods**: Fifty-two 23-gauge medium body gutta percha cartridges were evaluated by dipping 10 mm of the cartridge tip into a brain heart infusion (BHI) broth culture containing a set concentration of *E. faecalis*. One control and three test protocols using the Elements™ Obturation Unit were evaluated:

1. Bleed cartridge tip
2. Alcohol wipe of cartridge tip
3. Bleed + wipe

The cartridges were placed into a recovery tube and ultrasonic bath to remove viable bacteria. The broth was diluted and spread on BHI agar plates incubated at 37ºC overnight. The CFU/ml recovered was calculated. **Results**: Viable cultures of *E. faecalis* were unable to be recovered consistently from the control and the three protocols performed. **Conclusions**: Cross-contamination of root canals with *E. faecalis* with the Elements™ gutta percha cartridge is unlikely.

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LIST OF ABBREVIATONS

BHI	Brain Heart Infusion
C	Celsius
CFU	Colony Forming Units
CFU/ml	Colony Forming Units per mililiter
CW	Continuous Wave
hr	Hour
IFU	Instructions for Use
GP	Gutta percha
Min	Minute
ml	Milliliter
mm	Millimeter
sec	Seconds
VBNC	Viable But Not Culturable
µl	Microliter

CHAPTER 1: Introduction

BACKGROUND

The direct relationship between bacteria and pulpal-periradicular disease was initially demonstrated on rats by Kakehashi, Stanley, and Fitzgerald in 1965.[1] Successful endodontic treatment requires the reduction of bacterial load within the root canal during the cleaning, shaping and obturation processes.[2] Canals must remain clean during the instrumentation and obturation processes.[3] The combination of endodontic treatment and aseptic techniques are paramount in preventing reinoculation and cross-contamination of the root canal system,[4] especially when treating teeth with multiple canals or multiple teeth in a single appointment.[5]

Sundqvist showed that anaerobic bacteria were most closely associated with necrotic pulps.[6] Bae et al. determined that *Prevotella nigrescens* and *Prevotella intermedia* were the most common microorganisms associated with endodontic infections.[7] However, *Enterococcus faecalis* is undoubtedly one of the hardiest bacteria often found in failed root canals.[8] Love demonstrated that *E. faecalis* can invade dentin and bind to immobilized collagen within the root canal system, possibly shielding the bacteria from elimination.[9] Figdor and Davies studied the starvation-survival kinetics of *E. faecalis* in nutrient-deprived medium and found that the microbe can enter a Viable But Not Culturable state (VBNC) and persist for extended periods of time.[10] *E. faecalis* can resist the antimicrobial properties of root canal sealers and intercanal medicaments[11] and persist in the anatomic isthmuses and fins of teeth in biofilms.[12] It is therefore assumed that if *E. faecalis* can be eradicated in a root canal system, other less-persistent

bacteria should be eradicated as well.[13] The trifecta of endodontic therapy (cleaning, shaping and obturation) aims to eliminate as much of the bacterial load as possible. However, there are steps in the process that may potentially introduce bacteria into canals.

Obturation of the root canal system has the following goals: to eliminate all avenues of leakage from the oral cavity or periradicular tissues into the root canal system and to seal within the system any irritants that cannot be fully removed during canal cleaning and shaping procedures (i.e. bacteria).[14] A common obturation method is the continuous wave technique (CW).[15] It involves coating the canal with sealer and placing a well-fitting gutta percha (GP) cone into the canal. Using specialized instruments, the apical segment of the GP cone is heated and softened while the coronal GP is removed. The softened apical GP is compacted with hand instruments to adapt it to the shape of the canal. Thermoplasticized GP delivered from a heated cartridge is used to fill the remaining space in the canal. According to the manufacturer, these cartridges are not considered sterile and are intended for use in a single patient. The Kerr Endodontics Instructions For Use (IFU) does not include instructions regarding the need for cartridge disinfection prior to or during a root canal procedure. Providers often use these cartridges in multiple canals of a single tooth or multiple teeth on the same patient without disinfection in between uses. This creates the potential for bacteria to be transferred between canal systems. Only a few studies have investigated the potential of transferring bacteria to clean canals via GP cartridges. A 1987 study by Winford et. al tested the Obtura™ obturation system to determine whether bacteria could survive passage through the heated delivery tip after contamination with *Staphylococcus epidermidis*, *Escherichia*

coli or *Enterococcus* (*Streptococcus*) *faecalis*.[16] Although no organisms were able to be recovered, the study did not screen for hardier microorganisms that may have survived the process.[16] In 1990, Glickman et al. evaluated the same microbes in a similar obturation system using lower temperature and found that microorganisms were detectable on the tip of the cartridge at low heat even after an alcohol soak.[17] A 2013 thesis by Smart researched the possibility of cross-contamination of bacteria in multiple patients using the Calamus™ Flow System. However, the study was restricted to a single type of growth medium and did not screen for hardier microorganisms.[18]

PURPOSE

Kerr Dental Elements™ GP cartridges cannot be autoclaved or heat sterilized prior to use and are packaged as non-sterile. The goal of this study was to determine the potential for Elements™ GP cartridges to cross-contaminate root canals with *E. faecalis*. Also evaluated were Elements™ GP cartridge disinfection methods and the viability of *E. faecalis* in heated gutta percha.

CHAPTER 2: Materials and Methods

PILOT STUDY 1

The first pilot study used the Kerr Dental Elements™ GP obturation unit and four corresponding 23-gauge, medium body GP cartridges to determine if bacteria could be isolated from cartridges taken directly from the manufacturer's packaging.

The cartridges were aseptically removed from the packaging and placed into 50 ml vials with 3 ml of brain heart infusion (BHI) broth, which covered approximately 10 mm of the cartridge tip. The tips were sonicated for 5 minutes (min) in an ultrasonic water bath (Branson, model 3210), and 100 µl of the resulting solution was serially diluted and plated on BHI agar. Agar plates were incubated for 24 hours (hrs) at 37ºC. Colony forming units per ml (CFU/ml) was calculated for each cartridge.

PILOT STUDY 2

The second pilot study was designed to determine if exposure time was a factor in the amount of bacteria recovered from purposefully contaminated cartridges. *E. faecalis* OG1RF cultures at concentrations of 10^2, 10^4 and 10^6 CFU/ml were prepared. The cartridges were processed as described for Pilot Study 1 except that the cartridge tips were immersed in one of the prepared *E. faecalis* OG1RF cultures (described in the main study) for either 4, 10, 30, or 60 seconds (sec) after being removed from the manufacturer's packaging.

MAIN STUDY

The *E. faecalis* OG1RF strain, which was isolated from the human oral environment, was used to purposefully contaminate cartridges.[19, 20] *E. faecalis* colonies

were streaked for isolation on BHI agar from a glycerol stock maintained at -80°C. Agar plates were incubated for 24 hrs at 37ºC. After incubation, three isolated colonies were inoculated into 2 ml of BHI broth and incubated for 24 hrs at 37ºC.

After incubation, the culture was centrifuged for 1 min, and the resulting cell pellet was re-suspended in 1 ml of BHI broth. Serial dilutions were used to obtain cultures with target concentrations of 10^2, 10^4 and 10^6 CFU/ml of *E. faecalis*. Aliquots of the cultures were serially diluted and plated on BHI agar to enumerate CFU/ml.

Four treatment conditions were evaluated:

1. Control: cartridge tip was immersed in bacteria. This was used to confirm if bacteria could be isolated from the contaminated cartridge.

2. Test condition 1: cartridge tip was wiped with an alcohol swab (70% isopropyl alcohol) after immersion in bacteria.

3. Test condition 2: cartridge heated, and GP extruded while tip was immersed in bacteria inoculum.

4. Test condition 3: cartridge heated, and GP extruded while tip was immersed in bacteria inoculum, then the cartridge was removed and wiped with an alcohol swab (70% isopropyl alcohol).

For the control and alcohol wipe groups, 10 mm of the tip of the cartridge was placed into the *E. faecalis* inoculum (either 10^2, 10^4 or 10^6 CFU/ml) for 4 sec. For the control group, the cartridge was allowed to air dry for 5 min and then placed in a 50 ml centrifuge tube containing 3 ml of BHI broth and sonicated as described in Pilot Study 1. For the alcohol wipe treatment, the cartridge was allowed to air dry for 5 min, the length of the cartridge tip was wiped with a new alcohol swab, then the cartridge was placed in a

50 ml centrifuge tube containing 3 ml of BHI broth and sonicated as described above. After sonication, aliquots of the cultures were serially diluted and plated on BHI agar in technical duplicate to enumerate CFU/ml.

For the treatment involving the heated obturation device, the obturation unit was turned on and allowed to reach the set temperature of 100ºC, as described in the IFU. A new cartridge was aseptically placed in the extruder handpiece. 10 mm of the cartridge tip was placed into one of the three dilutions of *E. faecalis* inoculum (10^2, 10^4 or 10^6 CFU/ml), and gutta percha was extruded for 4 sec. For the heat only treatment, the cartridge was allowed to air dry for 5 min and then placed in a 50 ml centrifuge tube containing 3 ml of BHI broth and sonicated as described above. For the heat and alcohol wipe treatment, the cartridge was allowed to air dry for 5 min in the heated obturation unit, then the length of the cartridge tip was wiped with a new alcohol swab. The cartridge was placed into in a 50 ml centrifuge tube containing 3 ml of BHI broth and sonicated as described above. After sonication, aliquots of the cultures were serially diluted and plated on BHI agar in technical duplicate to enumerate CFU/ml.

Four biological replicates of each treatment condition were completed on separate days.

CHAPTER 3: Results

The results of Pilot Study 1 are shown in Table 1. A single bacterial colony (equivalent to 100 CFU/ml) grew on one of the two replicate agar plates for Cartridge 1. No bacteria grew on the other agar plate. No bacteria were detected after processing the additional three cartridges.

Table 1. Pilot Study: Recovered CFU/ml from GP cartridges removed directly from manufacturer's packaging

Sample Type	Treatment	CFU/ml
Cartridge 1	None	0
Cartridge 1	None	100
Cartridge 2	None	0
Cartridge 2	None	0
Cartridge 3	None	0
Cartridge 3	None	0
Cartridge 4	None	0
Cartridge 4	None	0

Pilot Study 2 evaluated the effect of cartridge exposure time on bacterial recovery. The only condition from which bacteria were recovered in the 10^2 CFU/ml concentration of *E. faecalis* was at the 10 sec exposure time (Figure 1). In the 10^4 CFU/ml *E. faecalis* concentration, the minimum number of detectable bacteria (100

CFU/ml) were recovered after 60 sec exposure (Figure 2). The 10^6 CFU/ml *E. faecalis* concentration was the only one in which consistent levels of bacteria were recovered from cartridges (~1000 CFU/ml) regardless of exposure time (Figure 3).

The main study evaluated the effect of four beforementioned treatment conditions on bacterial recovery. No bacteria were recovered from any of the treatment conditions in the 10^2 CFU/ml concentration of *E. faecalis* (Figure 4). In the 10^4 CFU/ml concentration of *E. faecalis*, the minimum number of detectable bacteria (100 CFU/ml) was recovered in one of the four replicates of the control treatment condition; no bacteria were recovered from the other treatment conditions (Figure 5). In the 10^6 CFU/ml concentration of *E. faecalis*, the minimum number of detectable bacteria (100 CFU/ml) was recovered in two of the four replicates of the control treatment condition, and 150 CFU/ml bacteria were recovered in one of the four replicates of the alcohol treatment condition (Figure 6). The remaining treatment conditions at 10^6 CFU/ml *E. faecalis* (of heat, and heat combined with an alcohol wipe) did not yield any detectable bacteria (Figure 6). Due to the lack of bacteria recovered, a final statistical analysis was not needed for this study.

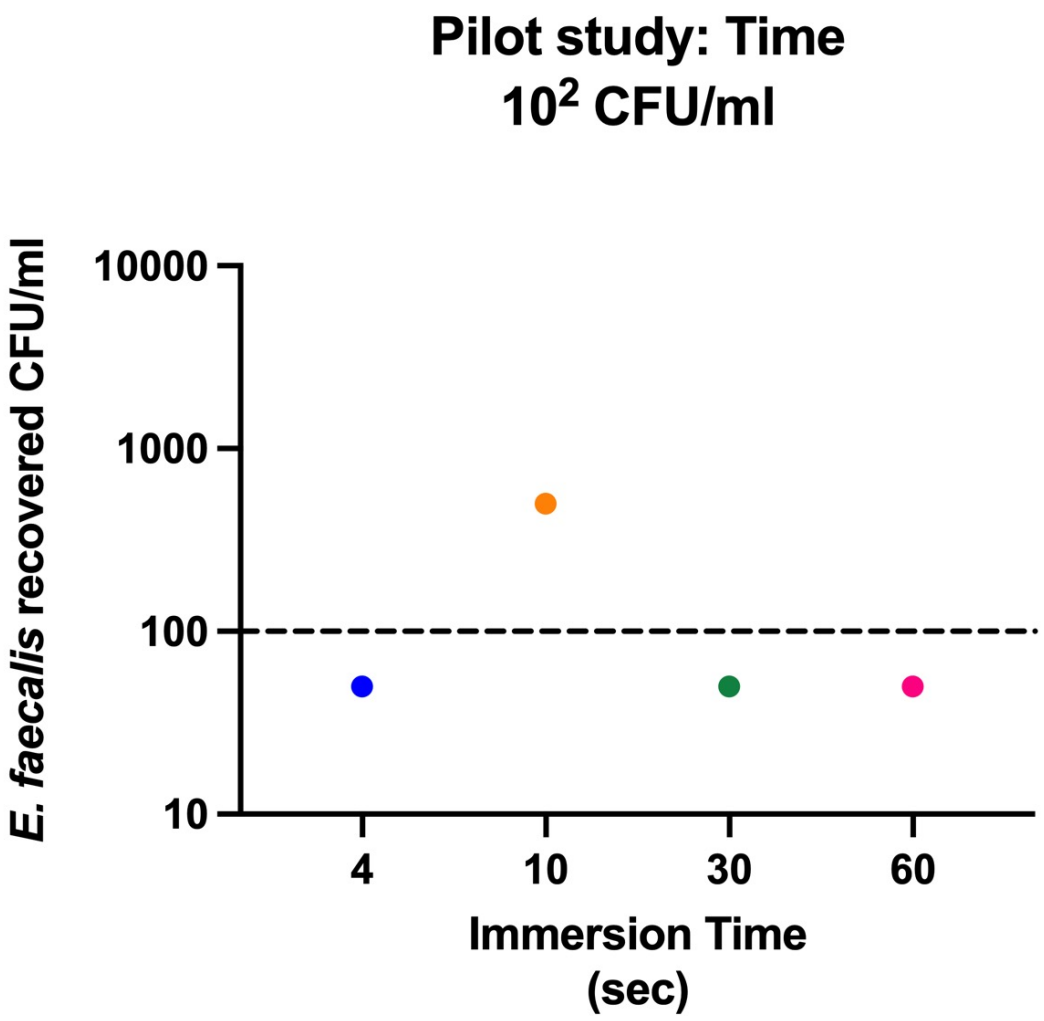

Figure 1. Time Pilot Study: Recovered CFU/ml from cartridges immersed in concentrations of 10^2 *E. faecalis*. The dotted line represents the limit of detection of the agar plating assay. Points below the line indicate that no colonies were recovered. A point on the line indicates that the minimum number of bacteria detectable by the assay were recovered.

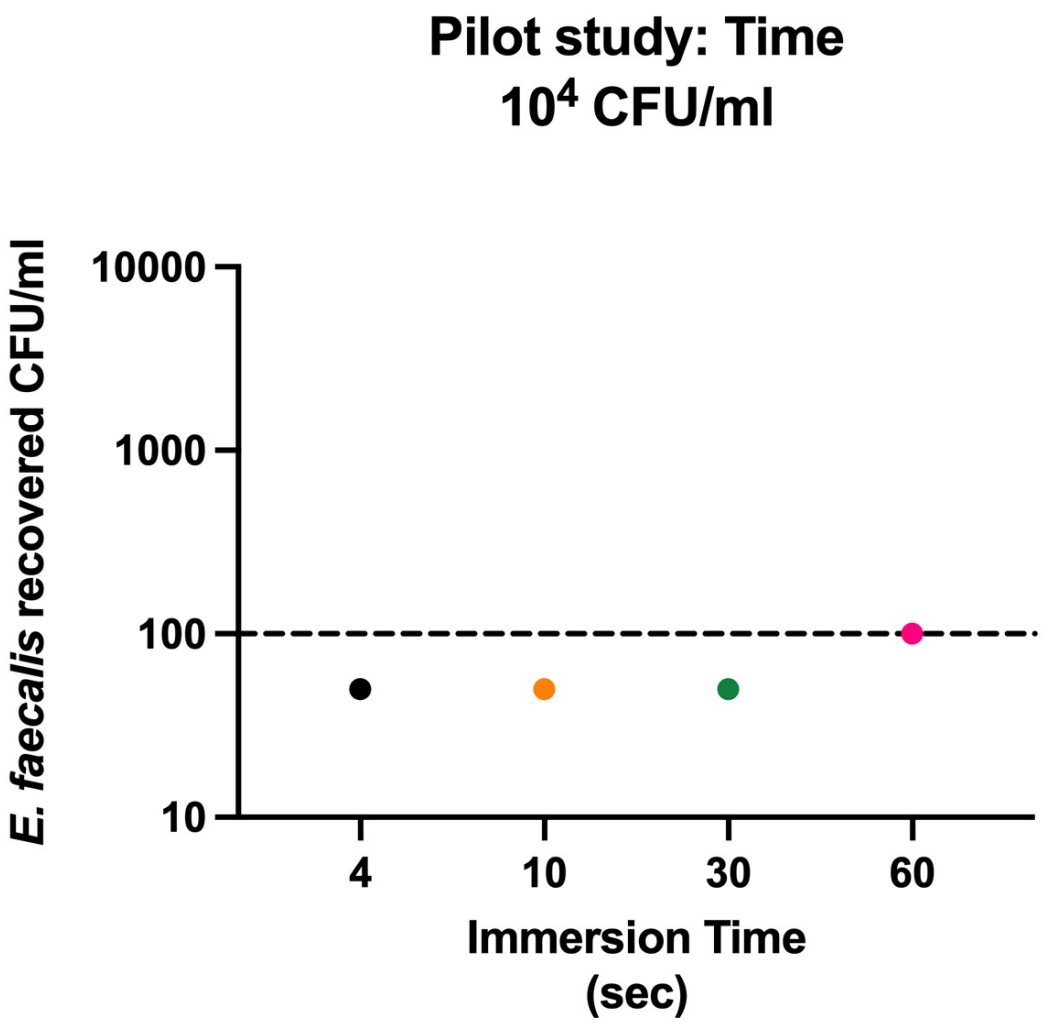

Figure 2. Time Pilot Study: Recovered CFU/ml from cartridges immersed in concentrations of 10^4 *E. faecalis*. The dotted line represents the limit of detection of the agar plating assay. Points below the line indicate that no colonies were recovered. A point on the line indicates that the minimum number of bacteria detectable by the assay were recovered.

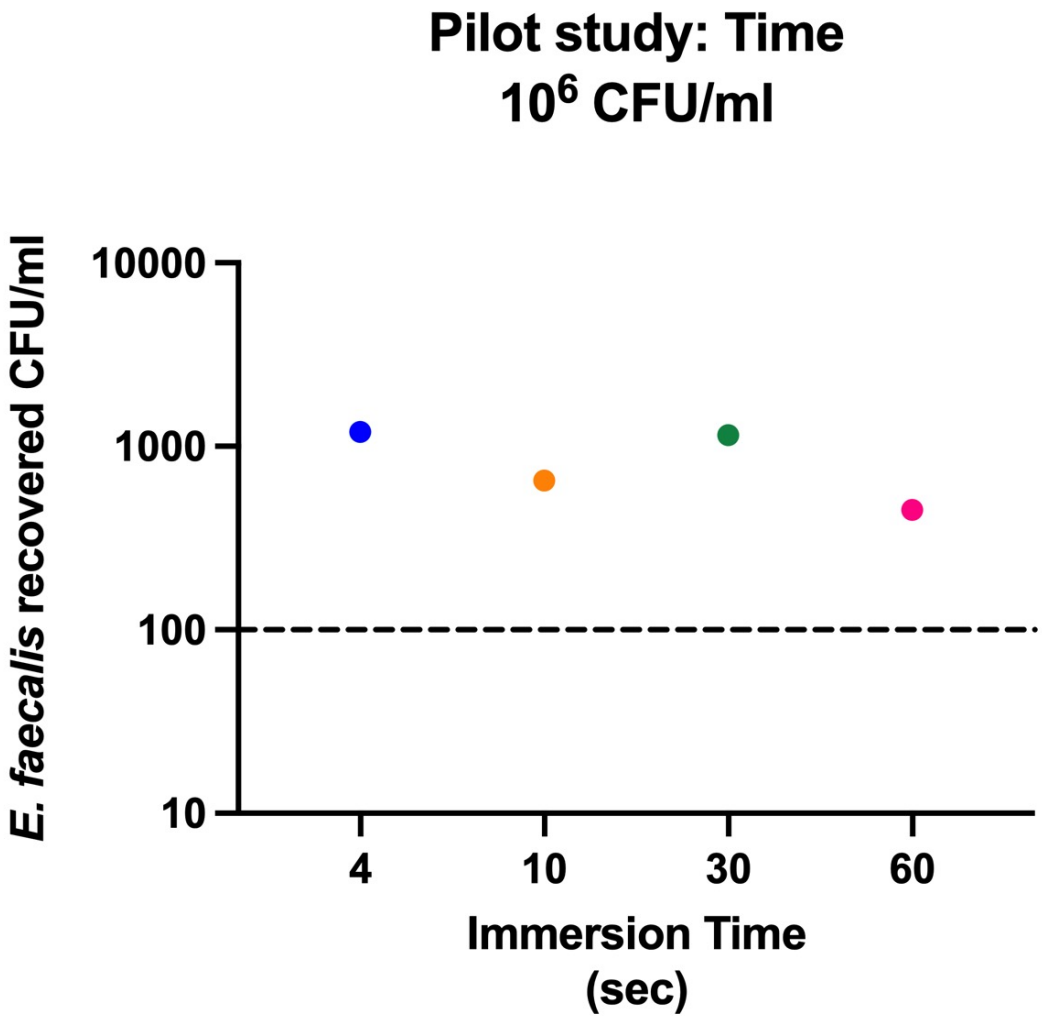

Figure 3. Time Pilot Study: Recovered CFU/ml from cartridges immersed in concentrations of 10^6 *E. faecalis*. The dotted line represents the limit of detection of the agar plating assay. Points below the line indicate that no colonies were recovered. A point on the line indicates that the minimum number of bacteria detectable by the assay were recovered.

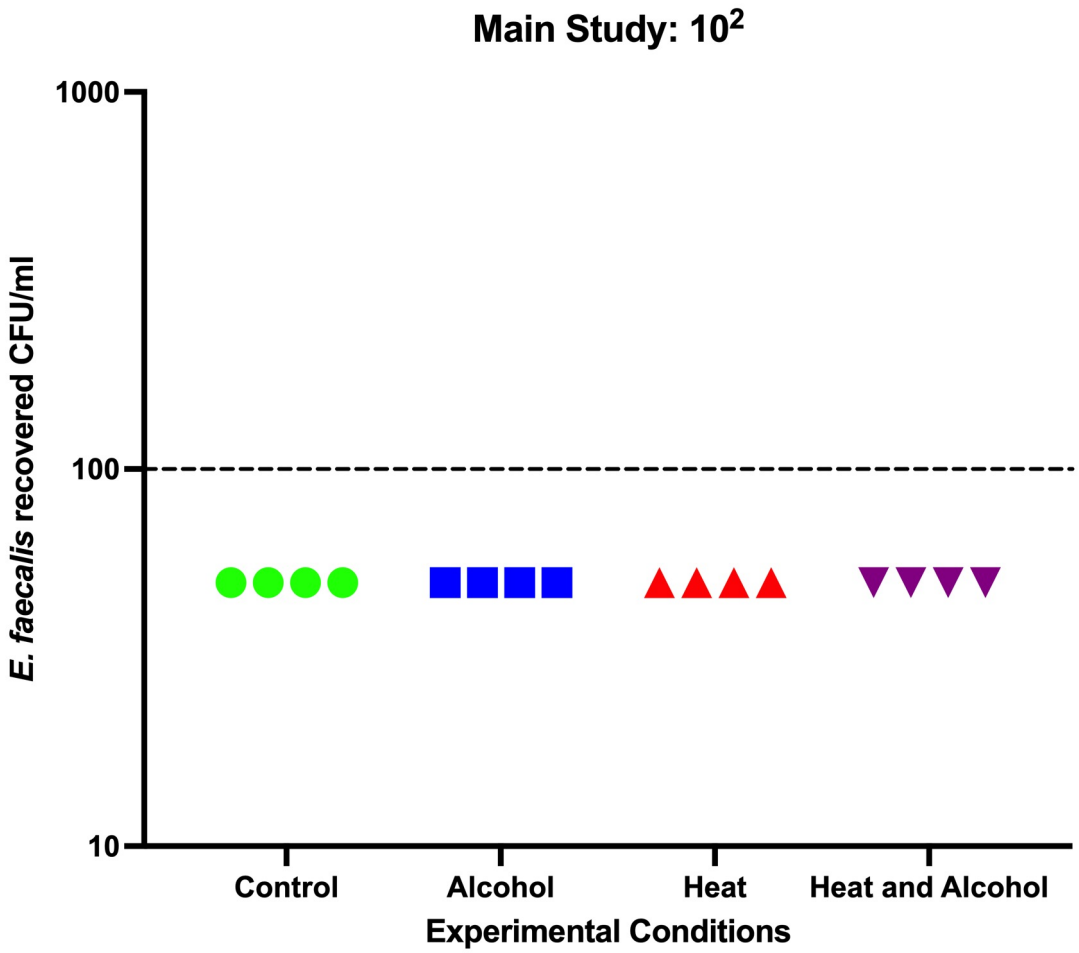

Figure 4. Recovered CFU/ml from protocols immersed in concentrations of 10^2 *E. faecalis*. The dotted line represents the limit of detection of the agar plating assay. Points below the line indicate that no colonies were recovered. A point on the line indicates that the minimum number of bacteria detectable by the assay were recovered.

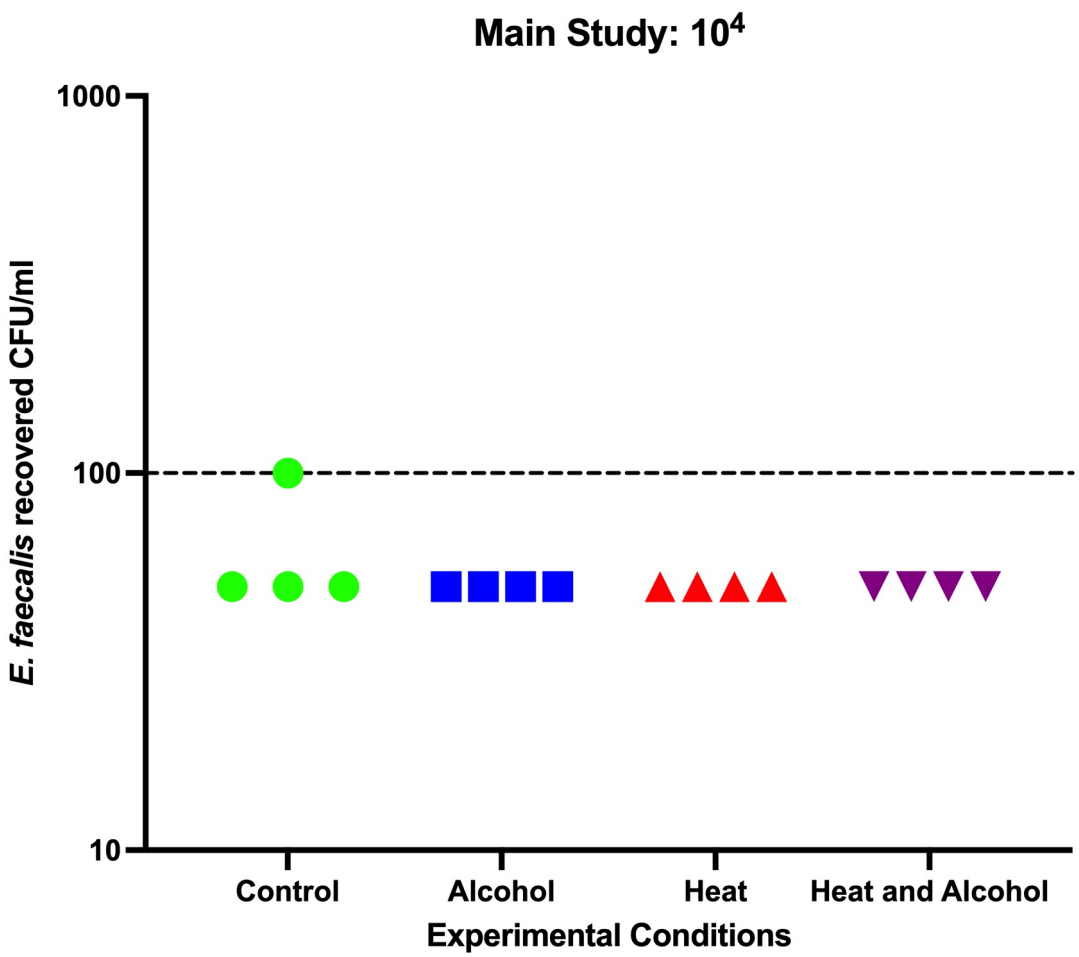

Figure 5. Recovered CFU/ml from protocols immersed in concentrations of 10^4 *E. faecalis*. The dotted line represents the limit of detection of the agar plating assay. Points below the line indicate that no colonies were recovered. A point on the line indicates that the minimum number of bacteria detectable by the assay were recovered.

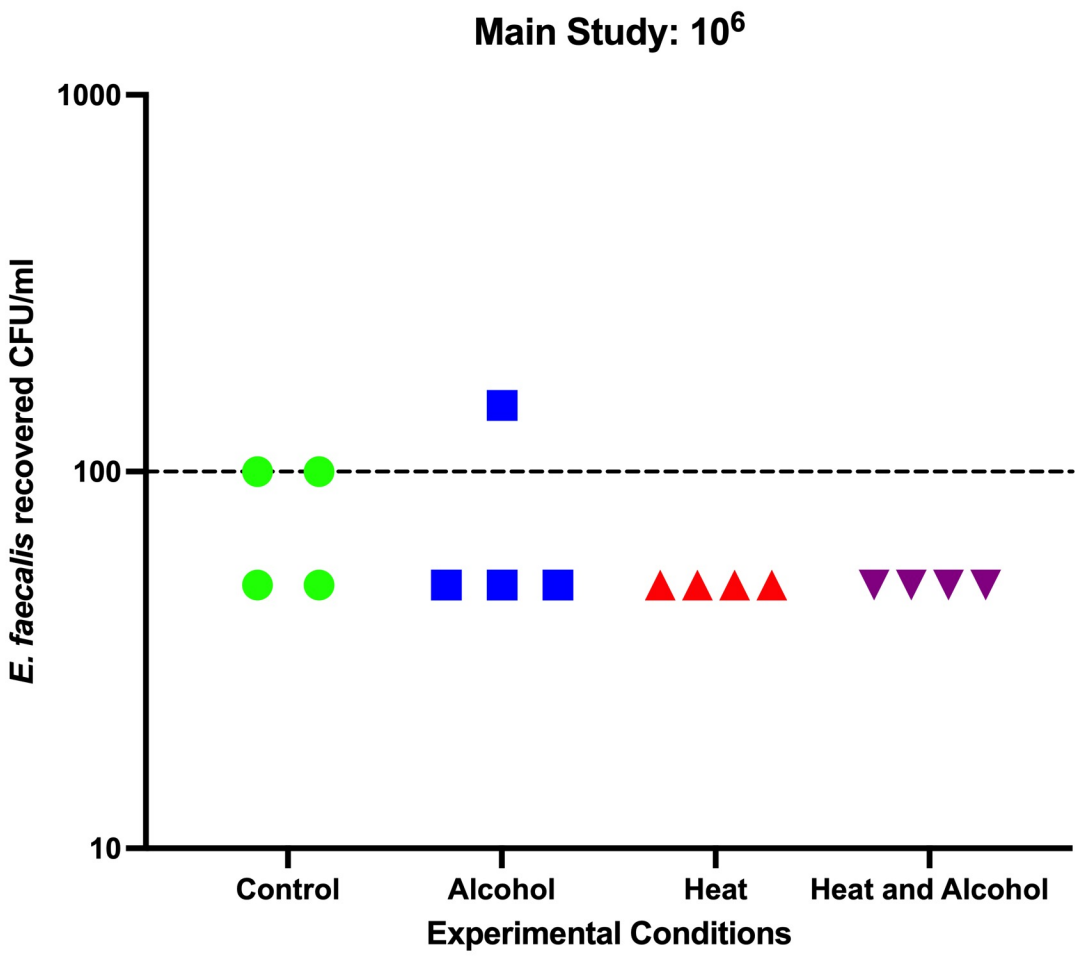

Figure 6. Recovered CFU/ml from protocols immersed in concentrations of 10^6 *E. faecalis*. The dotted line represents the limit of detection of the agar plating assay. Points below the line indicate that no colonies were recovered. A point on the line indicates that the minimum number of bacteria detectable by the assay were recovered.

CHAPTER 4: Discussion

Traditional non-surgical root canal treatment relies on a fundamental basic triad: debridement, disinfection and obturation. Removal of bacteria from the root canal system is paramount for successful outcomes. Cleaning or debridement opens the canal system to allow for disinfection. Irrigants are flushed throughout the root canal system to remove debris and reduce the bacterial load.[14] By the time the root canal is prepared for obturation, the majority of the bacteria in the main canal should have been eradicated. According to Sjögren, an expected concentration of bacterial cells is 10^2 to 10^8 prior to root canal therapy, and 10^3 bacterial cells after biomechanical preparation.[21] Higher numbers of remaining bacteria would be clinically significant, as that would indicate poor instrumentation and irrigation and raise the possibility of root canal treatment failure.

This study tested the hypothesis that GP cartridges have the potential to cross-contaminate root canals with *E. faecalis*. Overall, the results from this in vitro study demonstrate that *E. faecalis* OG1RF did not reliably and reproducibly contaminate GP cartridges when the cartridges were exposed to any of the set bacterial concentrations for only four seconds. The four second exposure time was chosen because that is the approximate duration a GP cartridge would be located within an instrumented root canal during a procedure. Although the four second exposure time of the 10^6 CFU/ml *E. faecalis* condition in the time pilot study resulted in a cartridge contamination level of ~1000 CFU/ml (Figure 3), similar levels of contamination were not achieved in the main study (Figures 4-6). More consistent levels of *E. faecalis* colonization on the cartridges may be attainable if the cartridges were exposed to higher concentrations of bacteria and/or for longer durations. However, such conditions would not be clinically relevant.

Another contributing factor for the lack of colonies recovered may be that only one strain of *E. faecalis* was tested. OG1RF was selected due to its ease of accessibility as a laboratory strain. However, there are many different genotypes of *E. faecalis* with various virulence and resistance capabilities and the OG1RF strain is one of many.[19, 20, 22, 23] Therefore, it is possible that different results would be obtained if one or more additional strains were tested for the ability to contaminate GP cartridges in a reproducible manner.

Methods of disinfecting GP cartridges were also evaluated in this study. However, the lack of cartridge contamination, as described above, suggests that it is unlikely for GP cartridges to cross-contaminate root canals with *E. faecalis*. As a result, conclusions cannot be drawn about the effectiveness of the GP cartridge disinfection techniques tested in this study.

Previous studies have noted that types of thermoplasticized GP delivery systems other than the one used in this work have applicator tip temperature ranges of 42.8 to 64.4 ºC (from base to tip). These temperatures were determined to provide sufficient heat to reduce microbial contamination.[16, 17] A review article by Fisher et. al noted that *E. faecalis* can survive heating at 60 °C for 30 min.[24] Therefore, the heat output of 100ºC of the Elements™ unit used in this study may serve as an antibacterial mechanism. Based on the results of this study, in part, it is hypothesized that the low concentrations of bacteria found in a well-instrumented canal combined with the heat of the obturation unit may prevent cross-contamination of microorganisms in other canals. This would be the case even if the same cartridge were to be used in multiple canals or in multiple teeth on the same patient.

The metallurgical properties of the GP tips may also have an antimicrobial role. Studies show that certain metals, such as copper and silver, have antimicrobial properties.[25-27] A study by Warnes et. al demonstrated the biocidal effects of copper on vancomycin-resistant *E. faecalis*.[28] The exact composition of the tips in this study have not been fully disclosed by Kerr Endodontics, but the ductility and conductive properties of the metal tips are common properties of copper.[29] Further research is needed regarding antimicrobial metals in dental instruments.

CHAPTER 5: Conclusions

In conclusion, within the limits of this study, the results indicate that there is a low risk for Elements™ gutta percha cartridges to cross-contaminate root canals with *E. faecalis*. The same can be presumed for cartridges from other manufacturers with similar composition and subjected to similar testing parameters. The results also suggest that cross-contamination *in vivo* under similar clinical working conditions would be unlikely to contribute negatively to endodontic outcomes.

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