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RESEARCH ARTICLE

Evaluation of the risk of *Enterococcus faecalis* cross-contamination of gutta-percha cartridges

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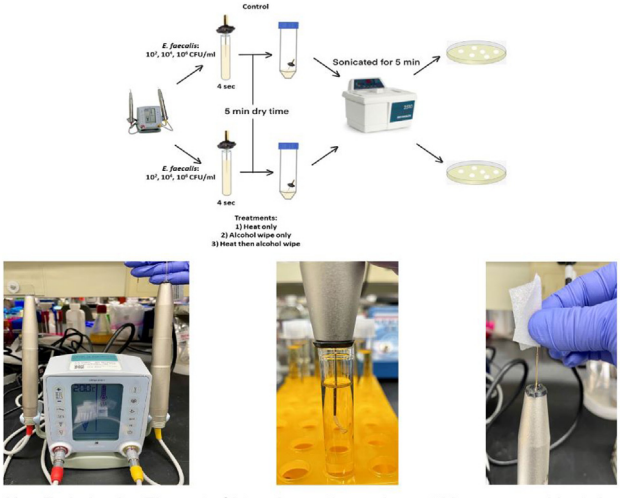

Kerr Endodontics Elements Obturation gutta percha cartridges were subjected to numerous contamination and disinfection protocols.

Why Is This Important?

There is a low risk of gutta-percha cartridges cross-contaminating root canals with *Enterococcus faecalis* when used in multiple canals or multiple teeth of the same patient.

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Abstract

Background. Gutta-percha (GP) cartridges are intended for a single patient and are not considered sterile. Providers may use these cartridges in multiple canals of a single tooth or multiple teeth on the same patient without disinfection between uses, creating the potential for bacteria to be transferred between canal systems. *Enterococcus faecalis* is a hardy bacterial species often isolated from failed root canal treatments. This study tested if GP cartridges have the potential to cross-contaminate root canals with *E. faecalis*. Methods for GP cartridge disinfection were also evaluated.

(Continued on next page)

Methods. Twenty-three–gauge medium-body GP cartridges placed in an obturation unit were purposefully contaminated with 1 of 3 concentrations of *E. faecalis* strain OG1RF. Contaminated cartridges were either (1) wiped with an alcohol swab, (2) heated with GP extrusion while in the bacterial culture, (3) heated with GP extrusion while in the bacterial culture and then wiped with an alcohol swab, or (4) not disinfected after removal from the bacterial culture (control). Bacteria were dislodged into a fresh medium by sonication, and the number of viable bacteria recovered from the tips was measured.

Results. Viable cultures of *E. faecalis* could not be recovered consistently from tips in the control and disinfection treatment conditions.

Conclusions. Cross-contamination of root canals with *E. faecalis* with the GP cartridge is unlikely.

Key Words. Gutta-percha; obturation; *Enterococcus faecalis*; root canal failure; endodontic treatment.

Introduction

Successful endodontic treatment requires the reduction of bacterial load within the root canal during the cleaning, shaping, and obturation processes.[1,2] The use of aseptic techniques is paramount in preventing reinoculation and cross-contamination of the root canal system,[3] especially when treating teeth with multiple canals or multiple teeth in a single appointment.[4] However, there are steps in the processes that have the potential to introduce bacteria into canals.

A common obturation method is the continuous wave technique.[5] This technique 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, and thermoplasticized GP delivered from a heated cartridge is used to backfill the remaining canal space. In general, these cartridges are intended for single patient use and are not sterile. Providers may use GP cartridges in multiple canals of a single tooth or multiple teeth on the same patient without disinfection in between uses, creating the potential for bacteria to be transferred between canal systems.

Few studies have investigated the potential for transferring bacteria to clean canals via GP cartridges. In 1 study, the Obtura obturation system (Young Specialties) was contaminated with *Staphylococcus epidermidis*, *Escherichia coli*, or *Enterococcus faecalis* to determine whether bacteria could survive passage through the heated delivery tip; no microbes were recovered.[6] In contrast, when the same microbes were studied in a similar obturation system using a lower temperature, bacteria were detectable on the tip of the cartridge at low heat, even after an alcohol soak.[7] Finally, an investigation of bacterial cross-contamination when a single GP cartridge in the Calamus Flow System (Dentsply Sirona Endodontics) was used on multiple patients concluded that this practice did not lead to an increased risk of bacterial transfer.[8]

E. faecalis is one of the hardiest bacteria often isolated from failed root canal treatment.[9] *E. faecalis* can invade dentin and bind to immobilized collagen within the root canal system, possibly shielding the bacteria from elimination.[10] Study of *E. faecalis* starvation-survival kinetics in a nutrient-deprived medium showed that the microbe could enter a viable but not culturable state and persist for extended periods.[11] Furthermore, *E. faecalis* can resist the antimicrobial properties of root canal sealers and intercanal medicaments and persist in the anatomic isthmuses and fins of teeth by forming biofilms.[12,13] 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.[14]

In general, GP cartridges cannot be autoclaved or heat sterilized before use and are packaged as nonsterile. The goal of this study was to test the hypothesis that Elements GP cartridges (Kerr Endodontics) have the potential to cross-contaminate root canals with *E. faecalis*. The viability of *E. faecalis* in heated Elements GP cartridges and disinfection methods for these GP cartridges were also evaluated.

Methods

Evaluation of GP cartridge sterility

Four 23-gauge, medium-body GP cartridges for the Elements GP obturation unit (Kerr Endodontics) were aseptically removed from the packaging and placed into 50 mL conical tubes 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 in an ultrasonic water bath (model 3210, Branson Ultrasonics). Aliquots of the sonication fluid were serially diluted in BHI broth, and 0.01 mL of each dilution was plated in technical duplicate on BHI agar. Agar plates were incubated for 24 hours at 37 °C. The number of colonies that grew on each technical duplicate agar plate was tallied, then the values of the duplicate plates were averaged. Colony-forming units (CFUs) per mL were calculated on the basis of the plating of 0.02 mL total volume, meaning that the minimum number of CFUs detectable per cartridge in this experiment was 50 CFUs/mL.

Bacterial strain and growth conditions

The *E. faecalis* OG1RF strain, which was originally isolated from the human oral environment, was used to purposefully contaminate GP cartridges.[15,16] *E. faecalis* colonies were streaked for isolation on BHI agar from a glycerol stock maintained at −80 °C. Agar plates were incubated for 24 hours at 37 °C. After incubation, 3 isolated colonies were inoculated into 2 mL of BHI broth and incubated for 24 hours at 37 °C in ambient air without shaking.

Inoculum preparation

The overnight culture was centrifuged for 1 minute, and the resulting cell pellet was resuspended in 1 mL of BHI broth. Serial dilutions were used to obtain cultures with target concentrations of approximately 10^2, 10^4, and 10^6 CFUs/mL of *E. faecalis*. Aliquots of the cultures were serially diluted and plated on BHI agar to enumerate CFUs/mL.

Disinfection conditions

The 4 disinfection conditions tested were (1) control: cartridge tip was immersed in bacteria; (2) cartridge tip was wiped with a 70% isopropyl alcohol swab after removal from immersion in the bacterial culture; (3) cartridge was heated, and GP was extruded while the tip was immersed in bacteria inoculum; and (4) cartridge was heated, GP was extruded while the tip was immersed in bacteria inoculum, and the cartridge was then removed from the inoculum and then wiped with a 70% isopropyl alcohol swab.

For the control and alcohol wipe conditions, 10 mm of the tip of the cartridge was placed into the *E. faecalis* inoculum (either approximately 10^2, 10^4, or 10^6 CFUs/mL) for 4 seconds. For the control condition, the cartridge was allowed to air dry for 5 minutes and then placed in a 50 mL conical tube containing 3 mL of BHI broth and sonicated as described in the evaluation of GP cartridge sterility section above. For the alcohol wipe condition, the cartridge was allowed to air dry for 5 minutes, and the length of the cartridge tip was wiped with a new alcohol swab and then placed in a 50 mL conical tube containing 3 mL of BHI broth and sonicated as described above. After sonication, aliquots of the cultures were serially diluted. 0.01 mL volumes of each dilution were plated on BHI agar in technical duplicate to enumerate CFUs/mL.

For the disinfection conditions involving the heated obturation device, the obturation unit was turned on and allowed to reach the set temperature of 100 °C. A new cartridge was aseptically placed in the extruder handpiece. Ten mm of the cartridge tip were placed into 1 of the 3 dilutions of *E. faecalis* inoculum, and GP was extruded for 4 seconds. For the heat-only condition, the cartridge was allowed to air dry for 5 minutes and then placed in a 50 mL conical tube containing 3 mL of BHI broth and sonicated as described above. For the heat and alcohol wipe condition, the cartridge was allowed to air dry for 5 minutes in the heated obturation unit, and then the length of the cartridge tip was wiped with a new alcohol swab. The cartridge was then placed into a 50 mL conical tube containing 3 mL of BHI broth and sonicated as described above. After sonication, aliquots of the cultures were serially diluted. 0.01 mL volumes were plated on BHI agar in technical duplicate to enumerate CFUs/mL.

Four biological replicates of each disinfection condition were completed on separate days. The number of colonies that grew on each technical duplicate agar plate was tallied, then the values of the duplicate plates were averaged. CFUs/mL were calculated on the basis of the plating of 0.02 mL total volume for each biological replicate, meaning that the minimum number of CFUs detectable per cartridge in this experiment was 50 CFUs/mL.

Results

Four GP cartridges taken aseptically from the manufacturer's packaging were tested for sterility. A single bacterial colony (equivalent to 50 CFUs/mL) grew from 1 cartridge. No bacteria were detected from the additional 3 cartridges.

Three cartridge disinfection methods were evaluated at 3 levels of bacterial contamination. No bacteria were recovered from the control, alcohol wipe only, and heat and alcohol wipe conditions in the 10^2 CFUs/mL concentration of *E. faecalis* (Figure A). Fifty CFUs/mL bacteria were recovered from 1 of the 4 biological replicates in the heat only condition. In the 10^4 CFUs/mL concentration of *E. faecalis*, low numbers of bacteria (50 CFUs/mL, 100 CFUs/mL) were recovered from 2 of the 4 biological replicates for the control condition. However, no bacteria were recovered from 3 test conditions at this bacterial concentration (Figure B). In the 10^6 CFUs/mL concentration of *E. faecalis*, 100 CFUs/mL were recovered in 2 of the 4 replicates of the control condition, 150 CFUs/mL were recovered in 1 of the 4 replicates of the alcohol wipe disinfection condition, and 50 CFUs/mL were recovered from 1 of the 4 replicates of the heat only condition (Figure C). The heat and alcohol wipe condition at 10^6 CFUs/mL of *E. faecalis* did not yield any detectable bacteria (Figure C).

Because of the overall lack of bacteria recovered across all conditions and bacterial concentrations, a final statistical analysis was not performed.

Discussion

Traditional nonsurgical root canal treatment relies on a basic triad: debridement, disinfection, and 3-dimensional obturation with GP or other materials. Reduction of the bacterial load in the root canal system is vital for successful outcomes. 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.[17] By the time the root canal is prepared for obturation, most of the bacteria in the main canal should have been eradicated. Infected canals may contain up to 10^8 bacterial cells before root canal therapy, and ideally, less than 10^3 bacterial cells after biomechanical preparation.[18] Higher numbers of remaining bacteria can be clinically significant, as that

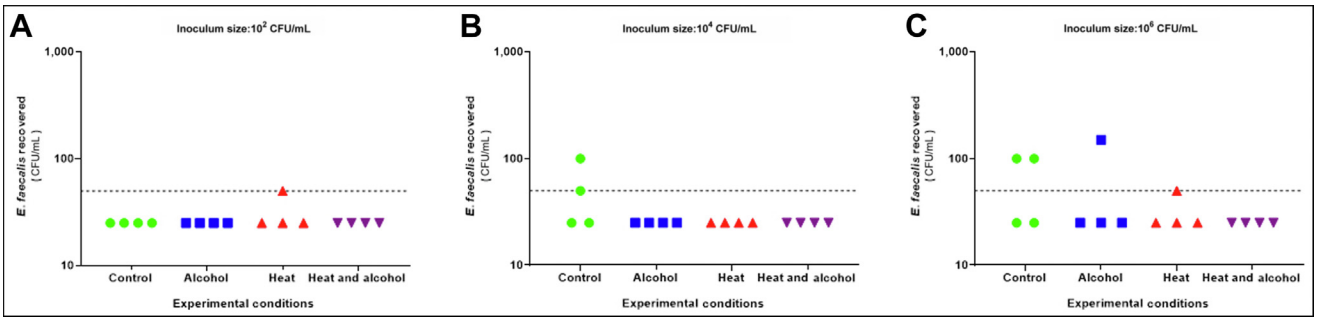

Figure The recovered CFUs/mL from cartridge disinfection protocols at *Enterococcus faecalis* inoculum levels of 10^2 (**A**), 10^4 (**B**), and 10^6 CFUs/mL (**C**). The dotted line represents the limit of detection of the agar plating assay (50 CFUs/mL). Points below the line indicate that no colonies were recovered. A point on the line indicates that the assay recovered the minimum number of bacteria detectable. CFUs, colony-forming units.

indicates poor debridement and disinfection and increases the possibility of root canal treatment failure. Three-dimensional obturation entombs any remaining bacteria within the dentinal tubules, where they cannot reinfect the main canal system. Therefore, it is essential that GP not serve as a vector for the introduction of bacteria into the canal system.

We tested the hypothesis that GP cartridges have the potential to cross-contaminate root canals with *E. faecalis*. Most of the GP cartridges tested were sterile when removed from the original packaging, although they were not labeled as sterile by the manufacturer. Overall, the results from our in vitro study showed that *E. faecalis* OG1RF did not reliably and reproducibly contaminate GP cartridges when the cartridges were exposed to any set bacterial concentrations for 4 seconds. The 4-second exposure time was chosen because that is the approximate duration a GP cartridge would remain within an instrumented root canal during obturation. A preliminary study conducted in our laboratory, wherein a cartridge was exposed to 10^6 CFUs/mL of *E. faecalis* for 4 seconds, resulted in a cartridge contamination level of approximately 1,000 CFUs/mL. However, similar contamination levels were not reproducibly achieved on further experimentation (Figure). More consistent levels of *E. faecalis* colonization on the cartridges might be attainable if the cartridges were exposed to higher concentrations of bacteria or for longer durations. However, such conditions would not accurately replicate conditions that are encountered clinically.

Another factor contributing to the lack of reproducible contamination of the cartridges, even at a bacterial concentration of 10^6 CFUs/mL, may be the strain of *E. faecalis* that was tested. The OG1RF strain was selected because of its accessibility as a laboratory strain. However, different strains, such as ones originally isolated from infected root canals, may yield different results.

Methods of disinfecting GP cartridges were also evaluated in this study. However, the inherent sterility of the cartridges on removal from the manufacturer packaging and the inability to contaminate them with *E. faecalis*, means that conclusions about the effectiveness of the GP cartridge disinfection techniques tested in our study cannot be drawn.

Thermoplasticized GP delivery systems other than the one used in our study have applicator tip temperature ranges from 42.8 through 64.4 °C (from base to tip), and these temperatures were determined to provide sufficient heat to reduce microbial contamination.[6,7] *E. faecalis* can survive being heated to 60 °C for 30 minutes.[19] Therefore, the heat output of 100 °C of the Elements unit used in our study may serve as an antibacterial mechanism. Based on the results of our study, in part, we hypothesize that the low concentrations of bacteria found in a well-disinfected canal, in combination with the heat of the obturation unit, may also prevent cross-contamination of canals.

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.[20-22] Copper has been shown to have biocidal effects on vancomycin-resistant *E. faecalis*.[23] The exact composition of the tips used in our study has not been disclosed by Kerr Endodontics; however, the ductility and conductive properties of the tips coincide with common properties of copper.[24]

An important limitation of this study is that only a single commercially available GP delivery system was tested. Warm vertical compaction is a widely used method for root canal obturation, and there are more than a dozen obturation systems that also use heated GP cartridges. Although our study cannot fully account for presumed differences in the precise metallurgy of the cartridges used by different manufacturers or the effects that such differences may have on the antimicrobial properties of the cartridges, it follows that the findings of this study have broad applicability across all of these systems.

Conclusions

Within the limits of our study, the results indicate a low risk for Elements GP cartridges to cross-contaminate root canals

with *E. faecalis*. The same can be presumed for cartridges from other manufacturers with similar compositions if they were to be subjected to similar testing parameters. The results also suggest that bacterial cross-contamination in vivo under similar clinical working conditions would be unlikely to contribute negatively to endodontic outcomes.

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