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Microbial Evaluation of the Calamus Heated Gutta-Percha Delivery System

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Microbial Evaluation of the Calamus Heated Gutta-Percha Delivery System

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

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Abstract

MICROBIAL EVALUATION OF THE CALAMUS™ HEATED GUTTA-PERCHA DELIVERY SYSTEM

By Christopher J. Smart, DDS

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If gutta-percha cartridges are contaminated with bacteria prior to obturation then contamination of the root canal system may result. Successful treatment not only depends on bacterial elimination but also depends on prevention of recontamination. This study was motivated by the observation that endodontists frequently use single use gutta-percha cartridges on multiple patients. The goal of this study was to determine if cross contamination occurred when a single gutta-percha cartridge in the Calamus™ Flow System was used on multiple patients. An effective recovery method was established during a pilot study using *Enterococcus faecalis* as a sample bacterium. Microbial recovery was obtained using standard media. The Calamus™ heated gutta-percha delivery system was evaluated to determine the percentage of cartridges with recoverable microorganism under four conditions. Results showed that for cartridges tested immediately after removal from manufacturer's packaging, the observed negative outcome was 0/10 (0%). For cartridges used on a single patient with an alcohol wipe after use, the negative

outcome was 0/20 (0%). For cartridges used on a single patient with no alcohol wipe after use, the negative outcome was 1/20 (5%). For cartridges used on three patients with an alcohol wipe after each use, the number of observed negative outcomes was 0/20 (0%). Using 95% score confidence intervals, the results indicate that the percentage of cartridges with recoverable microorganism under the four conditions is below 27.8% for cartridges sampled from manufacturer's packaging, 16.1% for cartridges used on a single patient with a alcohol wipe after use, 23.6% for cartridges used on a single patient with no alcohol wipe after use, and 16.1% for cartridges used on three patients with an alcohol wipe after each use. Evidence from this study indicates using Calamus™ flow cartridges on multiple patients vs. single patient does not lead to an increased contamination risk.

Introduction

The goal of root canal treatment is to eradicate microbiota infecting the root canal system creating a sterile environment and to obturate with a 3-D obturation that seals the canal from reinfection. Due to the complex anatomy of the root canal system this is difficult with current instruments and techniques. Bacterial elimination is accomplished by mechanical instrumentation techniques using hand and rotary files in combination with various chemical irrigants, interappointment medicaments, and obturation materials. Maintaining the chain of asepsis is extremely important to prevent bacterial contamination of the root canal system. Based on current infection control concepts, the instruments and materials used during endodontic treatment (including gutta-percha delivery systems) must be as bacteria-free as possible.

Role of Bacteria

Since the first discovery of oral bacteria or "animalcules" in the late 1600's, dentistry has made extensive progress in treating dental diseases (1). Miller (1894) discovered that bacteria could infect and persist in the pulpal tissues causing pulpal disease (2). This study helped change the way dentists looked at bacterial involvement with pulpal symptoms and pulpal disease in patients.

Kakehashi, et al. conducted a landmark study using an animal model involving one group of germ-free rats in which pulpal exposure was induced and a second group of standard germ-present rats in which pulpal exposure was induced. The germ-free rats with orally exposed

pulpal tissue and periapical tissues showed no signs of apical periodontitis, while standard rats showed signs of pulpal necrosis and apical periodontitis (3).

In a subsequent study, Sundqvist, showed that traumatized incisors with clinically intact crowns and necrotic pulp remained normal and that only the teeth that harbored bacteria developed apical periodontitis (4). Moller et al. showed that aseptically devitalized sealed teeth remained disease-free after a period of 6-7 months. However, teeth that were infected by oral flora developed apical periodontitis (5). These three studies by Kakehashi, Sundqvist, and Moller were the foundation of and furthered understanding of the bacterial etiology of periapical disease.

Later studies showed the effect of bacteria on the outcome of endodontic treatment. In a clinical study of 55 root canals, Sjogren et al. demonstrated that having a bacteria-free canal before obturation positively affected the success of endodontic treatment. Ninety four percent of cases that yielded a negative culture were healed at five years' recall. However, in cases that yielded positive samples prior to obturation, the success rate of treatment was only 68%. This study emphasized the importance of sterilization of the root canal for optimal outcome (6).

Microbiology of Endodontics

When the pulp succumbs to necrosis, bacteria begin to colonize it immediately. Facultative anaerobes dominate the initial stages of necrosis, but as necrosis progresses, obligate anaerobes take over (7). Major factors affecting bacteria in the necrotic pulp include oxygen tension, type and availability of nutrients, as well as bacterial interactions. Low oxygen tension is a result of the bacteria consuming the oxygen and the loss of blood supply to the pulp (8-10).

Saccharolytic bacterial species dominate initially. These species metabolize products of the pulp, proteins and other components of saliva and metabolic byproducts of other bacteria. As

the infection matures, asaccharolytic bacterial species dominate as the infection progresses metabolizing proteins into amino acids (11).

The specific bacteria most commonly isolated from primary endodontic infections include *Fusobacterium*, *Streptococcus*, *Porphyromonas*, *Prevotella*, *Eubacterium*, *Peptostreptoccus*, *Propionibacterium* and *Campylobacter* species (12). Classic studies have shown that black pigmented rods are more likely to be associated with clinical symptoms such as pain and swelling (13, 14).

Bacteria harboring in root canals can either be in a planktonic form, biofilm form, or both (15). Planktonic microbiota have been shown to be easily eradicated by different methods, whereas biofilm microbiota have been shown to be resistant. Biofilms are communities of organisms attached to root canal walls that consist of an extracellular polysaccharide matrix which helps protect bacteria from the harsh environment, allows for metabolic commensalism among different species, and aids resistance to antimicrobial agents (16-19).

Completely removing bacterial biofilms from the root canal system is difficult and could be one reason why apical periodontitis appears in secondary infections. Bacteria that are often associated with treatment failures are different from the bacteria in primary infections. Secondary infections contain fewer bacteria species and are dominated by gram-positive facultative anaerobes including *Streptococci, Lactobacilli, Propionibacterium* species, yeasts, *Enterococcus faecalis* and *Actinomyces* species (20-23). *E. faecalis* is one of the most frequently isolated bacteria from failing previously treated teeth.

Enterococcus faecalis

E. faecalis is a gram-positive facultative anaerobic coccus which is a normal inhabitant of human intestinal flora. It can cause life-threatening infections in humans, especially in the

nosocomial environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contributes to its pathogenicity (24).

Clinical studies have shown that *E.faecalis* has been isolated from oral cavity and gingival sulcus and can be present in untreated root canals with primary infections (25-28). However, it has been most commonly isolated from root canal-treated teeth with apical periodontitis. Several studies revealed that *E. faecalis* is the most frequent species isolated with prevalence values reaching up to 90% of cases in teeth with apical periodontitis (19, 20, 24, 27). It has also been estimated that root canal-treated teeth are nine times more likely to harbor *E. faecalis* than primary infections (19). It is a fastidious organism that is thought to invade root canals during treatment from the oral fluids and then persists due to its ability to adapt to ecological conditions and survive (30).

E. faecalis has been shown to be resistant to high pH calcium hydroxide (31, 32). This resistance seems to be related to its internal functional proton pump that acidifies the cell cytoplasm (33). The ability of *E. faecalis* to penetrate deep into dentinal tubules to escape from chemomechanical preparation and intracanal medicaments is another survival mechanism (34). *E. faecalis* is capable of entering a viable but non-cultivable (VBNC) state. This survival mechanism is utilized by other bacteria as well as *E. faecalis* when exposed to environmental stress (35, 36). In the VBNC state, bacteria are able to survive periods of starvation and then resume their growth when nutrients become available again (30). *E. faecalis* has been shown to survive starvation in root canal-treated teeth providing a nidus for subsequent infection (37). *E. faecalis* has also demonstrated that it can form coaggregates with other bacterial species resulting in biofilms (38). The presence of *E. faecalis* in the root canal system, its fastidious survival mechanisms, and its association with secondary infections make it essential that the

antimicrobial measures taken before obturation be as effective as possible against this microorganism.

Gutta-Percha

The success of treatment not only depends on bacterial elimination but also depends on the prevention of recontamination after cleaning and shaping the root canal system. Therefore, obturation should not introduce additional irritants into the root canal system that could ultimately decrease successful treatment outcomes.

Material used for obturation of root canals has changed as the field of endodontics has evolved. Obturating materials have included both pastes and solid materials such as silver cones. Gutta-percha, carrier-based gutta-percha, and Resilon are the materials that are primarily used today, with gutta-percha being the most commonly used obturation material.

Gutta-percha fulfills most of the ideal characteristics originally outlined by Grossman (1940). The ideal characteristics of an obturating material are: 1) easily introduced into the root canal, 2) seals the canal laterally as well as apically, 3) does not shrink after being inserted, 4) is impervious to moisture, 5) is bacteriostatic or at least does not encourage bacterial growth, 6) is radiopaque, 7) does not stain tooth structure, 8) does not irritate periradicular tissues, 9) is sterile, or easily and quickly sterilized, immediately before the insertion, and 10) is removed easily from the root canal, if necessary (39).

Gutta-percha (*Palaquium*) is a genus of tropical trees native to Southeast Asia and northern Australasia. The milky exudate from the trees is evaporated and the resulting material is called gutta-percha. Chemically, gutta-percha is a polyterpene, a polymer of isoprene, or polyisoprene, specifically the trans-1,4-polyisoprene (40). It is the dominant isomer in both gutta-percha and balata.

Gutta-percha was introduced as a root canal filling material in 1847 by Hill who developed the material and called it "Hill's-stopping". It was composed of a mixture of bleached gutta-percha and carbonate of lime and quartz (40). Bowman (1867) was the first to use gutta-percha for a root canal filling on an extracted molar (40). Later Perry (1883) used pointed gold wire wrapped with soft gutta-percha which he rolled and packed into the canal (40).

The first commercial manufacture of gutta-percha points was by S.S White Company in 1887 (40). A newer mixture of gutta-percha was introduced by Rollins in 1893. He added pure oxide of mercury to the gutta-percha. This product was removed later due to dangerous levels of mercury (40). In 1914 Callahan introduced softening and dissolution of gutta-percha with the use of rosins in obturation (40). In 1959 Ingle and Levine were the first to propose standardization of root canal instruments and filling materials. Standardized gutta-percha was introduced to the profession in 1959 (41).

Gutta-percha exists in two crystalline forms α and β. The mechanical properties of the α and β forms are the same except when heated. Heating gutta-percha results in volumetric difference (42, 43, 44). When in β form, gutta-percha is compactable. When heated, it changes to α phase and becomes pliable and therefore, can be made to flow. When α phase gutta-percha cools normally, it crystallizes to the β form with a slight shrinkage of between 1 and 2% (45).

Gutta-percha has both advantages and disadvantages. Shrinkage is one disadvantage. Another is its inability to bond to dentin. The major advantages of gutta-percha are its plasticity, ease of manipulation, minimal toxicity, radiopacity, and ease of removal with heat or solvents (44). Gutta-percha cones consist of approximately 20% gutta-percha, 65% zinc oxide, 10% radiopacifiers, and 5% plasticizers (46). These chemical additives alter the material behavior compared to pure gutta-percha (47).

Obturation Methods

There is very little scientific evidence to support one method of obturation versus another in terms of outcomes of success or failure. Lateral compaction, warm vertical compaction, continuous wave compaction, warm lateral compaction and carrier based compaction are the methods available.

Currently there are multiple thermoplasticized injectable gutta-percha obturating systems used today. The concept of obturating root canals using thermoplasticized injectible gutta-percha was introduced by Ye et al (48). Thermoplascticized injectible gutta-percha may be used as a primary technique or, as it is used most often today, as a back-filling technique to obturate the remaining canal space after initial compaction technique.

Thermoplasticized injectable gutta-percha obturating systems include pellet based units and cartridge based units. The Obtura (Obtura Spartan, Earth City, MO) and HotShot (Discus Dental, Culver City, CA) use gutta-percha pellets. These units require gutta-percha pellets to be inserted into the delivery system gun, and then the gutta-percha pellet is heated to 150^{o} to 200^{o}C prior to injection into the canal. The Calamus (DENTSPLY Tulsa Dental Specialties, Tulsa, OK), Elements (SybronEndo, Orange, CA), and Ultrafill 3D (Coltene/Whaledent, Cuyahoga Falls, OH) use gutta-percha cartridges which are inserted into the system. The Ultrafill™ system is a low heat system that heats preloaded gutta-percha cartridges from 70^{o} to 90^{o}C prior to injection into the canal. The Elements™ and Calamus™ systems heat preloaded gutta-percha cartridges from 60^{o} to 200^{o}C prior to injection into the canal. These systems have been developed with minimal guidelines for clinical asepsis. The only scientific evidence available for these units comes from studies completed on the Obtura™ and the Ultrafill™ systems (49, 50). The focus of the present study is based on the Calamus™ system.

The Calamus™ unit cartridges come in manufacturing packages that give no information on how the product is prepared. The Calamus™ directions outline the use for cartridges and are as follows:

1. Cartridges are for single patient use.
2. Prior to using a cartridge on a patient, wipe the cartridge tip with alcohol or a disinfectant.
3. Store cartridges at room temperature.
4. Do not immerse the cartridges into any liquid.
5. Do not use cartridges after the expiration date.
6. Cartridges CANNOT be re-heated and/or re-used.

Clinicians can assume that if the gutta-percha pellets or cartridges are contaminated with bacteria prior to obturation the contamination of the root canal system may result. Furthermore, some clinicians may use these pellets and single use cartridges on multiple patients resulting in cross-contamination. The success of treatment not only depends on bacterial elimination but also depends on the prevention of recontamination after cleaning and shaping the root canal system. Therefore, obturation should not introduce additional irritants into the root canal system that could ultimately decrease successful treatment outcomes.

This study was motivated by the observation that endodontists frequently use single use gutta-percha cartridges on multiple patients. The goal of this study was to determine if cross contamination occurred when a single gutta-percha cartridge in the Calamus™ Flow System was used on multiple patients.

Materials and Methods

All non-surgical root canal treatment (NSRCT) (vital and necrotic) or non-surgical retreatment cases treated in the VCU School of Dentistry Graduate Endodontic Practice are obturated using warm continuous wave obturation which utilizes thermoplasticized gutta-percha cartridges or pellets. Each cartridge contains enough gutta-percha to obturate 2-4 cases. These cartridges are discarded after use. All cases are treated using a standard protocol for cleaning and shaping, irrigation and obturation. This study included cases treated during Spring Semester 2012.

The gutta-percha cartridges used on patients that presented for NSRCT at VCU School of Dentistry were evaluated after continuous wave obturation for the existence of microorganisms under four conditions:

1. 10 cartridges immediately removed from manufacturer's packaging,
2. 20 cartridges used on single patients cleaned with an alcohol wipe after use,
3. 20 cartridges used on single patients with no alcohol wipe after use,
4. 20 cartridges (1 cartridge used per 3 patients) with an alcohol wipe after each use.

All materials and methods were under the control of one operator. The media used for recovery of microorganisms was sterile saline and the media used for growth of microorganisms included brain-heart infusion broth (BHIB) and brain-heart infusion agar (BHI agar) plates. BHIB and BHI agar are highly nutritious general-purpose growth media used for culturing fastidious and nonfastidious microorganisms. One Calamus™ Dual unit was used throughout the study (Fig. 1). Calamus™ cartridges from only unopened manufacturer's storage packages

were used in this study. The Calamus™ gutta-percha cartridge is an aluminum prefilled capsule that contains gutta-percha with a silver cartridge tip (Fig. 2).

Pilot Study Sample Preparation

The pilot study sample preparation is shown in Figure 3. Cartridges used to evaluate the recovery method used were inoculated with an overnight culture of *E. faecalis*. An overnight stock culture of *E.faecalis* was prepared by thawing the culture in the biological safety cabinet and pipetting a10 ul aliquot of *E.faecalis* into 5 ml of BHI broth. The culture was incubated at 37^{o}C for 24hrs. The cartridges were prepared by removing them from packaging with sterile forceps and gloves, wiped with a 2x2 alcohol wipe, allowed to dry in the biological safety cabinet, and pre-weighed on a sterile laboratory scale. Next, all tips were inoculated by the same protocol by inserting the tip of the cartridge to the hub in the overnight culture with sterile forceps and holding it there for 10 seconds. Tips were then removed in the biological safety cabinet, dried, and reweighed.

After drying, tips were treated in three ways. One tip was placed into a tube containing 3 ml of sterile saline with forceps and allowed to incubate at room temperature for 24hrs. The second tip was placed into another tube containing 3ml of sterile saline with forceps and was vortexed in preparation for immediate recovery. The third tip was held with sterile forceps and swabbed with a sterile cotton swab soaked in sterile saline. The swab was then inserted and twirled into a tube containing 3ml of sterile saline. This 3 ml volume was sufficient to cover the entire silver cartridge tip but only partially cover the aluminum cartridge of the gutta-percha. Tubes were labeled according to how the sample was treated.

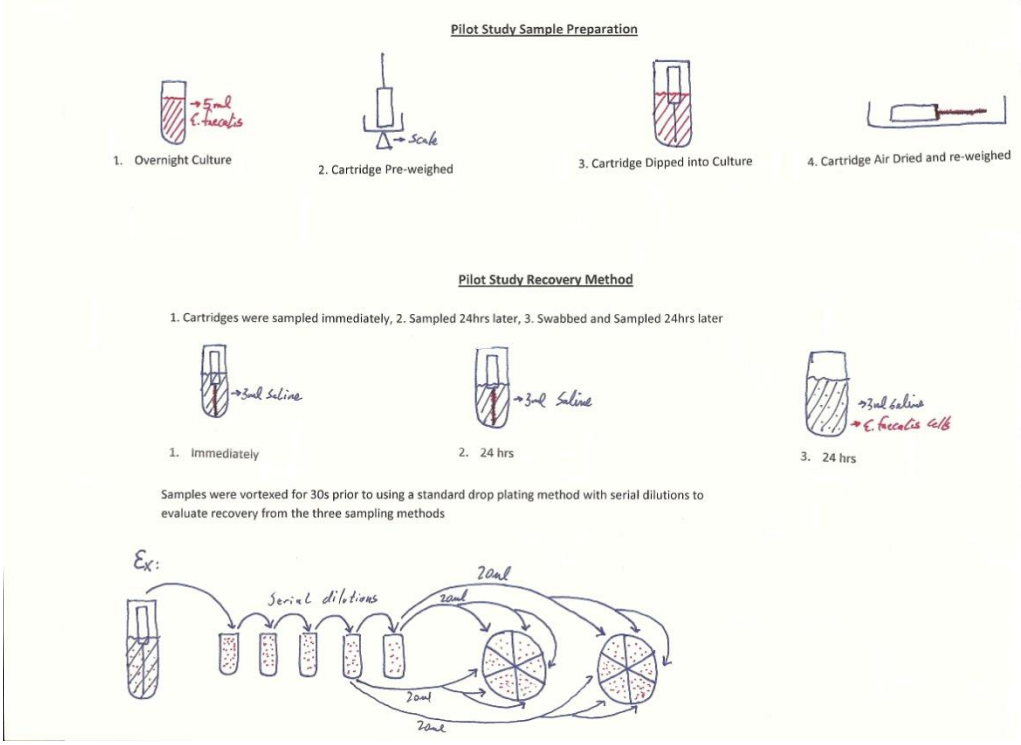

Figure 3. Pilot Study Sample Preparation and Recovery Method

Preparation of Manufacturer's and Patient Samples

The preparation of manufacturer's and patient samples is given in Figure 4. In all patient cases in this study, hand and rotary instrumentation in combination with antimicrobial solutions of 5.25% sodium hypochlorite (NaOCl) and 17% etheylenediamine tetraacetic Acid (EDTA) were used prior to obturation. If a case was non-vital, 2% chlorhexidine (CHX) was added as an antimicrobial irrigant. If the case was a non-surgical retreatment the addition of chloroform was used prior to obturation. In multiple visit cases calcium hydroxide ($Ca(OH)_2$) was used as an intracanal intraappointment medicament prior to obturation.

Preparation of new samples prior to use followed manufacturer's instructions and utilized the following protocol. Cartridges were removed with sterile forceps and gloves, wiped with a 2x2 alcohol wipe, and placed into the flow hand piece prior to patient usage. After cleaning and shaping procedures were completed, master cones were fitted and down packed with the pack

hand piece. When down packing was completed the Calamus™ flow hand piece with the inserted cartridge was used to backfill the canals. After the last usage of the flow hand piece the cartridge tip was treated in two ways, it was wiped with a 2x2 alcohol wipe or was not wiped. After the patient was dismissed from the operatory, the cartridge was removed from the flow hand piece with sterile gloves and forceps and placed into a test tube containing 3 ml of sterile saline and labeled according to how it was treated (alcohol wipe or no alcohol wipe).

Preparation of the samples for multiple cases on patients followed single patient procedures with minor differences. After each case was obturated and before its next use, the cartridge tip was wiped with a 2x2 alcohol wipe. After the final usage the cartridge tip was placed aseptically into a 3ml of sterile saline.

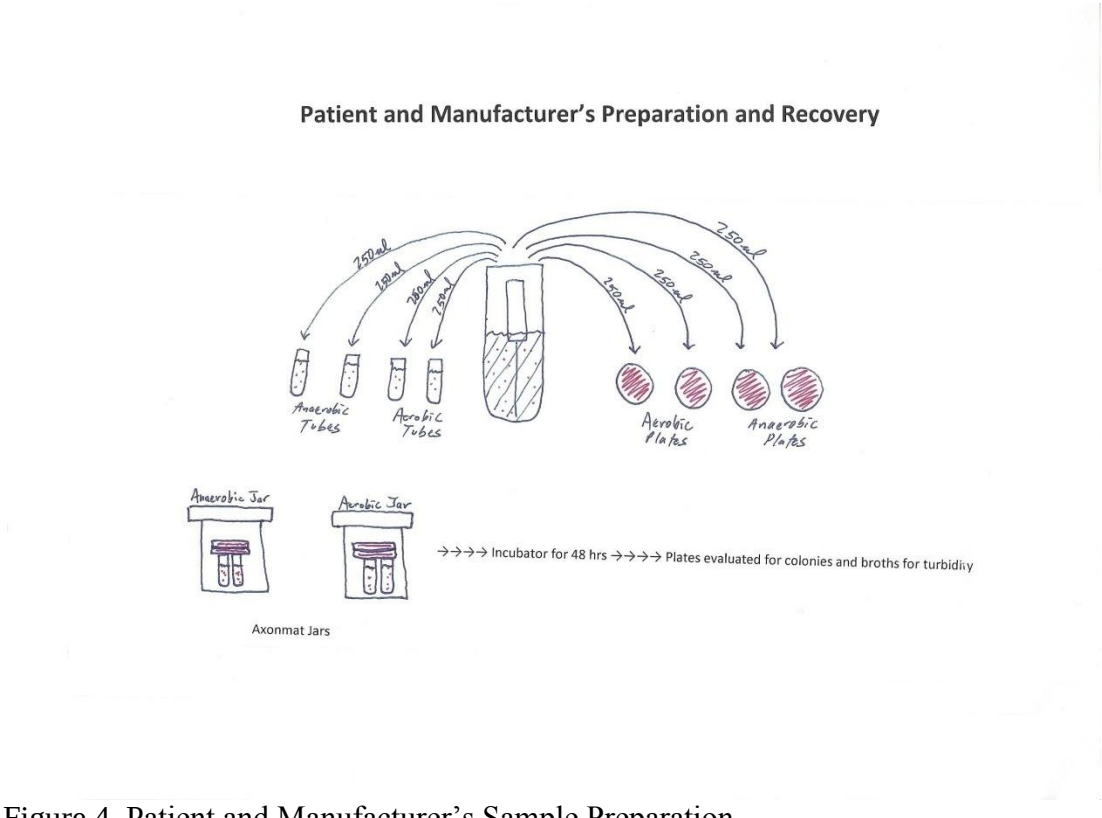

Figure 4. Patient and Manufacturer's Sample Preparation

Pilot Study Recovery Method

The recovery method used in the pilot study is shown in Figure 3. A standard Drop Plating method on BHI plates was used to evaluate the number of bacteria from the overnight cultures and the number of bacteria recovered from Calamus™ cartridge tip samples.

Overnight culture determination was done according to the following protocol. A 10 ul aliquot of overnight culture was pipetted into a centrifuge tube containing 990ul of BHI broth labeled tube L and vortexed. From tube L, a 100 ul aliquot was pipetted into another tube containing 900 ul of BHI broth labeled M and vortexed. From tube M, a 250 ul aliquot was pipetted into another tube containing 750 ul of BHI broth labeled N and vortexed. Dilutions were carried out in tubes labeled O, P, Q, and R using the same method as from tube M to tube O. Twenty ul aliquots from tubes M through R were drop plated in triplicate onto BHI plates. All plating was done in duplicate. The two Calumus™ tip samples and the sterile cotton swab sample followed the same protocol but differed in the fact that one of the Calamus™ tip sample was treated 24hrs later to mirror patient treatment. A 20 ul aliquot was taken directly from the sample drop plated on the BHI plate. A 250 ul aliquot was pipetted into a centrifuge tube containing 750 ul of BHI broth labeled B and vortexed. From tube B, a 250 ul aliquot was pipetted into another tube containing 750 ul of BHI broth labeled C and vortexed. Dilutions were carried out in tubes labeled D, E, and F using the same method as from tube B to tube C. The 20 ul aliquots from tubes B through C were drop plated into triplicate onto BHI plates (Fig. 4). All plating was done in duplicate.

Patient and Manufacturer's Samples Recovery Method

The patient and manufacturer's samples recovery method is shown in Figure 4. All test tube samples from patient usage and manufacturer's packaging were labeled accordingly. The

samples remained at room temperature for 24-48hrs. After 24-48hrs samples were taken to a microbiology lab for sampling in a biological safety cabinet. The following protocol was utilized for all samples after remaining at room temperature for 24-48hrs:

1. Samples containing the cartridges were vortexed for 30 seconds to free any adhering microorganisms from exposed surfaces.
2. Two 250 ul aliquots from the sample were directly plated and streaked onto two separate BHI agar plates labeled anaerobic culturing.
3. Two 250 ul aliquots from the sample were directly plated and streaked onto two separate BHI agar plates labeled aerobic culturing.
4. Two 250 ul aliquots from the sample were pipetted into two test tubes containing 9.0 ml of BHIB labeled anaerobic culturing and were vortexed.
5. Two 250 ul aliquots from the sample were pipetted into two test tubes containing 9.0 ml of BHIB labeled aerobic culturing and were vortexed.
6. The BHI agar plates and BHIB test tubes were placed in two separate jars labeled aerobic or anaerobic of the Anoxomat system. The Anoxomat system is a system for culturing anaerobes and micro-aerobes in any laboratory, clinical or industrial setting. It is a complete and unique system creating rapidly and automatically anaerobic, micro-aerophilic or capnophilic conditions in an anaerobic jar. The anaerobic jar conditions included 80% N2, 10% CO2, 10% H2, and 0 % O2. The aerobic jar conditions included 80% N2, 10% CO2, 10% H2, and 6 % O2.
7. When the jars completed their cycles to create this environment they were then incubated at 37^{o}C for 24-48 hrs.

8. After 24-48hrs BHI agar plates and BHIB test tubes were visually evaluated for the presence of microorganisms. If colonies were not visualized on the BHI agar plates or the BHIB test tube was not turbid then the samples were considered negative. If colonies were visualized on the BHI agar plates or the BHIB test tube appeared turbid then the samples were considered positive.

Results

An effective recovery method for E. faecalis inoculated gutta-percha cartridges was established during a pilot study. This method effectively recovered inoculated bacteria from the cartridge tips allowing the use of this method on patient samples. Figure 5 shows that tips sampled after 24 hours resulted in the highest recovery of bacteria at 4.97% followed by tips tested immediately at 4.52%. The cotton swabs recovered the lowest amount of bacteria at 3.19%.

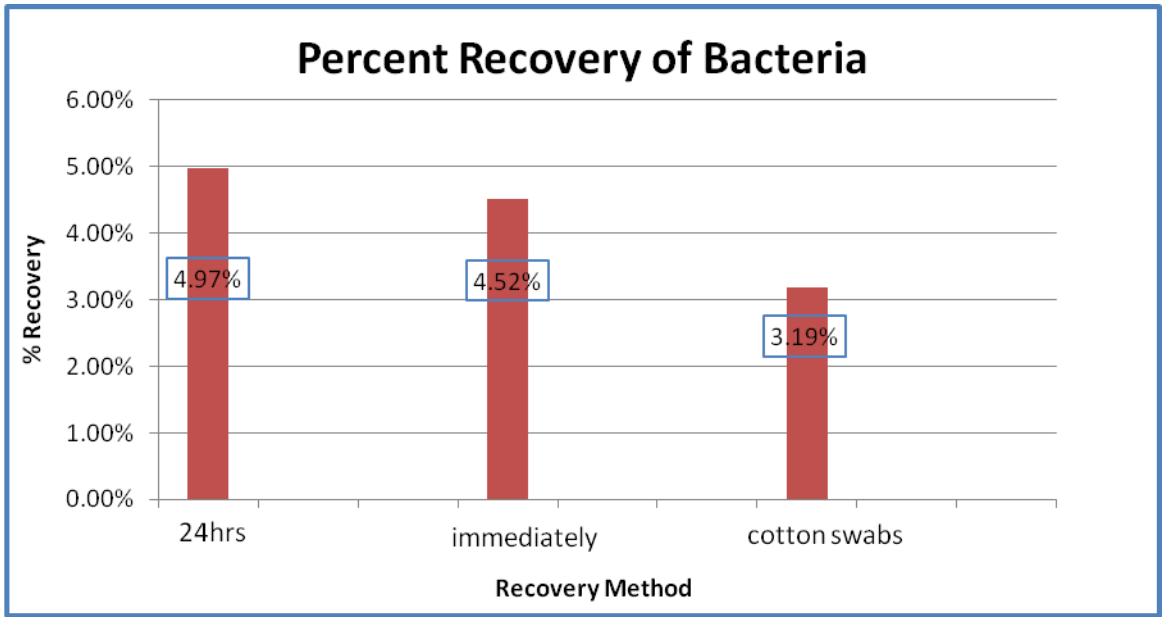

Figure 5. Pilot Study Percent Recovery Graph

Following the pilot recovery studies, gutta-percha cartridges used in the Calamus™ Flow Gutta-Percha Delivery System were evaluated to determine if cross contamination occurred when a single use gutta-percha cartridge was used on multiple patients. Gutta-percha cartridges observed negative outcomes were determined under four conditions:

1. 10 cartridges immediately removed from manufactures packaging,
2. 20 cartridges used on single patients cleaned with an alcohol wipe after use,
3. 20 cartridges used on single patients with no alcohol wipe after use,
4. 20 cartridges (1 cartridge used per 3 patients) with an alcohol wipe after each use.

The observed negative outcome was 0/10 (0%) for cartridges tested immediately after removal from manufacturer's packaging. The observed negative outcome for cartridges used on a single patient cleaned with an alcohol wipe after use was 0/20 (0%). The observed negative outcome for cartridges used on a single patient with no alcohol wipe after use was 1/20 (5%). The observed negative outcome for cartridges used on three patients (60 patients) with an alcohol wipe after each use was 0/20 (0%).

Using 95% score confidence intervals, results from Figure 6 indicates that the percentage of cartridges with recoverable microorganism under the four conditions is below 27.8% for cartridges taken directly out of manufacturer's packaging, 16.1% for cartridges used on a single patient cleaned with an alcohol wipe, 23.6% for cartridges used on a single patient with no alcohol wipe, and 16.1% for cartridges used on three patients with an alcohol wipe after each use.

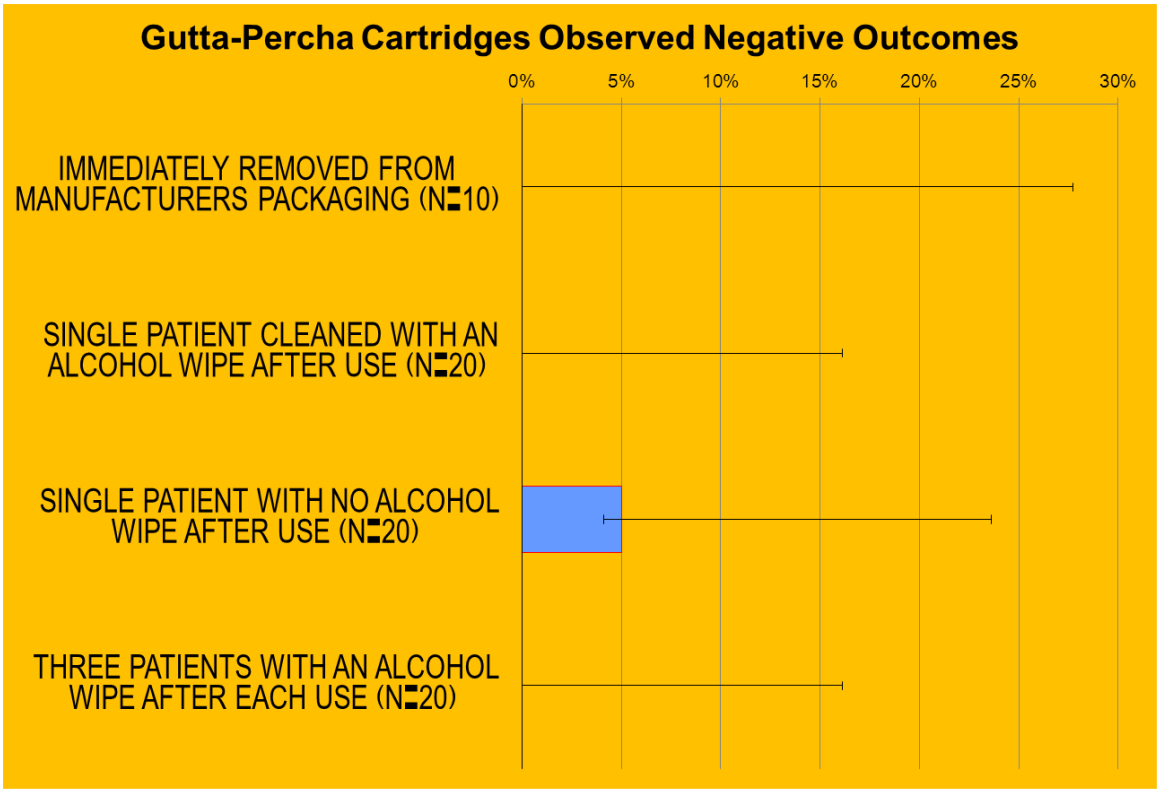

Figure 6: 95% Confidence Intervals of Observed Negative Cartridges

Discussion

As stated earlier, Calamus™ gutta-percha cartridges are marketed as single use to prevent cross contamination between patients. This study was motivated by the observation that endodontists frequently use these single use gutta-percha cartridges on multiple patients. The purpose of this study was to determine if cross contamination occurred when a single gutta-percha cartridge in the Calamus™ Flow System was used on multiple patients.

Microbial recovery from cartridges among patient samples in this study was all negative for 99 out of 100 patients (60 total cartridges). The high number of negative cultures may be due to

the effects of mechanical instrumentation techniques in combination with various chemical irrigants and interappointment medicaments prior to obturation. Furthermore, the temperature (170^{o}C) of the obturation unit may have been lethal to remaining bacteria on or in the cartridge tip prior to use (49).

Prior to this study there has only been one study that completed microbiological evaluation of high heat gutta-percha delivery systems. Winford et al. tested the Unitek Obtura™ heated gutta-percha system to determine whether microorganisms have the ability to survive passage through the mechanism or to survive on the applicator tip of the instrument during clinical use. No organisms tested survived the passage through the heated chamber or were demonstrable on the applicator tip during clinical usage patterns. The authors suggest that the use of NaOCl, the temperature of the applicator tip, and alcohol wiping the tip as being sufficient to control the microflora tested (50). Their results are similar to the findings in this study. The use of 5.25% NaOCl, 17% EDTA, 2% CHX, Ca $(OH)_2$, multiple alcohol wipes, and the 170^{o}C temperature of the Calamus™ unit may also have contributed to the lack of positive cultures.

When looking at the results of this study it appears that a clinician may have a higher risk of obtaining a positive culture when using a new cartridge out of manufacturer's packaging versus using a used one on multiple cases (27.8% for cartridges out of manufacturer's packaging and 16.1% for cartridges used on multiple patients). The results appear this way because only 10 new cartridges were sampled resulting in a smaller sample size compared to the number of cases sampled (20). One can assume if a higher sample size was used then the confidence intervals would have decreased thus making the risk of a positive culture equal to or below that of cases treated with alcohol wipes.

In conclusion, evidence from this study indicates using a single use Calamus™ cartridge on multiple patients versus one patient does not lead to an increased cross-contamination risk. Prior to adopting the use of single use patient cartridges on multiple cases as a standard of care the use of a single gutta-percha cartridge for multiple patients requires additional microbial studies to evaluate the Calamus™ flow gutta-percha cartridges under different clinical conditions.

Study variables need to be controlled for case type, i.e. vital, non-vital, and retreatment. Sample size needs to be increased for all case types. Increasing the sample size would have likely decreased the risk of obtaining positive cultures. Statistical analysis across and among case type should be completed.

The use of a single researcher added bias to the study. Any future studies should attempt to remove this bias by adding more researchers.

The use of a different culturable microorganism, the use of different growth media, and the utilization of more robust identification methods such as polymerase chain reaction methods might more reliably allow for prediction of cross-contamination.

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Appendix

Figure 1. Calamus Unit

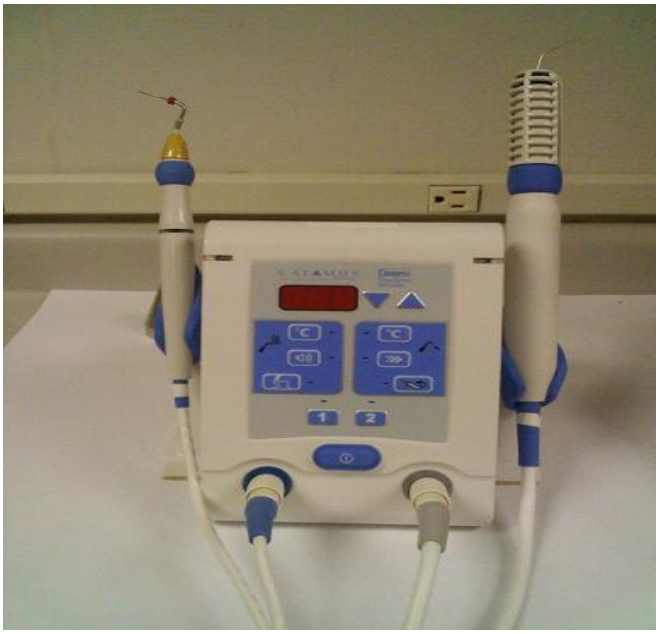

Figure 2. Cartridge

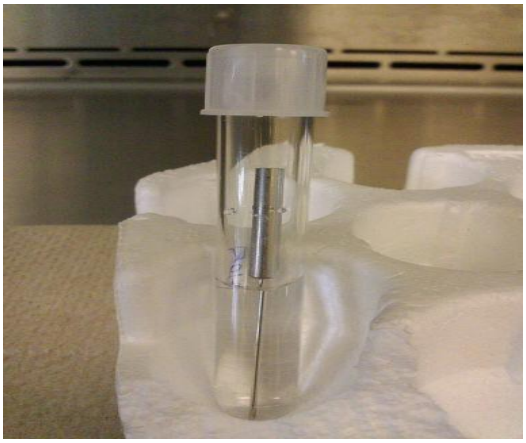

Table 1. Percent Recovery of Pilot Study

Tips after 24hr	Tips Immediately	Cotton swabs immediately
0.026	0.026	0.0077
0.059	0.081	0.009
0.038	0.11	0.059
0.059	0.085	0.11
0.037	0.02	0.05
0.14	0.02	0.02
0.03	0.012	0.057
0.059	0.15	0.03
0.035	0.023	0.11
0.085	0.11	0.013
0.049799513	0.045234761	0.031925007
4.97%	4.52%	3.19%

Table 2. Raw Data of Observed Negative Outcomes

Raw Data Collection of Observed Negative Outcomes			
Singe use with alcohol wipe	Single use with no alcohol wipe	Multiple use with alcohol wipe	Package Samples
0	1	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	
0	0	0	
0	0	0	
0	0	0	
0	0	0	
0	0	0	
0	0	0	
0	0	0	
0	0	0	
0	0	0	

Vita

Dr. Christopher John Smart was born on December 8, 1981, in Morgantown, West Virginia, and is an American citizen. He graduated from Northwood School, Lake Placid, New York in 2000. He received his Bachelor of Science in Nutrition and Food Science from the University of Vermont, Burlington, Vermont in 2005 followed by his Master of Science in Nutrition and Food Science with a concentration in Food Microbiology from the University of Vermont in 2007. He obtained his Doctor of Dental Surgery from West Virginia University School of Dentistry in 2011. Dr. Smart then enrolled in the Advanced Specialty Program in Endodontics at Virginia Commonwealth University School of Dentistry. Dr. Smart is currently a member of the AAE, ADA, and Vermont State Dental Society. Dr. Smart will enter private practice in Burlington, Vermont upon graduation. He will graduate from VCU with a Master of Science in Dentistry and a Certificate in Endodontics.