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# Decontamination of Endodontic Gutta-percha: an In-vitro Study

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# **Decontamination of Endodontic Gutta-percha: an In-vitro Study**

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# APPROVAL PAGE

Master of Science Thesis

Decontamination of Endodontic Gutta-percha: an In-vitro Study

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## Introduction

Apical periodontitis is an infectious disease and thus asepsis, antisepsis and disinfection are paramount in endodontic treatment. Development of aseptic techniques dates back to 1847 when Ignaz Philipp Semmelweis showed that hand washing prior to delivery of babies significantly reduced puerperal fever. 20 years later, Joseph Lister was the first to introduce the use of phenol as an antiseptic to reduce surgical infection rates.

In endodontics major efforts are made during instrumentation and debridement of the root canals to eliminate bacteria from the infected pulp space. Because of this, maintaining the chain of asepsis is extremely important to prevent bacterial contamination of the root canal system. Based on modern-day infection control concepts, the instruments and materials used during endodontic treatment (including gutta-percha cones), need to be free of contaminating microorganisms.

### *The Role of Bacteria*

A primary goal of endodontic treatment is the prevention and treatment of apical periodontitis. Apical periodontitis is the body's response to endodontic infection and is characterized by the destruction of the bone and periodontium around the apex of the tooth. Apical periodontitis develops as a consequence of caries, trauma, periodontal disease and iatrogenic restorative procedures.

W.D. Miller (1894) was the first to demonstrate the link between the presence of bacteria in the necrotic dental pulp and apical periodontitis.

However, it would be another seventy years before the importance of the role of

bacteria in endodontic infection would be understood. That understanding was first indicated in a landmark study by Kakehashi *et al.* (1969). Using conventional and germ-free rats, they demonstrated that pulp necrosis and apical periodontitis developed only in conventional animals whose molar pulps were exposed in oral cavity. In contrast, germ-free rats only developed minimal pulpal inflammation and no apical periodontitis was observed. Germ-free rats also developed dentinal bridges over the exposure sites, showing a capacity for healing in the absence of bacterial infection.

By extending the work of Kakehashi *et al.*, Sundqvist (1976) was able to gain further understanding of the relationship between bacterial infection and apical periodontitis. In a study of traumatized incisors with clinically intact crowns and necrotic pulp, he demonstrated that only the teeth that harbored bacteria developed apical periodontitis.

In a subsequent study in monkeys, Møller *et al.* (1981) showed that aseptically devitalized and sealed teeth remained disease-free after a period of 6-7 months. However, teeth that were infected by oral flora showed inflammatory reactions in the apical tissues both clinically and radiographically. These three landmark studies furthered our understanding of the bacterial etiology of periapical disease.

Later studies showed the effect of bacteria on the outcome of endodontic treatment. Sjögren *et al.* (1997) demonstrated in a clinical study of 55 root canals, that having a bacteria-free canal before obturation positively affects the success of endodontic treatment. They showed that 94% of cases that yielded

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 completely eliminating bacteria from the root canal for optimal outcome.

### Portal of entry

The most common pathways for bacteria to invade the pulp are via caries, restorative procedures and fractures. As a result of trauma, small openings in the dental hard tissues become pathways for bacterial ingress into the pulp. In addition, it has been proposed that bacteria can enter the pulp complex through the exposed dentinal tubules on the root surface or through lateral or accessory canals in the periodontal pocket. Langeland *et al.* (1974) showed that even though pulpal changes occur in the presence of periodontal disease, necrosis develops only when the periodontal pocket involves the apical foramen. It has also been shown that endodontic pathogens can cause an inflammatory reaction in the pulp even without penetrating directly into the pulpal space. This occurs by means of infected dental tubules adjacent to the pulp (Bergenholtz and Lindhe, 1975; Warfinge and Bergenholtz, 1986). Bacteria have also been isolated from traumatized teeth with seemingly intact crowns (Bergenholtz 1974, Sundqvist 1976). A process called anachoresis has been proposed as one of the explanations for this phenomenon (Gier and Mitchel 1968; Tziafas 1989). Anachoresis supposedly occurs when blood-borne microorganisms are transported and seeded into the areas of inflammation. This theory has been disputed, however (Müller *et al.* 1981; Delivanis and Fan, 1984), and current

research suggests that the portal for pulpal infection in these cases is through enamel cracks that develop after the trauma (Love 1996).

### Microbial identification methods

Given the negative impact bacteria have on root canal treatment, and the many ways that different bacteria can gain access to the pulp, identification and closer examination of the bacteria responsible for apical periodontitis is warranted. To date, bacterial culture and molecular techniques have helped identify more than 500 bacterial species in the oral cavity (Paster *et al.* 2001, Aas *et al.* 2005).

Microbial culture technique has been used for studying endodontic pathogens for many years. It allows for identification of new species and quantification of major viable microorganisms (Ingle *et al.* 2008). Culture studies are commonly used for evaluation of new therapeutic techniques and materials.

For all of their advantages, however, culture studies have been shown to have some drawbacks. Those include the inability to grow a large number of bacterial species existing in a sample, low sensitivity and specificity, dependence on mode of transport, slow provision of results and being too labor intensive. It's not possible, in other words, to cultivate all microorganisms in the laboratory setting. It's been shown that 50% of bacteria present in the oral cavity represent uncultivable bacteria due to the fact that the nutritional and growth conditions for these microorganisms are currently unknown (Aas *et al.* 2005).

In addition to culturing, various molecular biology methods have been introduced in the last twenty years. These methods are based on investigation of

bacterial DNA and RNA. The most common techniques include PCR method, DNA-DNA hybridization and fluorescence in situ hybridization (FISH). These tests have high sensitivity and specificity and can identify both cultivable and as-yet-uncultivated bacterial species (Siqueira and Rocas, 2009). However, their high sensitivity and ability to detect non-viable cells can be a limitation as well. By detecting dead bacteria at the site of the infection, a wrong assumption about their role in the infection may be made. But, these limitations can be considered an advantage when studying endodontic infections because, theoretically, any pathogen found in the root canal *can* cause endodontic infection (Sundqvist, 1994), and the use of these tests can reduce the risk of overlooking a potentially relevant bacterial species.

### Endodontic microflora

As soon as dental pulp becomes necrotic, it is quickly colonized by bacteria. In the initial stages of endodontic infection, facultative anaerobes predominate, but as infections progress, the unique environment of the root canal selects for obligate anaerobes (Fabricius *et al.* 1982). This shift in bacterial flora has been studied in monkeys (Müller *et al.* 1981, Fabricius *et al.* 1982). The researchers infected monkey root canals with endemic oral bacteria and found that after an interval of up to 1,060 days, 98% of bacteria isolated were obligate anaerobes.

The major factors that affect the bacterial makeup in the infected root canal include oxygen tension, the type and availability of nutrients, and bacterial interactions. Low oxygen tension develops in the necrotic canal as a

consequence of consumption by facultative bacteria and loss of blood supply to the pulp (Loesche 1968; Loesche *et al.* 1983; Carlsson *et al.* 1977).

The main sources of nutrients in the necrotic root canal result from the breakdown products of the pulp, proteins from tissue fluid and exudate, components of saliva, and the metabolic byproducts of other bacteria. This is why the early stages of infection are dominated by saccharolytic species. As the infection progresses, they are soon outnumbered by asaccharolytic species that are capable of fermenting proteins into amino acids, especially in the apical portion of the root canal (Sundqvist *et al.* 2003).

Antagonistic and symbiotic interactions also occur in the bacterial ecosystem and influence the survival of one bacteria over another. Certain bacterial species obtain the essential nutrients for their growth from the metabolism of other bacteria (Marsh 1989), for example, black-pigmented anaerobic rods such as *Prevotella* and *Porphyromonas* depend on vitamin K and hemin for their development. Bacterial species *Veillonella* and *Campilobacter* produce vitamin K and hemin as products of their metabolism, which can then be utilized by *Prevotella* and *Porphyromonas* species for their growth (Gibbons *et al.* 1964; Grenier *et al.* 1986).

Bacteria that have been most consistently isolated from primary endodontic infections belong to the genera *Fusobacterium*, *Streptococcus*, *Porphyromonas*, *Prevotella*, *Eubacterium*, *Peptostreptococcus*, *Propionibacterium* and *Campylobacter* (Sundqvist 1994). Several studies demonstrated that black-pigmented rods are more likely to be associated with clinical symptoms such as

pain and swelling (Sundqvist 1976; Sundqvist *et al.* 1989; Hashioka *et al.* 1992). Chavez de Paz (2002) showed that *F. nucleatum* was associated with the most severe pain in endodontic flare-ups, and suggested that the combination of *F. nucleatum*, *Prevotella* and *Porphyromonas* may, when acting synergistically, increase the risk factor for endodontic flare-ups.

Furthermore, these bacterial species do not exist in the canal as separate colonies but rather overlap their growth in communities known as biofilms. These were described by Nair (1987) as “coaggregating” communities with palisade structures that adhere to the dentinal wall of the root canal. The formation of biofilms protects the bacteria from the harsh environment, allows for metabolic commensalism among different species, and amplifies resistance to antimicrobial agents (Costerton *et al.* 2003).

The difficulty of completely removing bacterial biofilms from the root canal system may be a key reason for apical periodontitis that appears in secondary infections. The bacterial flora that are associated with treatment failures are different from the ones found in primary infections. Secondary infections are composed of fewer species and are mostly dominated by Gram-positive facultative anaerobes that include streptococci, lactobacilli, *Propionibacterium* species, yeasts, *Enterococcus faecalis* and *Actinomyces* species (Sundqvist *et al.* 1998; Pinheiro *et al.* 2003; Chavez de Paz *et al.* 2004; Siqueira and Rocas, 2004). *E. faecalis* is one of the most frequently isolated bacteria from root-treated teeth.

### *Enterococcus faecalis*

*E. faecalis* is a Gram-positive facultatively anaerobic coccus that is a normal part of human intestinal flora. *E. faecalis* cells are ovoid in appearance and can grow in single cells and in chains. It is the second most common cause of nosocomial infections in the United States and can cause urinary tract infections, prosthetic joint infections, infective endocarditis and abdominal and pelvic infections (Richards *et al.* 1999).

Clinical studies have shown that *E. faecalis* also inhabits the oral cavity and gingival sulcus (Sedgley *et al.* 2004; Zhu *et al.* 2010) and can be present in untreated root canals with primary infections (Siquera *et al.* 2002; Sedgley *et al.* 2006). However, it has been most commonly isolated from root-treated teeth with apical periodontitis (Sundqvist *et al.* 1998; Molander *et al.* 1998; Siqueira *et al.* 2004; Rocas *et al.* 2004).

Figdor *et al.* (2003) suggested that *E. faecalis* is inoculated during root canal treatment from the oral cavity and then persists due its ability to survive. *E. faecalis* has been shown to be present in multiple oral samples from six of the eight patients undergoing endodontic treatment (Gold *et al.* 1975) and was detected in oral rinses of 11% of 100 endodontic patients and 1% of dental students with no history of endodontic treatment (Sedgley *et al.* 2004).

Oral prevalence of *E. faecalis* may vary according to periodontal condition; it was detected in greater numbers from patients with periodontal disease compared to those with healthy gingiva (Sedgley *et al.* 2006).

The two methods used for identification of this bacteria are the culture and molecular assays. Using culture studies, it was demonstrated that *E. faecalis* was present in 30-38% and even 64% of root-treated teeth with apical periodontitis, respectively (Sundqvist *et al* 1998; Hancock *et al* 2001; Peciuliene *et al.* 2001). However, a more sensitive polymerase chain reaction technique detected *E. faecalis* in as many as 77% of failing root canal treated teeth (Siqueira *et al.* 2004) or as few as 22% (Fouad *et al* 2005) or 12.1% (Kaufman *et al.* 2005). There appears to exist a marked difference in detection of the *E. faecalis* in root canal samples by culture technique and PCR. A study, comparing the prevalence of *E. faecalis* using both techniques, showed that *E. faecalis* was detected in 10.2% and 79.5% of root canals samples by culture and qPCR, respectively (Sedgley *et al.* 2006).

Other studies, however, show that this bacteria is not as strongly associated with secondary root canal infections as previously thought. Studies by Cheung *et al.* (2001) and Rolph *et al.* (2001), for example, failed to recover this bacteria from retreatment cases at all. Sedgley *et al.* (2006) showed that *E. faecalis* was recovered from 67.5% of teeth with primary infections. More recent studies using 16S rDNA found it to not be an important contributor. For example, Kaufman *et al.* (2005) compared the root-filled teeth with and without signs of apical periodontitis and found that *E. faecalis* was present in only 6% of teeth with lesions and 23% of teeth without lesions. Zoletti *et al.* (2006) detected the presence of *E. faecalis* in 81% of 27 root-filled teeth with normal periapex and 78% of 23 root-filled teeth with apical periodontitis. While the authors of both

studies concluded that it is possible that *E. faecalis* does not cause infections in root-filled teeth, they suggest that more research is needed.

Given the studies above, it is probable that the recovery of *E. faecalis* from previously root-treated teeth is not related as much to its pathogenicity as its simple ability to survive the harsh environment of a root canal during endodontic treatment.

*E. faecalis* can colonize root canals as a single infection, but may also be able to potentiate the pathogenicity of other bacteria. For example, in a monkey study by Müller *et al.* (2004), when *E. faecalis* was inoculated together with other bacterial strains into monkey root canals, it not only was the only microorganism isolated from all 24 teeth, it also increased the survival and pathogenicity of other bacterial strains.

*E. faecalis* is a fastidious bacteria (Figdor *et al.* 2003). It has the ability to resist the high pH of the most commonly used antimicrobial medication: calcium hydroxide (Haapasalo *et al.* 1987, Orstavik *et al.* 1990). This is due to its ability to regulate its internal pH with a proton pump (Evans *et al.* 2002). It has been shown to have low sensitivity against sodium hypochlorite (Orstavik *et al.* 1990) and be resistant to antibiotics, such as  $\beta$ -lactams, most aminoglycosides, and clindamycin (Molander *et al.* 1990, Sedgley *et al.* 2005).

Another survival mechanism is the ability of *E. faecalis* to penetrate deep into dentinal tubules to protect itself from chemomechanical preparation and intracanal medicaments (Haapasalo *et al.* 1987). *E. faecalis* has also been shown to be capable of entering and recovering from a viable but non-cultivable

(VBNC) state, a survival mechanism utilized by bacteria when exposed to environmental stress (Lleo *et al.* 2001; Lleo *et al.* 2005). In this state, the bacteria are able survive periods of starvation and then resume their growth when nutrients once more become available (Figdor *et al.* 2003). In an *ex-vivo* study, Sedgley *et al.* (2005) showed that *E. faecalis* can also survive starvation in root-filled canals, providing a locus for subsequent infection. *E. faecalis* has also been shown to generate stress proteins when exposed to stressful environmental factors such as sodium hypochlorite (Laplace *et al.* 1997).

Another survival mechanism was found in recent studies, such as the work by Johnson *et al.* (2006) which demonstrated that *E. faecalis* can form coaggregates with other bacterial species such as *F. nucleatum*. Another study on extracted teeth demonstrated that *E. faecalis* is capable of forming biofilms on root dentin (Kishen *et al.* 2006). This can also provide the means for survival in root-treated teeth.

Finally, researchers recently identified some virulence factors that help the survival of this bacteria in root canals. These consist of enterococcus surface protein (Esp), collagen-binding protein (Ace), gelatinase, and toxins such as cytolysin (Sedgley *et al.* 2005). These traits allow *E. faecalis* to be selected over the other bacteria in the root canal environment.

Given the presence of *E. faecalis* in the root canal system, its diverse survival mechanisms, and research that supports its contribution to secondary infections in root canal treatments, it is essential that any antimicrobial measures taken before obturation be effective against this bacteria.

## Sodium Hypochlorite

Use of an irrigating solution is an essential part of the root canal treatment. It acts as a lubricant for instrumentation, removes pulp remnants and dentinal debris and disinfects the canal. Sodium hypochlorite is the most widely used irrigating solution for these purposes.

In terms of its antimicrobial activity, sodium hypochlorite is a broad-spectrum antibacterial agent that is effective against both Gram-positive and Gram-negative bacteria, yeast, fungi and viruses (Shih *et al.* 1970, Bystrom *et al.* 1985). It is mostly used in concentrations that vary from 0.5% to 6% (McDonnell and Russell, 1999). Sodium hypochlorite was first introduced by Dakin in 1915 as an antiseptic for disinfecting wounds and used in the buffered form at pH 9 and concentration of 0.5%.

Sodium hypochlorite's mode of action is as follows: in water, NaOCl ionizes to produce  $\text{Na}^+$  and the hypochlorite ion,  $\text{OCl}^-$ . Between pH 4 and 7, the active moiety exists as hypochlorous acid (HClO), and above pH 9, as  $\text{OCl}^-$  (McDonnell and Russell, 1999). Hypochlorous acid provides antibacterial action by disrupting oxidative phosphorylation and DNA synthesis (McKenna *et al.* 1988, Barrette *et al.* 1989).

Sodium hypochlorite is highly effective at dissolving both vital and necrotic pulp tissue and collagen, which is the organic component of the dentin (Hand *et al.* 1978; Baumgartner and Cuenin, 1992). Increasing the temperature of lower concentrations of NaOCl increases its antimicrobial and tissue-dissolving capability (Cunningham and Joseph, 1980).

Much debate has taken place over the optimal concentration of sodium hypochlorite for endodontic irrigation. Spangberg *et al.* (1973) recommended only 0.5% NaOCl for endodontic irrigation due to its low toxicity in periapical tissues while still providing maximum antimicrobial effect. Bystrom and Sundqvist (1983) showed that concentrations of 0.5% are just as clinically effective at achieving negative cultures as concentrations of 5.25%. In several subsequent *in-vitro* studies, the antibacterial effect of various concentrations of NaOCl was demonstrated against endodontic pathogens (Waltimo *et al.* 1999, Barnard *et al.* 1996, Portenier *et al.* 2005, Haapasalo *et al.* 2000).

In comparison to *in-vitro studies*, *in-vivo* studies showed sodium hypochlorite to have poorer antibacterial performance. For example, a study evaluating the antibacterial effect of 0.5% NaOCl in 15 single-rooted teeth showed that only 12 out of 15 teeth were rendered free of bacteria after several appointments (Bystrom and Sundqvist, 1983). Another *in-vivo* study in root-filled teeth with apical periodontitis demonstrated the presence of bacteria (including *E. faecalis*) in 10 out of 40 teeth after chemomechanical preparation (Peciuliene *et al.* 2001).

The studies above focus on disinfection of the root canal, but for optimal results, ensuring a bacteria-free environment also requires the prevention of re-entry of microorganisms into the root canal during treatment. One of the ways bacteria can enter the root canal is through contaminated endodontic materials. Therefore, the disinfection and/or antimicrobial activity of the materials used for

obturation, such as gutta-percha and various sealers, becomes one of the methods to ensure a bacteria-free root canal environment.

### Gutta-Percha

Grossman (1940) identified the following criteria for the ideal root canal filling material:

- 1) It should be easily introduced into the root canal.
- 2) It should seal the canal laterally as well as apically.
- 3) It should not shrink after being inserted.
- 4) It should be impervious to moisture.
- 5) It should be bacteriostatic or at least not encourage bacterial growth.
- 6) It should be radiopaque.
- 7) It should not stain tooth structure.
- 8) It should not irritate periradicular tissues.
- 9) It should be sterile, or easily and quickly sterilized, immediately before the insertion.
- 10) It should be removed easily from the root canal, if necessary.

Historically, various materials have been used for obturation of root canals, including both pastes and solid materials such as silver cones, gutta-percha, carrier-based gutta-percha and Resilon. Currently, gutta-percha is the most commonly used root filling material and satisfies most of the criteria outlined by Grossman.

Gutta-percha was first introduced by a Connecticut dentist, Dr. Asa Hill, in 1847, as an option for a plastic restorative material. He mixed it with carbonate of

lime and quartz and called it “Hill’s stopping”. In 1867, Dr. G.A. Bowman claimed that he used gutta-percha to fill canals in a first molar (Gatewood 2007).

Gutta-percha is produced from rubber trees that are native to Malaysia, Borneo, Indonesia and Brazil. Gutta-percha is the trans isomer of polyisoprene,  $C_5H_8$  (rubber). It exists in two crystalline forms,  $\alpha$  and  $\beta$ . Raw gutta-percha comes in  $\alpha$  form, and commercially manufactured gutta-percha points exist in  $\beta$  crystalline form.

The mechanical properties of the two different forms are the same; however, there are some thermal and volumetric differences (Goodman *et al.* 1974). When gutta-percha is in solid  $\beta$  form, it’s easy compactable. When gutta-percha in  $\beta$  form is heated above  $46^\circ C$ , it changes to  $\alpha$  phase and becomes pliable and can be made to flow. This is very useful when thermoplastic techniques are utilized. When  $\alpha$  phase gutta-percha cools normally, it crystallizes to  $\beta$  form with a slight shrinkage of between 1 and 2% (Schilder *et al.* 1974). As thermoplastic techniques become more and more common, more gutta-percha products in  $\alpha$  phase have been introduced to the market (e.g., Thermafil and Microseal).

Implantation studies in animals have shown that gutta-percha generally has low toxicity and good biocompatibility with the periapical tissues when compared with root canal sealers (Spangberg 1969, Seltzer *et al.* 1975). Other studies, however, did not support this finding and showed that overextension of gutta-percha can be associated with periapical radiolucency (Sjögren *et al.* 1990, Nair *et al.* 1990). Spangberg *et al.* (1990) showed that gutta-percha composition

has an effect on its biocompatibility with the periapical tissues. They found through *in-vitro* study that gutta-percha was cytotoxic due to leaching of zinc oxide. Sjögren *et al.* (1995) demonstrated in an *in-vivo* implantation animal study that larger gutta-percha particles caused less inflammation and were more encapsulated when compared to small particles. The smaller particles induced an inflammatory reaction in guinea pigs, with massive accumulation of mononucleated and multinucleated macrophages.

Gutta-percha cones consist of approximately 20% gutta-percha, 65% zinc oxide, 10% radiopacifiers, and 5% plasticizers (Friedman *et al.* 1977). Moorer *et al.* (1982) showed that gutta-percha cones exhibit antimicrobial activity due to their zinc oxide content, however, this activity may be too weak to be effective.

### *Gutta-percha Combined with Antibacterial Medicaments*

Researchers have attempted to combine gutta-percha cones with various antibacterial medicaments such as chlorhexidine (Roeco active point, Coltene/Whaledent, Germany), Ca(OH)<sub>2</sub> (Roeco calcium hydroxide, Roeco; Hygenic calcium hydroxide points, Coltene/Whaledent, Germany), iodoform (MGP, Medidenta International, Inc., Woodside, NY) and tetracycline (Martin, Rockwell, MD) for increased antibacterial activity. An *in-vitro* study comparing the antibacterial effect of calcium hydroxide and chlorhexidine containing gutta-percha points showed that chlorhexidine impregnated points had a better antibacterial effect (Lin *et al.* 2006). However, it is important to note that the cones used in this study are only intended for temporary use as carriers of antibacterial medication.

An *in-vitro* study on the effects of iodoform containing gutta-percha showed that it was inhibitory against most endodontic pathogens except *E. faecalis* and *E. coli* (Shur *et al.* 2003). A later study by Bodrumlu *et al.*, (2006) comparing iodoform impregnated gutta-percha (MGP) with regular, non-medicated gutta-percha, showed that MGP gutta-percha inhibited all endodontic pathogens for up to 24 hours and was more effective than regular gutta-percha.

Gutta-percha containing tetracycline inhibited all tested endodontic pathogens (Bodrumlu *et al.* 2008) and a study comparing tetracycline containing gutta-percha and iodoform containing gutta-percha showed that tetracycline impregnated gutta-percha was superior in inhibition of all organisms tested (Melker *et al.* 2006).

Overall, the incorporation of antimicrobials into the obturation materials can be useful for prevention of secondary infection after completion of root canal treatment. However, gutta-percha cones containing medicaments are new on the market and *in-vivo* studies are needed to evaluate both their toxicity and their antibacterial and antifungal effects.

### Sealers

Sealers are used in combination with semisolid materials like gutta-percha for obturation of the root canal. Their purpose is to fill the voids between the root canal walls and the gutta-percha. Orstavik (2005) demonstrated the importance of this when he showed that gutta-percha root fillings used without a sealer were frequently associated with clinical and radiographic signs of apical periodontitis. A study by Wu *et al.* (2000), that compared leakage in root canals obturated with

gutta-percha alone and gutta-percha with sealer, showed that even though leakage was evident in all of the teeth, it was significantly worse in root canals filled only with gutta-percha.

According to Grossman (1976), root canal sealer should provide a tight seal, be tissue compatible, and have antimicrobial properties, amongst other parameters.

AH 26 is the most commonly used sealer, and belongs to the epoxy resin group of sealers. It contains a bis-phenol epoxy resin that releases formaldehyde upon setting for up to two days (Spangberg *et al.* 1993). AH 26 exhibits low shrinkage, low solubility, radiopacity, good adhesion to dentin and gutta-percha (Lee *et al.* 2002), and tissue compatibility (Spangberg and Pascon, 1988). AH26 has also been shown to exhibit cytotoxicity that is contributed to the release of formaldehyde (Spangberg, 1969). A newer formulation, AH Plus, was formulated to prevent release of formaldehyde while maintaining the advantages of AH26 (Leonardo *et al.* 1999).

Despite proper chemomechanical preparation and placement of antibacterial medicaments, bacteria can still survive in the dentinal tubules (Molander, 1999). Therefore, it is desirable that endodontic sealers also have antimicrobial activity.

Various sealers have been reported to have antimicrobial properties. For example, it has been shown that sealers containing eugenol and formaldehyde (such as AH 26) are the most effective against bacteria (Kaplan *et al.* 1999). However, conflicting results have been found regarding the antimicrobial activity

of AH Plus sealer which has significantly less formaldehyde compared with the original AH26 formulation. A study of four endodontic sealers demonstrated that AH Plus had no antimicrobial activity (Mickel *et al.* 2003), however, a study by Kayaoglu *et al.* (2005) demonstrated that AH Plus exerted a strong antimicrobial activity on *E. faecalis*. The contradictory findings of these studies suggest that more work is needed in this area.

### Disinfection of Gutta-percha

Commercially available gutta-percha cones come in pre-sterilized packages. However, some studies have shown that 5-8% of the cones from sealed packages can be contaminated with bacteria (Montgomery *et al.* 1971, Gomes *et al.* 2005). Also, gutta-percha cones can be contaminated by handling, when exposed to the dental operatory environment and during storage (Linke *et al.* 1983, da Motta *et al.* 2001).

Given these conditions, and the importance of preventing cross-contamination of the root canal during endodontic treatment, it has been recommended that gutta-percha cones be sterilized prior to obturation. Because gutta-percha cones cannot be sterilized by conventional autoclaving, different chemicals have been suggested for use in decontamination of cones. The following agents have been recommended: Zephirin, Zephirin chloride, untinted tincture of Metaphen, thimerosal, povidone-iodine, alcohol, formaldehyde gas, and glutaraldehyde (Montgomery *et al.* 1971, Doolittle 1975, Senia *et al.* 1975, Senia *et al.* 1977, Frank *et al.* 1983, Stabholtz *et al.* 1987). In recent years, other agents such as chlorhexidine and MTAD have also been suggested (Gomes *et*

al. 2005, Pang *et al.* 2007, Royal *et al.* 2007). Finally, sodium hypochlorite, the most widely used agent for irrigation, has become the material of choice for chairside chemical decontamination of gutta-percha.

### Disinfection of Gutta-percha with Sodium Hypochlorite

The most efficient and reliable technique for disinfecting gutta-percha cones was first proposed by Senia *et al.* (1975). They suggested disinfecting gutta-percha cones by placing them into 5.25% NaOCl for at least one minute. They contaminated gutta-percha cones with cultures of *Staphylococcus epidermalis*, *Carynebacterium xerosis*, *E. coli*, *E. faecalis* and spores of *B. subtilis*, immersed them in 5.25% NaOCl, and found that all microorganisms were killed after one minute. Sequiera *et al.* (1998) and Gomes *et al.* (2005) found similar results when showing that *B. subtilis* spores were eliminated by 5.25% NaOCl. Royal *et al.* (2007) showed that disinfection with 5.25% NaOCl was effective against *E. faecalis* contaminated cones. Other studies using lower concentrations of NaOCl, such as 2.5%, also showed it to be effective against spore forming genus *Bacillus* (da Motta *et al.* 2001, Ozalp *et al.* 2006). A study done on the effectiveness of Dakin's solution on decontamination of gutta-percha showed that this lower concentration can kill *Staph. aureus*, *E. coli*, and spores of *B. subtilis* if cones are placed in it for 5 minutes (Cardoso *et al.* 1999). Another study showed that 0.5% NaOCl required 30 min to eliminate *B. subtilis* spores, *E. faecalis* and *S. aureus* from contaminated gutta-percha (Gomes *et al.* 2005).

But, decontamination of gutta-percha has been shown to have potential, unintended drawbacks. For example, Short *et al.* (2003) recently showed, in a

scanning electron microscope study, that after decontamination of gutta-percha in 5.25%NaOCl, cuboidal chloride crystals formed on the cones that could impede the obturation seal. They recommended rinsing disinfected gutta-percha cones in 96% ethyl alcohol, 70% isopropyl alcohol, or distilled water to remove them.

Another potential drawback is the discovery of changes in the physical properties of gutta-percha cones after disinfection with chemical agents. This was first described by Müller and Orstavik (1985). In an atomic force microscopy study, Valois *et al.* (2005) reported that a 1 minute treatment with 5.25% NaOCl increased the elasticity of gutta-percha cones in comparison with untreated cones. The authors also found topographic changes after gutta-percha was placed in 5.25% NaOCl for 5 minutes. However, a lower concentration of 0.5% NaOCl did *not* cause any topographic or elasticity changes to the gutta-percha cones. The authors concluded that 0.5% NaOCl could be an effective alternative to full-strength bleach for disinfection of gutta-percha cones.

### Purpose of this Study

Dentists are occasionally faced with the problem of secondary infection after completion of root canal treatment. Prevention of bacterial contamination is essential to prevent such infections. One key way to achieve this is through disinfection of gutta-percha cones and/or use of obturation materials with antimicrobial properties. Dakin's solution has been shown to be effective against a variety of Gram-negative, Gram-positive pathogens, fungi and spores. It has also been shown to combine maximum antibacterial effect with minimal toxicity to

the tissues. However, there seems to be very little research on the disinfection of gutta-percha cones using Dakin's solution, especially looking at its effectiveness against *E. faecalis*. Only one study cited above (Gomes *et al.* 2005) addresses this, and suggests a 30-minute immersion of cones. This is something that is not practical in clinical settings. To further explore the use of sodium hypochlorite solution in the disinfection of gutta-percha cones this study was undertaken. The study had five goals.

- 1) To assess the presence of contamination in commercially available gutta-percha points.
- 2) To assess the presence of contamination from the environment in a previously opened box of gutta-percha points.
- 3) To assess the effectiveness of 0.5% NaOCl and 5.25% of NaOCl followed by a rinse in 100% ethyl alcohol for decontamination of gutta-percha points contaminated with *E. faecalis* and saliva.
- 4) To assess the minimum concentration of NaOCl and time required for disinfection of gutta-percha points contaminated with *E. faecalis*.
- 5) To assess the antimicrobial properties of AH26 sealer, if any, on gutta-percha points contaminated with *E. faecalis* and saliva.

## Materials and methods

All trials used size 30 gutta-percha cones manufactured by Premier Dental Products Co. (Plymouth Meeting, PA) and randomly selected from five new, sealed manufacturer's boxes. All trials were performed in duplicate.

### *E. faecalis* suspension

*E. faecalis* (strain ATCC) suspension was grown in 8mm culture tubes containing thioglycollate medium, vitamin K-1 and hemin (BBL™ Becton, Dickinson and Company, Sparks MD) and incubated at 37°C for approximately 23 hours; the optical density at 600 nm wavelength was monitored until the late exponential growth phase was reached (OD600 value of  $1 \pm 0.05$ ).

The bacteria were identified and images were acquired using a Zeiss Axioimager M1 microscope and a 63X NA 0.5 oil immersion objective. Bacteria were Gram-stained and color images were taken.

### Cone Contamination Procedure

Two contaminants were used: *E faecalis*, and saliva. *E. faecalis* suspension was prepared as described above and saliva was collected from healthy volunteers. Gutta-percha points for both contaminants were taken directly from sealed manufacturer's boxes and immersed in 1ml of contaminant for 1 min. They were subsequently transferred for air drying to sterile dishes containing sterile 4x4 gauze pads.

### Cone Decontamination Procedure

All contaminated cones were aseptically transferred and fully immersed in 2ml of two concentrations of NaOCl: 0.5% (pH<9.0, Dakin's solution, Century Pharmaceuticals Inc., Indianapolis, IN) and 5.25% (A1 bleach, James Austin Company, Mars, PA) and allowed to soak for 1 min.

Once removed from the NaOCl solution, cones were rinsed in 100%ethyl alcohol (Pharmco Products Inc., Brookfield, CT), air dried and transferred into tubes containing the bacterial culture medium described below.

### Bacterial Culture Medium

All cones were placed into tubes containing 8ml of BBL medium. All samples were incubated at 37°C for 7 days and observed periodically for turbidity. Samples that demonstrated turbidity at the end of 7 days were deemed culture positive. Culture tubes that remained clear at the end of 7 days were deemed culture negative. The same procedure was performed for controls.

### Evaluation of contamination in commercially available gutta-percha boxes

#### (Group 1)

Group 1 consisted of 20 cones each selected from five sealed manufacturer's boxes and placed in the bacterial culture medium.

### Evaluation of contamination of gutta-percha from the environment in a previously opened box of gutta-percha cones (Group 2)

Group 2 consisted of 40 cones divided into 4 subgroups. Ten new cones taken from a sealed manufacturer's box constituted group 2A. The open gutta-

percha box was then appropriately labeled and placed in the dental student clinic at University of Connecticut School of Dental Medicine for dental students' use. Once a week over the course of a month, 10 cones were taken from this box and placed in the bacterial culture medium described above to assess contamination. This yielded groups 2B, 2C and 2D respectively.

*Evaluation of the effectiveness of 0.5% NaOCl and 5.25%NaOCl for decontamination of gutta-percha cones contaminated in E. faecalis (Group 3)*

Group 3 consisted of 20 gutta-percha cones contaminated with *E. faecalis* as described above and divided into two subgroups. Cones in group 3A were decontaminated with 0.5% NaOCl as described above, and points in group 3B were decontaminated in 5.25% NaOCl also as described above. The cones were then placed in the bacterial culture medium to assess the antimicrobial effectiveness of different concentrations of NaOCl. Five cones selected from the manufacturer's box, placed in sterile culture medium for 1 min and decontaminated with either 0.5% or 5.25% NaOCl followed by a rinse with ethyl alcohol served as the negative control for each subgroup. Five points contaminated with *E. faecalis* and placed in sterile culture medium served as the positive control for each subgroup.

Evaluation of the effectiveness of 0.5% NaOCl and 5.25%NaOCl for decontamination of the gutta-percha cones contaminated in saliva (Group 4)

Group 4 was divided into two subgroups of 10 points each and both contaminated with saliva as described above. Cones in group 4A were decontaminated with 0.5% NaOCl as described above, and cones in group 4B were decontaminated in 5.25% NaOCl. The cones were then placed in the bacterial culture medium. Five cones selected from the manufacturer's box and placed in sterile culture medium for one minute and decontaminated with either 0.5% or 5.25% NaOCl followed by a rinse in ethyl alcohol, served as the negative control for each subgroup. Five cones contaminated with saliva and placed in sterile culture medium served as the positive control for each subgroup.

Serial dilutions (Group 5)

To determine the minimum concentration of NaOCl solution that still effectively disinfects contaminated cones, several lower concentrations of NaOCl were tested. Concentrations of NaOCl tested were the following: 0.25%, 0.05%, 0.005%, 0.0005%, 0.00005% and 0.000005%. To prepare the desired concentrations, 0.5% NaOCl was diluted with sterile water to the desired concentrations.

Ten cones contaminated with *E. faecalis* were subjected to the decontamination procedure using each serial dilution concentration of NaOCl followed by a rinse in ethyl alcohol. To control for any decontaminating effects of ethyl alcohol, another 10 cones contaminated with *E. faecalis* were subjected to

the same procedures, but were not rinsed in ethyl alcohol. All cones were then transferred into a bacterial culture medium.

To provide negative controls for each group concentration, 2 cones were transferred directly from the manufacturer's box into a sterile culture medium for 1 min, then decontaminated in serial dilution concentration of NaOCl for 1 min and subsequently either rinsed in alcohol or not rinsed.

To provide positive controls, 2 cones contaminated with *E. faecalis* were transferred directly to the bacterial culture medium.

#### Minimum decontamination time (Group 6)

To assess the minimum time required to effectively decontaminate gutta-percha cones in 0.5% NaOCl, 10 cones contaminated with *E. faecalis* were decontaminated in 0.5% concentration of NaOCl for 30 seconds. One group was rinsed in 100% ethyl alcohol and one group was not, and then transferred to the bacterial culture.

For the negative controls, 2 cones, transferred directly from the manufacturer's box into a sterile culture medium for 1 min, were subjected to the same timed decontamination procedure.

Two cones contaminated with *E. faecalis* and transferred directly to the bacterial culture medium served as the positive controls.

#### Evaluation of antimicrobial activity of ethyl alcohol (Group 7)

To control for the antimicrobial activity of ethyl alcohol, ten cones of each contamination type were rinsed in ethyl alcohol and transferred to the bacterial

culture medium. For negative controls, 2 cones were transferred directly from the manufacturer's box into a sterile culture medium for 1 min, rinsed in ethyl alcohol and placed in the bacterial culture medium. For positive controls, 2 cones of each contamination type were placed directly into the bacterial culture medium.

*Assessment of the antimicrobial properties of AH26 sealer on gutta-percha points contaminated with E. faecalis and saliva (Group 8)*

Group 8 was subdivided into two subgroups of 10 cones each. Group 8A consisted of new cones taken from a sealed manufacturer's box, contaminated with *E. faecalis* as described above, then coated with AH26 sealer (Dentsply Maillefer, Johnson City, TN) and transferred into the bacterial culture medium. Group 8B consisted of new cones, contaminated with saliva as described above, coated with AH26 sealer and transferred into the bacterial culture medium in the same way as group 8A. This served to assess the antimicrobial properties of AH26 sealer. Five cones selected from the manufacturer's box, placed in sterile culture medium for 1 min and coated with AH26 served as the negative controls for each subgroup, and five cones contaminated with either *E. faecalis* or saliva served as the positive controls for each subgroup.

A summary of all the groups can be found in Table 1.

**Table 1. Summary of the experimental groups**

Group 1	gutta-percha cones taken from new manufacturer's box	
Group 2	gutta-percha cones from new manufacturer's box, opened and placed in the circulation in dental students' clinic and tested once a week over one month period	
Group 3	3A	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated with 0.5% NaOCl
	3B	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated with 5.25% NaOCl
Group 4	4A	gutta-percha cones contaminated with saliva decontaminated with 0.5% NaOCl
	4B	gutta-percha cones contaminated with saliva decontaminated with 5.25% NaOCl
Group 5	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated with decreasing concentrations of NaOCl	
Group 6	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated with 0.5% NaOCl for 30 seconds	
Group 7	7A	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated only with ethyl alcohol
	7B	gutta-percha cones contaminated with saliva decontaminated only with ethyl alcohol
Group 8	8A	gutta-percha cones contaminated with <i>E. faecalis</i> coated with AH26 sealer
	8B	gutta-percha cones contaminated with saliva coated with AH26 sealer

*Elapsed time between decontamination of gutta-percha points and appearance of positive bacterial culture for low concentrations of NaOCl*

The time necessary to obtain a positive culture for four different concentrations of NaOCl: 0.5% NaOCl, 0.0005%, 0.00005% and 0.000005% was also evaluated. Ten gutta-percha cones for each group were contaminated as described above with *E. faecalis* and then subjected to the decontamination

procedure described above using their respective NaOCl concentration levels. Culture tubes were visually inspected hourly over a 24-hour period, and time of appearance of turbidity noted. For positive controls, 10 gutta-percha cones were contaminated with *E. faecalis* and then transferred directly to the bacterial culture medium. To provide negative controls for each group, 2 cones were transferred directly from the manufacturer's box into a sterile culture medium for 1 min, then decontaminated in each group's NaOCl concentration for 1 min.

### Data analysis

Statistical analyses were performed on all data using Fisher's Exact test. The following experimental conditions were compared for significant differences: 0.5% NaOCl and 5.25% NaOCl for decontamination of cones contaminated in *E. faecalis* and saliva (groups 3A and 3B; groups 4A and 4B), AH26 sealer on gutta-percha cones contaminated with *E. faecalis* and saliva (groups 8A and 8B), and alcohol and no-alcohol conditions for each of the serial dilutions.

## Results

### Evaluation of the contamination of gutta-percha cones in manufacturer's boxes (Group 1)

None of the cones (100 cones total) removed from five different brand new boxes were culture positive.

### Evaluation of the contamination of gutta-percha from the environment in a previously opened box of gutta-percha points (Group 2)

None of the cones (80 cones total) taken from a previously opened box, placed in the circulation, and checked weekly over a four week period were culture positive.

### Evaluation of the effectiveness of 0.5% NaOCl and 5.25% NaOCl for decontamination of the cones contaminated in *E. faecalis* (Group 3).

Both 0.5% NaOCl and 5.25%NaOCl were equally able to eliminate *E. faecalis* from the gutta-percha cones after 1 minute of contact. Both groups had 0 positive cultures and 20 negative cultures. Using Fisher's exact test, no significant difference between the groups was found ( $p = 1.0$ ).

### Evaluation of the effectiveness of 0.5% NaOCl and 5.25%NaOCl for decontamination of the cones contaminated in saliva (Group 4)

Both 0.5% NaOCl and 5.25%NaOCl were equivalent in their ability to remove bacterial contamination present following submersion in saliva. One minute of contact with either concentration of NaOCl yielded zero positive

cultures. Using Fisher's exact test, no significant difference between the groups was found ( $p = 1.0$ ).

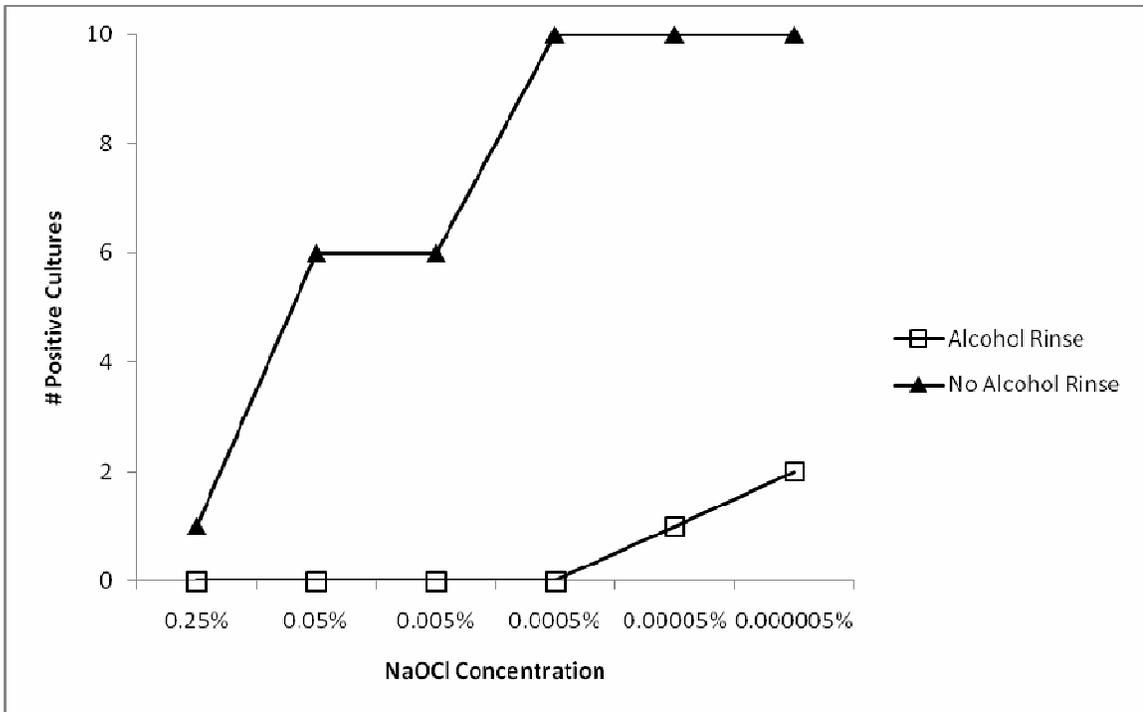
Serial dilutions (Group 5)

Following serial dilutions with alcohol rinse, positive cultures were only found with the 0.00005% concentration, where 1 positive culture was obtained, and the 0.000005% concentration, where 2 positive cultures were obtained. However, the no-alcohol rinse group showed 1 positive culture at 0.25% concentration, 6 at 0.05%, 6 at 0.005% and 10 (out of 10) positive cultures at 0.0005%, 0.00005% and 0.000005%. Comparison of the alcohol and no-alcohol conditions for each serial dilution yielded a significant difference for all dilutions except 0.25%. See Table 2 and Figure 1.

**Table 2. Positive culture results for serial concentrations of NaOCl with and without alcohol rinse (n=10).**

	NaOCl concentration					
	0.25%	0.05%	0.005%	0.0005%	0.00005%	0.000005%
<b>Alcohol</b>	0	0	0	0	1	2
<b>No-Alcohol</b>	1	6	6	10	10	10
<b>Fisher's Exact Test p-values</b>	1.0	0.01084*	0.01084*	0.00001083****	0.0001191***	0.0007145***

\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ , \*\*\*\* $p < .0001$



**Figure 1. Positive cultures of *E. faecalis*-contaminated gutta-percha points after disinfection with descending concentrations of NaOCl solution.**

In addition to differences between the alcohol and no alcohol conditions for each serial dilution, significant differences were found between serial dilutions within the no-alcohol group. Specifically, the 0.25% dilution was significantly different from the 0.0005%, 0.00005%, and 0.000005% dilutions, each with the same p value of 0.0001 (Table 3).

**Table 3. Fisher's exact test p-values for serial dilution comparisons in the no-alcohol group.**

	0.25%	0.05%	0.005%	0.0005%	0.00005%	0.000005%
0.25%	1.00	0.05728	0.05728	0.0001191	0.0001191	0.0001191
0.05%		1.00	1.00	0.08669	0.08669	0.08669
0.005%			1.00	0.08669	0.08669	0.08669
0.0005%				1.00	1.00	1.00
0.00005%					1.00	1.00
0.000005%						1.00

### Minimum decontamination time (Group 6)

*E. faecalis* was found to be extremely sensitive to 0.5% NaOCl and was eliminated (no positive cultures) after 30 seconds in both the alcohol and no alcohol groups. Therefore, there was no significant difference between using 0.5% NaOCl for 30 seconds and using it for 60 seconds (Fisher's exact test,  $p = 1.0$ ).

### Evaluation of antimicrobial activity of ethyl alcohol (Group 7)

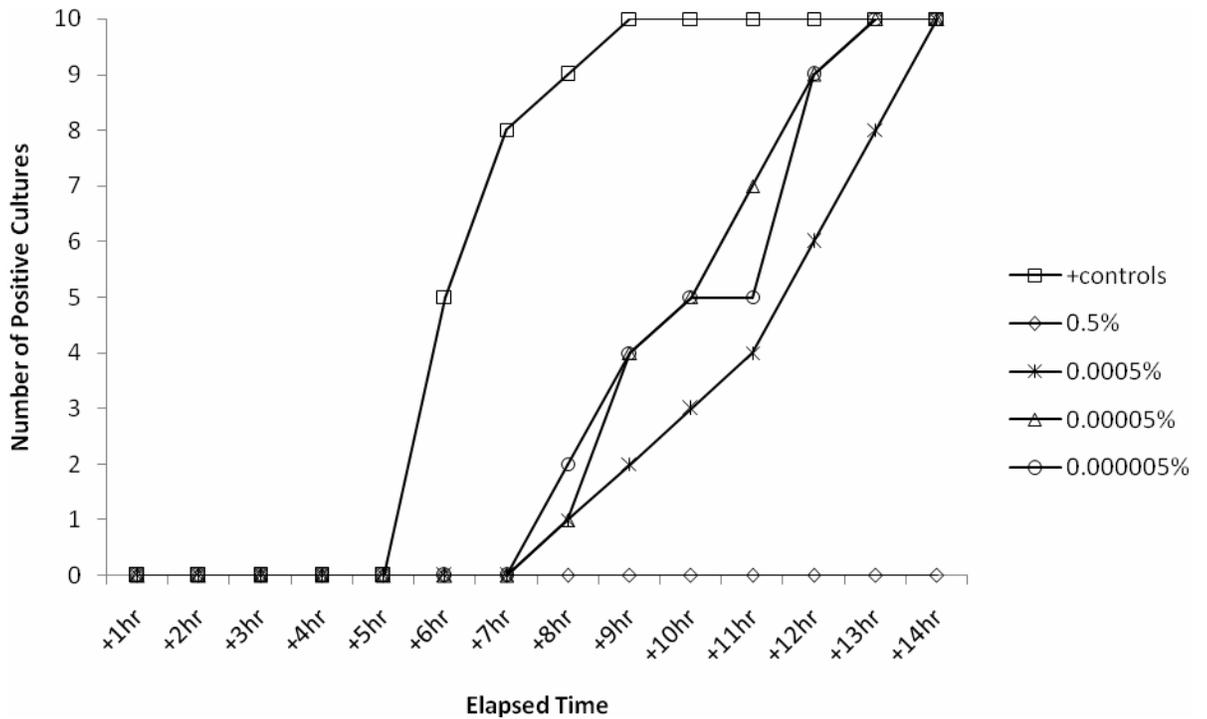
For the groups attempting to isolate antimicrobial effects of alcohol alone on with either saliva or *E. faecalis*, 9 out of 10 cultures showed bacterial growth, indicating that alcohol had little to no antimicrobial effect. In addition, comparison of differences between alcohol's antimicrobial effect on *E. faecalis* and saliva showed no significant differences, with Fisher's exact test producing a  $p$  value of 1.00.

### Evaluation of the effectiveness of the antimicrobial properties of AH26 sealer on gutta-percha cones contaminated with *E. faecalis* and saliva (Group 8)

Tests showed that AH26 sealer had no antimicrobial properties against *E. faecalis* and saliva. Both groups had 20 positive cultures and zero negative cultures, thus using Fisher's exact test, there was no significant difference between the AH26's effect on saliva and its effect on *E. faecalis* ( $p = 1.0$ ).

Elapsed time between decontamination of gutta-percha points and appearance of positive bacterial culture for low concentrations of NaOCl

The graph in Figure 2 shows the elapsed time from decontamination of gutta-percha cones with different concentrations of NaOCl and appearance of positive bacterial culture. No positive cultures appeared for 0.5% NaOCl solution, whereas the three lower concentrations eventually did display a positive culture within the 14 hour span of monitoring. None of the three lower concentrations produced positive cultures as quickly as the positive controls. For example, positive cultures appeared at the +6hr mark, whereas 0.0005% concentration did not produce positive cultures until the +8hr mark.



**Figure 2. Elapsed time from decontamination of gutta-percha points with NaOCl solution to appearance of positive bacterial culture**

## Discussion

The placement of gutta-percha cones in a prepared root canal is the final step in the root canal treatment procedure. For the treatment to succeed, it is imperative that a breakdown in the asepsis chain doesn't occur. Therefore, the sterility of gutta-percha is critical.

Other studies have shown that 5-8% of gutta-percha cones from sealed packages can be contaminated with bacteria (Montgomery *et al.* 1971, Gomes *et al.* 2005). Still other studies have shown that gutta-percha cones can be easily contaminated when exposed to the dental operatory environment, during storage and when manipulated incorrectly by the operator (Gomes *et al.* 2005). Because gutta-percha cones cannot be sterilized by heat, use of an effective chemical agent has been the preferred decontamination method. While different chemical agents have been suggested for this purpose (Montgomery *et al.* 1971, Doolittle 1975, Senia *et al.* 1975, Senia *et al.* 1977, Frank *et al.* 1983, Stabholtz *et al.* 1987), NaOCl has been the preferred choice (Senia *et al.* 1975).

The results of this study showed that none of the brand new gutta-percha cones that were cultured showed bacterial contamination as evaluated by culture. This corroborates others that also found no contamination in sealed new gutta-percha cones (Dolittle 1975, Pereira *et al.* 2010). However, our study is in contradiction to several studies (Mongtomery *et al.* 1971, Gomes *et al.* 2005) which did find contamination on gutta-percha cones from new packaging. A possible explanation for these differences is that our study only tested gutta-

percha manufactured by Premier Dental Products Co. while the studies cited above that did show contamination tested different brands (Mongtomery *et al.* 1971, Gomes *et al.* 2005). Therefore, it is possible that these inconsistencies may be attributable to variations in the manufacturing and packaging technology.

In this study, gutta-percha cones exposed to the environment of an endodontic clinic over a one-month period did not become contaminated. This is in disagreement with prior studies showing that 5.5% and even 19.4% of cones in a clinic were contaminated (Linke *et al.* 1983, Gomes *et al.* 2005, Pang *et al.* 2007). These differences could be attributed to the fact that my experiment was conducted over a one-month period whereas other studies, such as Gomes *et al.* (2005) conducted the experiment over a two-year period.

In this experiment, *E. faecalis* and saliva were used as contaminants. Many previous studies on disinfection of gutta-percha cones tested bacterial spores as a target microorganism (Senia *et al.* 1975, Siqueira *et al.* 1998, da Motta *et al.* 2001). *E. faecalis* was chosen in this experiment as it is a highly resistant microorganism and is prevalent within the root canal space (Haapasalo *et al.* 1987, Sundqvist *et al.* 1998, Siquera *et al.* 2002, Figdor *et al.* 2003). *E. faecalis* has become an ideal bacteria with which to test different irrigants and medicaments in-vitro due to its ability to grow under almost any laboratory conditions and the idea that this organisms implicated in secondary endodontic infection. However, it may not reflect the actual bacterial flora found on gutta-percha cones contaminated either in their boxes or from the operatory environment. Microorganisms most commonly isolated from gutta-percha cones

taken from opened boxes in endodontic clinics belong to *Staphylococcus* species (Gomes *et al.* 2005, Pang *et al.* 2007). These microorganisms are common inhabitants of the flora found on human skin, oral mucosa and in saliva.

Saliva was chosen as a second contaminant because of its high bacterial density (10<sup>8</sup> bacterial cells/ml) and the fact that it contains several different bacterial species, including *E. faecalis* and *Staphylococcus* species (Siqueira *et al.* 1998). It's also one of the most common ways to contaminate the root canal during treatment.

Various studies confirmed that gutta-percha cones can be effectively decontaminated with 5.25% NaOCl (Senia *et al.* 1975, Frank 1983, Sequiera *et al.* 1998, Cardoso *et al.* 1999, Gomes *et al.* 2005, Royal *et al.* 2007). In our study, NaOCl was found to be a potent disinfectant even in low concentrations. Specifically, 0.5% NaOCl was just as effective as 5.25% NaOCl in disinfecting against *E. faecalis* and saliva. These results were in disagreement with previous studies showing that it took up to 30 minutes to eradicate *E. faecalis* from contaminated gutta-percha in 0.5% NaOCl (Gomes *et al.* 2001, Cardoso *et al.* 1999). However, the results were in agreement with Haapasalo *et al.* (2000), who demonstrated that *E. faecalis* was rapidly eliminated by even a low concentration of 0.3% NaOCl.

It is possible that these conflicting results were due to differences in preparation of disinfectant. In this study, serial dilutions of NaOCl were prepared immediately before the experiment which corresponds with the recommendation

of Portenier *et al.* (2005), who showed that freshly prepared NaOCl in a low concentration quickly lost its activity in contact with air.

Disinfection methods used in this study mirrored the University of Connecticut Dental School accepted protocol for disinfection of gutta-percha: 1 minute immersion in 0.5% NaOCl followed by rinse in 100% ethyl alcohol. No statistically significant difference between immersion in 5.25% NaOCl followed by a rinse in ethyl alcohol and 0.5% NaOCl followed by a rinse in alcohol were found in this study. Alcohol rinse was first proposed as a countermeasure to the formation of NaOCl crystals formed on the surface of gutta-percha after decontamination with NaOCl (Spangberg 1994, Short *et al.* 2003). Given the effective antimicrobial activity of the lower concentration of 0.5%, and the fact that no studies have yet been done to determine if NaOCl crystals form at this concentration, it seems that further research should be done to determine the necessity of the alcohol rinse in clinical protocols that use it.

Even though ethyl alcohol has its own antimicrobial properties, this study showed that it was ineffective on its own in eliminating *E. faecalis* from gutta-percha cones. This was in agreement with Linke *et al.* (1983) and Sequiera *et al.* (1998), who showed that it cannot be solely used to disinfect gutta-percha cones. It has been shown that the antimicrobial action of alcohol is greater in the presence of water (Murray *et al.* 1984), however, in this study 100% ethyl alcohol was used both to minimize post-disinfection drying time of the gutta-percha cones and to match University of Connecticut Dental School accepted protocols.

Given the result above, a seemingly contradictory result emerged when testing very low concentrations of NaOCl, such as 0.00005%. Low concentrations, when used in combination with ethyl alcohol rinse, were more effective in eliminating *E. faecalis* from gutta-percha cones while these low concentrations used without alcohol rinse were not. This would suggest a synergistic antibacterial effect of NaOCl and alcohol. NaOCl provides antibacterial action by disrupting oxidative phosphorylation and DNA synthesis (McKenna *et al.* 1988, Barrette *et al.* 1989). Alcohol is a lipid solvent and its method of action is through disrupting the lipid structure of membranes and denaturing cellular proteins (Murray *et al.* 1994). It seems possible that alcohol potentiates the action of NaOCl on *E. faecalis*.

The results of this study also showed that AH26 sealer did not have independent antimicrobial properties against *E. faecalis* on contaminated gutta-percha cones. This result contradicts previous studies showing that AH26 does have antibacterial properties due to release of formaldehyde (Kaplan *et al.* 1999). However, the different culturing technique used in each study may account for this disparity.

In conclusion,

- 1) There was no contamination present in new sealed boxes of gutta-percha cones and gutta-percha cones are usually sterile during storage.
- 2) 0.5% NaOCl followed by a rinse in 100% ethyl alcohol was just as effective for decontamination of gutta-percha cones as 5.25% NaOCl followed by a rinse in alcohol against *E. faecalis* and saliva.

- 3) Concentrations of NaOCl at or below 0.5% may be effective for decontamination when used in combination with ethyl alcohol rinse.
- 4) Thirty seconds of immersion in 0.5% NaOCl was sufficient for decontamination of gutta-percha cones contaminated with *E.faecalis* and saliva.
- 5) AH26 did not have antimicrobial properties when tested using culture.

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