

Assessment of the efficacy of two concentrations of NaOCl and Chlorhexidine as disinfectant of Gutta-Percha contaminated with *Enterococcus Faecalis* (In vitro Study)



Medical Science

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ABSTRACT

Aim:

The aim of the present study is to assess the efficacy of different chemical disinfectants 5.25% NaOCl, 0.5% NaOCl, and Chlorhexidine 2% as an antimicrobial agent to decontaminate the Gutta-Percha cones used during obturation in endodontic procedure.

Materials and Methods:

Sixty four Gutta - Percha cones which were freshly extracted from freshly sealed packs are collected and used as three groups. Each group is composed of 20 cones and 4 cones are used as positive and negative controls. Depending upon the type of antibacterial agents used for sterilization. Group I - Gutta-percha immersed in NaOCl (5.25 %). Group II - Gutta-percha immersed in NaOCl (0.5%). Group III - Gutta-percha immersed in Chlorhexidine (2%).

A total of 64 gutta-percha cones (No. 30 Dentsply maillefer) were selected for this study, from freshly opened sealed pack. For each group, 20 cones were used. A total of 4 cones were used as controls. The following controls were used.

Positive control: A total of 2 gutta-percha cones were used as positive control exposed to the *E.Faecalis* bacteria. The cones were directly transferred to the culture media without disinfection.

Negative control: A total of 2 gutta-percha cones were taken from freshly sealed pack. The cones were then transferred to culture media without disinfection.

Results:

NaOCl in both concentrations 5.25% and 0.5% were excellent in eradication of *E.Faecalis* while Chlorhexidine 2% was good in disinfection of the gutta-percha cones.

Conclusions:

Gutta -percha that were freshly extracted from a sealed pack is not contaminated while those who were contaminated with *E.Faecalis* were best disinfected by NaOCl but Chlorhexidine is a good disinfectant agent for gutta-percha but not as good as NaOCl.

Introduction

Apical periodontitis is an infectious disease and thus asepsis, antisepsis and disinfection are paramount in endodontic treatment. 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.

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.

Obturation is one of the most important procedures which determine the success of endodontic therapy. The main purpose of the obturation is to promote healing and prevent percolation or ingress of microorganisms into the periapical area.¹

This can be achieved by following strict asepsis protocol during endodontic therapy. The practitioner must be concerned not only with endogenous oral microbial flora, but with exogenous bacterial contamination as well.²

An established fact that the success of endodontically treated teeth depends on the aseptic techniques followed during endodontic procedures.³ Gutta-percha points are the most commonly used endodontic filling material.⁴ They become accessible to potential contamination by variety organisms such as cocci, rods, and yeasts present in the air or contacting objects once the box of gutta-percha points are exposed to dental chair side clinical environment.⁵

Gutta-percha points, due to their chemical and physical properties are not amenable to the usual methods of physical sterilization in an autoclave or hot air oven. Therefore, chemical sterilization is mandatory for effective sterilization of gutta-percha points.⁶

A large variety of chemical disinfectants have been used to sterilize GP cones before root canal filling. These include sodium hypochlorite (NaOCl), glutaraldehyde, alcohol, chlorhexidine (CHX), hydrogen peroxide, polyvinylpyrrolidone iodine, and MTAD.⁽³⁻¹¹⁾

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.^(12,13)

Later studies showed the effect of bacteria on the outcome of endodontic treatment. 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.¹⁴

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.¹⁵

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 .¹⁶

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 ^(17,18, 19, 20). *E. faecalis* is one of the most frequently isolated bacteria from root treated teeth.

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. ^(17, 21,22)

However, a more sensitive polymerase chain reaction technique detected *E. faecalis* in as many as 77% of failing root canal treated teeth ²⁰ or as few as 22%.²³

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 PCR, respectively.²⁴

NaOCl shows antiseptic properties due to the formation of hypochlorous acid and the subsequent release of chlorine, which is a very active bactericide ²⁵. Free chlorine in NaOCl dissolves necrotic tissue by breaking down proteins into amino acids; to obtain this effect concentrations ranging from 0.5% to 5.25% have been recommended.²⁵

A study showed that concentrations of 0.5% are just as clinically effective at achieving negative cultures as concentrations of 5.25%.²⁶. In several subsequent *in-vitro* studies, the antibacterial effect of various concentrations of NaOCl was demonstrated against endodontic pathogens. ^(27,28, 29, 30)

Chlorhexidine is widely used in dentistry because of its broad spectrum of antimicrobial activity ³¹. However, its effectiveness in decontaminating gpc has been found to differ in some studies. ^(32, 33, 34, 35)

Aim of the study

To assess the efficacy of using different antimicrobial agents (NaOCl 5.25%, NaOCl 0.5%, and Chlorhexidine 2%) in disinfection of Gutta-Percha cones contaminated with *E.Faecalis* (the commonest cause of secondary infections in endodontic procedures).

Materials and Methods

In the present study, the gutta-percha cones were allotted to 3 groups depending upon the type of antibacterial agents used for sterilization. Group I – Gutta-percha immersed in NaOCl (5.25 %).Group II – Gutta-percha immersed in NaOCl (0.5%).Group III – Gutta-percha immersed in Chlorhexidine (2%).

A total of 64 gutta-percha cones (No. 30 Dentsply maillefer) were selected for this study, from freshly opened sealed pack. For each group, 20 cones were used. A total of 4 cones were used as controls.

The following controls were used.

Positive control: A total of 2 gutta-percha cones were used as positive control exposed to the *E.Faecalis* bacteria. The cones

were directly transferred to the culture media without disinfection.

Negative control: A total of 2 gutta-percha cones were taken from freshly sealed pack. The cones were then transferred to culture media without disinfection.

Test group: Test group was selected from gutta-percha taken from freshly sealed pack. A total of 20 gutta-percha cones were included in each group.

E. faecalis (strain ATCC) suspension was grown in 8mm culture tubes containing thioglycollate medium, vitamin K-1 and hemin and incubated at 37oC for approximately 24 hours.

The bacteria were identified and images were acquired using a Zeiss microscope and a 63X NA 0.5 oil immersion objective. Bacteria were Gram-stained and examined for documentation of the purity of bacteria.

After *E. faecalis* suspension was prepared as described above, Gutta-percha cones 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.

These cones were then treated with each solution of disinfectant for an immersion time of 1min. All the cones were subsequently transferred to tubes containing 8 ml of thioglycollate media.

The inoculated media were incubated at 37°C and observed after 24, 48 and 72 hrs. of incubation for presence of growth of microorganisms by comparing the turbidity with that of the uninoculated medium, which was clear. Samples that demonstrated turbidity at the end of 3 days were deemed culture positive. Culture tubes that remained clear at the end of 3 days were deemed culture negative. The same procedure was performed for controls.

Table 1.Summary of the experimental groups

Group 1	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated with 5.25% NaOCl
Group 2	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated with 0.5% NaOCl
Group 3	gutta-percha cones contaminated with <i>E. faecalis</i> decontaminated with 2% Chlorhexidine

Table 2.Summary of control group

Negative control	gutta-percha cones taken from freshly sealed pack and transferred directly to sterile culture media without contamination or disinfection
Positive control	gutta-percha cones contaminated with <i>E. faecalis</i> and transferred directly to sterile culture media without decontamination

Results

Evaluation of the contamination of gutta-percha cones in manufacturer’s boxes Negative control

None of the cones (2 cones total) removed from brand new freshly sealed boxes were culture Positive.

Evaluation of the contamination of gutta-percha cones with E. faecalis Positive control

None of the cones (2 cones total) that were not decontaminated were culture Negative.

Evaluation of the effectiveness of 5.25% NaOCl and 0.5% NaOCl for decontamination of the cones contaminated in *E. faecalis* (Group 1 and Group 2).

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 (0%) positive cultures and 40 (100%) negative cultures.

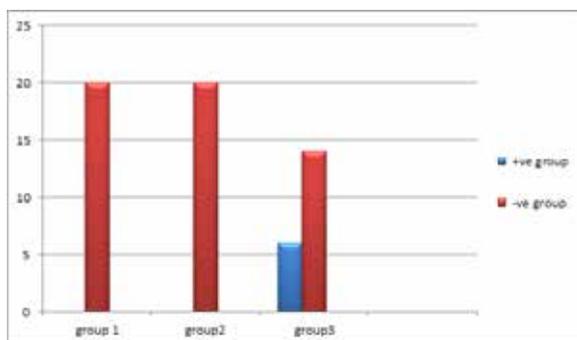
Evaluation of the effectiveness of 2% Chlorhexidine for decontamination of the cones contaminated in *E. faecalis* (Group 3)

Chlorhexidine showed good antimicrobial activity against *E. faecalis* that 14(70%) cones were negative cultures and 6 (30%) cones were positive cultures after decontamination for 1 min.

Table 3. Results

Group	Positive culture	percent-age	Negative culture	percent-age	Total
Group 1	0	0%	20	100%	20
Group 2	0	0%	20	100%	20
Group 3	6	30%	14	70%	20
Total	6	10%	54	90%	60

Figure 1. Showing results of all three groups



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.

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 (36, 6, 2, 37, 4), NaOCl has been the preferred choice. 6

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.

E. faecalis was chosen in this experiment as it is a highly resistant microorganism and is prevalent within the root canal space. (38, 17, 16, 39)

Various studies confirmed that gutta-percha cones can be effectively decontaminated with 5.25% NaOCl (6, 37, 1, 9, 40, and 41). 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-per-

cha in 0.5% NaOCl (42, 9). However, the results were in agreement with another study by Haapasalo *et al.*, who demonstrated that *E. faecalis* was rapidly eliminated by even a low concentration of 0.3% NaOCl.30 there was no difference between groups 1 and 2. P =1.0

Chlorhexidine, because of its broad spectrum of antimicrobial activity, substantivity, and hypoallergenic property is probably the biocide most used in the formulation of antiseptics, particularly those destined for hand washing and antisepsis of the oral cavity 31. Despite its pronounced bactericidal activity, the results of our study indicate that it is not as effective as NaOCl.

Our results are inconsistent with the results of 43 which showed complete eradication of *E. faecalis* by Chlorhexidine 2%.but this may be due to the fact that they used Chlorhexidine gel while we used solution. However our results are consistent with the results of (Menezes et al. 2003, Siren et al. 2004, Oztan et al. 2006) (44,45,46,47,48) ,which indicate that Chlorhexidine is a good disinfecting agent against *E. faecalis*. There was significant difference between group 1and 3, P=0.008 and significant difference between group 2 and 3, P=0.008

Table 3.showing groups results and the P value

Groups	cultures		P values
	Positive culture	Negative culture	
Group 1	0(0.0%)	20(100.0%)	0.008
Group 3	6(30.0%)	14(70.0%)	
Groups			
Group 2	0(0.0%)	20(100.0%)	0.008
Group 3	6(30.0%)	14(70.0%)	

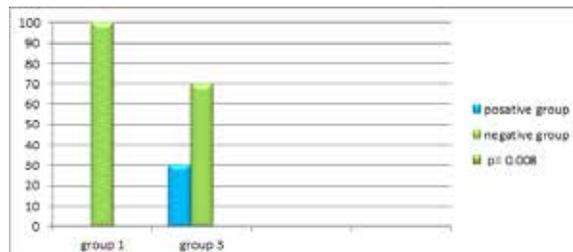


Figure 2. comparison of results between groups 1 and 3

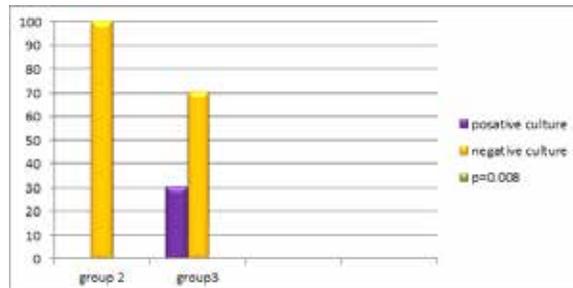


Figure 3. Comparison between results of groups 2 and 3

Conclusion

- 1) There was no contamination present in new freshly sealed boxes of gutta-percha cones and gutta-percha cones are usually sterile during storage.
- 2) 0.5% NaOCl is just as effective for decontamination of gutta-percha cones as 5.25% NaOCl against *E.faecalis*
- 3) Chlorhexidine is a good antimicrobial agent against *E.faecalis* but NaOCl is superior to it.

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