A Comparative Evaluation of Different Chemical Agents and Herbal Products in Disinfecting Gutta-Percha Cones: An In Vitro Study

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Abstract

Introduction The main aim of this study was to compare and evaluate different chemical agents and herbal products in disinfecting gutta-percha (GP) cones through an in vitro study.

Materials and Methods For this study, 160 GP cones were taken in test tubes, which were contaminated with *Enterococcus faecalis* inocula, prepared by dissolving it in Brain Heart Infusion (BHI) broth. The GP cones were then taken from the test tubes and dried on Whatman filter paper no. 1 and were divided into four groups according to the decontaminant used for the study—group 1 (5% sodium hypochlorite [NaOCl]), group 2 (2% chlorhexidine), group 3 (propolis), and group 4 (*Aloe vera*)—which were further subdivided into subgroups on the basis immersion time periods of 1 and 10 minutes. The GP cones were then again dried and taken in the test tubes containing freshly prepared BHI broth to check the turbidity.

Results It was found that 2% chlorhexidine was the most effective disinfectant against *E. faecalis*, 5% NaOCl was the second best disinfectant followed by propolis, while *Aloe vera* had not shown any effect as GP disinfectant.

Conclusion Since 2% chlorhexidine showed better disinfection efficacy against *E. faecalis* than 5% NaOCl, it can be recommended for chairside disinfection of GP. One-minute immersion of GP in 2% chlorhexidine is sufficient for elimination of *E. faecalis*.

Keywords
- chemical agents
- *Enterococcus faecalis*
- herbal products

Introduction

Gutta-percha (GP) is the most commonly used material for root canal obturation. GP is a dried coagulated extract of plants of *Palaquium* Blanco genus of Sapotaceae family, and was introduced to dentistry in 1847 by Edwin Truman.[1] GP cones are supplied in sealed packets and they can easily get contaminated during storage, handling, and even by exposure to the clinical environment resulting in breach of aseptic chain.[2] Therefore, GP must be decontaminated before being placed in root canal. GP cannot be sterilized by conventional methods like moist heat, or dry heat, because of its thermoplastic properties. Therefore, cold sterilization is recommended.

Various chemical agents have been proposed as GP disinfectants, including sodium hypochlorite (NaOCl), glutaraldehyde, alcohol, iodine compounds, and hydrogen peroxide.[3]

NaOCl has oxidizing, hydrolyzing, and proteolytic properties. NaOCl is bactericidal and virucidal because of its proteolytic properties. However, these properties deteriorate with time, temperature, exposure to light, and contamination with metallic ions.[4]

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NaOCl is commonly used to sterilize GP, but due to strong oxidizing effect it changes the structure of GP.[5] It also causes crystal deposition on GP surface and within the canals hampering the bond of sealers with canal walls after obturation and thus leading to microleakage.

Chlorhexidine, a cationic bisbiguanide, is clinically used as antimicrobial agent. It acts by adsorbing onto the microorganism's cell wall and causing intracellular component leakage. It has antibacterial properties with broad spectrum and relatively low toxicity.[6]

Nowadays various herbal products are also gaining popularity to decontaminate the GP points. Various herbal products included are neem, ginger, turmeric, *Aloe vera* juice, and propolis.

Propolis (bee glue) is a byproduct of honeybees that is widely used in alternative medicine.[7] Propolis is a resinous, yellow brown to dark brown substance that is collected by honey bees (*Apis mellifera*) from tree buds to seal their hives.

Aloe vera (synonym: *Aloe barbadensis* Miller) belongs to the Liliaceae family.[8] Total leaf extracts contain anthraquinones, which have antibacterial properties. *Aloe vera* gel has inhibitory effects on *Streptococcus pyogenes* and *Enterococcus faecalis* because of anthraquinone.[9]

This present in vitro study was undertaken to compare disinfection ability of chemicals (5% NaOCl and 2% chlorhexidine) and herbal products (*Aleo vera* and propolis) against *E. faecalis* present on the GP cones.

Materials and Methods

Bacteriological Media Used in the Study

Brain Heart Infusion broth (Hi Media) was used for cultivation of *E. faecalis* as well as to analyze the disinfection attained for GP after immersion in disinfecting agents.

Procurement of Microorganism

The microorganism, *E. faecalis*, used in this study was procured in freeze-dried form in an air tight glass tube from Institute of Microbial Technology, Chandigarh.

Contamination of Gutta-Percha cones

Presterilized test tubes were taken and with the help of micropipette, 5 mL of prepared inocula containing activated *E. faecalis* was poured in each test tube and were then incubated at 37°C for 30 minutes. The GP cones were taken out from sealed packets with the help of sterilized tweezer and were added into each test tube containing activated E. faecalis **(►Fig. 1).** These test tubes were then incubated in an incubator at 37°C for 30 minutes.

Disinfection of Gutta-Percha Cones

In the study, 160 GP cones were used. These were divided into four equal groups depending upon chemical agents or herbal products used to disinfect the GP cones.

Group 1 (n = 40): 5% NaOCl was used to disinfect the GP cones. This group was further subdivided into two subgroups depending upon the time required to disinfect the GP cones.

Group 1a (n = 20): GP cones were disinfected for 1 minute.

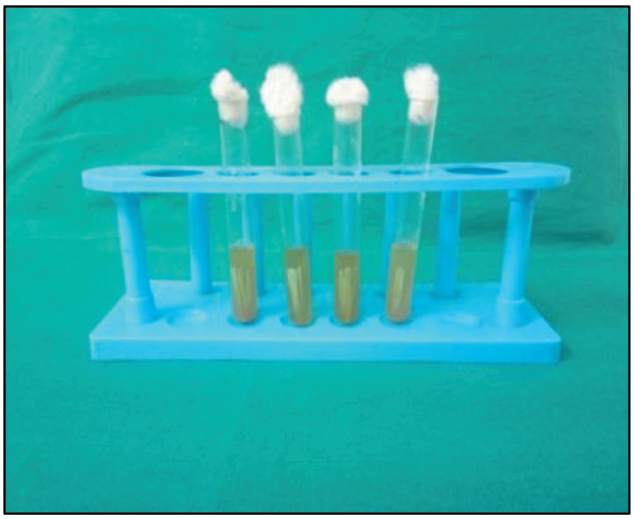

Fig. 1 Gutta-percha contamination with *Enterococcus faecalis*.

Four test tubes having 4 mL of 5% NaOCl in each was taken so as to fully submerge the GP cones. Five GP cones were placed in each test tube.

Group 1b (n = 20): GP cones were disinfected for 10 minutes.

Four test tubes having 4 mL of 5% NaOCl in each was taken so as to fully submerge the GP cones. Five GP cones were placed in each test tube.

Group 2 (n = 40): 2% chlorhexidine was used to disinfect the GP cones. This group was further subdivided into two subgroups depending upon the time required to disinfect the GP cones.

Group 2a (n = 20): GP cones were disinfected for 1 minute.

Group 2b (n = 20): GP cones were disinfected for 10 minutes.

Group 3 (n = 40): Propolis was used to disinfect the GP. This group was further subdivided into two subgroups depending upon the time required to disinfect the GP cones.

Group 3a (n = 20): GP cones were disinfected for 1 minute.

Group 3b (n = 20): GP cones were disinfected for 10 minutes.

Group 4 (n = 40): *Aloe vera* was used to disinfect the GP. This group was further subdivided into two subgroups depending upon the time required to disinfect the GP cones.

Group 4a (n = 20): GP cones were disinfected for 1 minute.

Group 4b (n = 20): GP cones were disinfected for 10 minutes.

After drying, each GP cone was individually inserted into test tubes containing Brain Heart Infusion broth and incubated at 37°C for 72 hours. The presence of bacterial growth was analyzed by turbidity of the medium. These test tubes were checked for turbidity after 24, 48, and 72 hours. Presence of turbidity indicated bacterial growth **(►Fig. 2).**

Results

Overall, 2% chlorhexidine was found to be the best disinfectant. No turbidity was observed after 24, 48, and 72 hours at 1- and 10-minute immersion times. Also, 5% NaOCl showed

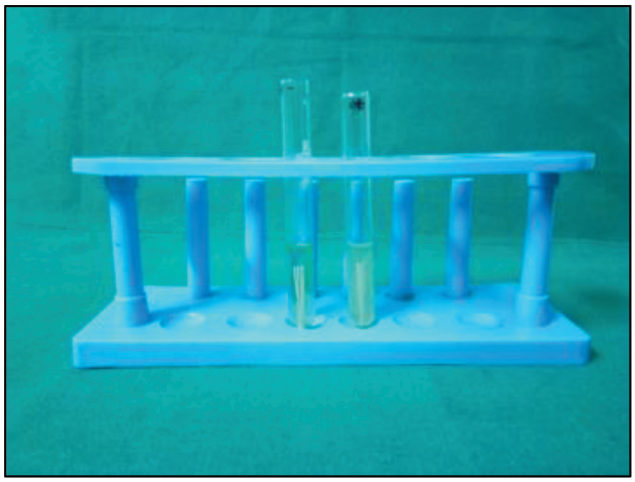

Fig. 2 Turbidity signifying bacterial growth: negative (–) tube showing absence of bacterial growth, and positive (+) tube showing presence of bacterial growth.

better disinfection when immersion time was 10 minutes. Propolis showed minimum disinfection than 2% chlorhexidine and 5% NaOCl irrespective of immersion time. *Aloe vera* did not show any antimicrobial activity. The results were obtained by using squared ranks and Wilcoxon signed rank tests, and the results were found to be statistically significant ($p < 0.05\%$) (►**Table 1**).

Discussion

GP cones are widely used and accepted material to obturate the root canals.[10] GP was introduced to endodontics by Bowman in 1867. The GP cones can be easily contaminated by physical contact, aerosols, gloves handling, and other sources during storage.

Nan-Shim Pang et al[11] conducted a study in which they found that 19.4% of GP contaminated after exposure to clinical environment. Montgomery[12] found that 8% of the commercially available cones had bacterial growth. The common contaminants of GP includes *Bacillus subtilis*, *Staphylococcus aureus*, and *E. faecalis*. Molander et al[13] found that *E. faecalis* is one of the most resistant microorganisms to root canal instrumentation and irrigation procedures. *Enterococci* are normal human commensals, which can survive in nutrient-enriched, oxygen-depleted, ecologically complex environment of the oral cavity, gastrointestinal tract, and vaginal vault. It forms catalase negative creamy white colonies that can survive at 6°C for 30 minutes and can also tolerate pH of up to 11.1.[14] It has been found that there is a high prevalence of *E. faecalis* in failed endodontic cases (24–70%),[15] as it has the ability to survive harsh environmental conditions present in the root canals of endodontically treated teeth. In the present study also, *E. faecalis* had been used to contaminate the GP cones.

GP should be sterilized before using it to maintain the chain of asepsis in root canal treatment. The thermoplastic nature of GP does not allow sterilization by autoclaving as it may cause deformation. GP is normally sterilized by ethylene oxide.[16]

Many chemicals, which include thiomersal solution (0.19%), paraformaldehyde, tincture of benzalkonium chloride, NaOCl, hydrogen peroxide, quaternary ammonium, glutaraldehyde, and chlorhexidine, have been suggested to decontaminate GP cones.[17] In our study, 2% chlorhexidine was found to be a better disinfectant than 5% NaOCl, propolis, and *Aloe vera*, irrespective of immersion time of GP, against *E. faecalis*. Gomes et al[18] stated that 2% chlorhexidine takes less than 30 seconds to completely eliminate *E. faecalis* from contaminated GP cones. Cardoso et al[10] also found that 2% chlorhexidine for 1 minute was more effective GP disinfectant than 1% NaOCl, 2% glutaraldehyde, 6% hydrogen peroxide, and 10% polyvinyl pyrrolidone-iodine. The results of our study are similar to above investigators.

Subha et al[19] showed that the time required for antimicrobial property of NaOCl is inversely proportional to its concentration: 1% NaOCl removes *E. faecalis* in 20 minutes whereas 5.25% NaOCl takes less than 1 minute. This might be the reason that 5% NaOCl took more time to completely disinfect the GP.

Propolis showed minimum antimicrobial effect against *E. faecalis* in 1-minute immersion time; however. more antimicrobial effect was observed when immersion time was 10 minutes. McHugh et al suggested that high pH (10–11) is necessary for growth retardation of *E. faecalis*.[20] Ineffectiveness of propolis in our study may suggest that the pH of propolis was not up to the desired level, which resulted in its failure as GP disinfectant.[21]

Table 1 Results

Groups	Immersion time	Test tubes showing turbidity after 24 h	Test tubes showing turbidity after 48 h	Test tubes showing turbidity after 72 h
Sodium hypochlorite (group 1)	1 min	50%	75%	100%
	10 min	0	25%	75%
Chlorhexidine (group 2)	1 min	0	0	0
	10 min	0	0	0
Propolis (group 3)	1 min	75%	100%	100%
	10 min	25%	75%	100%
Aloe vera (group 4)	1 min	100%	100%	100%
	10 min	100%	100%	100%

Kusuma et al compared the antimicrobial activities of neem, *Aloe vera*, calcium hydroxide, and 2% chlorhexidine. They found that *Aloe vera* had minimal antimicrobial activity whereas 2% chlorhexidine showed maximum antimicrobial activity against *E. faecalis*.[22]

Duman et al[23] suggested that poor antimicrobial activity of *Aloe vera* may be due to lesser acidic component and low number of monomeric anthocyanins. The results of our study show that the chemical agents, that is, 2% chlorhexidine and 5% NaOCl, are more effective in disinfection of GP than herbal products. Thus, 2% chlorhexidine was found to be the best among all in disinfecting the GP. Herbal products may hold promising future but more research on the use of particular concentration of these products to achieve maximum therapeutic effect is required.

Conclusion

In this study, 2% chlorhexidine showed better disinfection efficacy against *E. faecalis* than 5% NaOCl. Thus, 2% chlorhexidine can be recommended for chairside disinfection of GP. One-minute immersion of GP in 2% chlorhexidine is sufficient for elimination of *E. faecalis*.

Conflict of Interest

None declared.

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