Original Research Article

Rapid chairside disinfection and surface alterations of gutta-percha cones with 1% Alexidine, 2% Chlorhexidine and 5.25% sodium hypochlorite against *Enterococcus faecalis* - An *in vitro* comparative study

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Abstract

Background: During endodontic intervention, major efforts are made to eliminate bacteria from the infected root canal system. Along these lines, keeping up the chain of asepsis is critical to anticipate sterile root canal system. Currently, gutta-percha (GP) is the most commonly used root canal core filling material.

Objective: The purpose of this study was to compare the efficacy of 1% Alexidine, 2% Chlorhexidine, 5.25% Sodium Hypochlorite and saline against *E.faecalis* in disinfecting gutta-percha cones and to analyze the surface topography of gutta-percha cones after the rapid chemical disinfection procedure.

Materials and methods: Gutta-percha cones were contaminated with *E.faecalis* and immersed in 1% ALX, 2% CHX, 5.25% NaOCl and 0.9% saline for 30 and 60 sec. The disinfected cones were immersed in test tubes and incubated at 37°C for 72 hours. Bacterial growth was evaluated by the presence of turbidity in the broth. Results were confirmed by sub culturing the bacterial colony. For topographical examination, the gutta-percha cones were analyzed under Scanning electron microscopy (SEM). Data was statistically analyzed using one way ANOVA.

Results: ALX and NaOCl did not show growth of the tested microorganism for any observed period. CHX completely eliminated E. faecalis after 60 sec. Saline showed no antimicrobial activity. The results of SEM showed some deposits after disinfection procedure by NaOCl and CHX test solutions used.

Conclusion: ALX and NaOCl were found to be the most efficient disinfecting agent in eliminating *E.faecalis* at different time intervals tested. The topographical examination revealed that ALX and CHX left little residue than as compared to NaOCl after 1 minute.

Key words

Alexidine, Chlorhexidine, Sodium Hypochlorite, Disinfection, Guttapercha, Scanning electron microscopy, *E.faecalis.*

Introduction

During endodontic intervention, major efforts are made to eliminate bacteria from the infected root canal system. Along these lines, keeping up the chain of asepsis is critical to anticipate sterile root canal system. Currently, gutta-percha (GP) is the most commonly used root canal core filling material. Although GP cones are manufactured under aseptic conditions, manufacturers do not claim it to be sterile and they can also be contaminated by aerosols, improper storage and physical handling during use [1].

Studies have revealed the presence of microorganisms in freshly opened boxes [2, 3]. *E.*faecalis is organism that is found to be the most resistant intracanal organism in failed root canals that serves as a gold standard bacterium in endodontic research [4]. Due to their superior virulence, they were selected in this study to represent the organisms that may contaminate the GP cones.

GP cones cannot be sterilized by moist or dry heat due to its thermoplastic nature. Thus, rapid cold sterilization is required. A desired disinfectant should provide faster chair side disinfection without modifying the structure of the cone. Alexidine (ALX) is a bisguanide disinfectant that has greater affinity for the major virulence factors such as bacterial lipopolysaccharide and E. faecalis lipoteichoic acid [5]. Chlorhexidine (CHX) is a strong disinfectant which is used in root canal irrigation. It has also been used as GP disinfectant [6]. 5.25% Sodium Hypochlorite (NaOCl) is a broad spectrum antimicrobial agent. However, studies have reported crystal deposition with various irrigants used, hampering the bond of the sealers with GP cones, leading to microleakage [7].

Literature has unfolded no study to date comparing these irrigants as disinfecting solutions for GP cones and their effect on its topography. Hence, the aim of the present study was to evaluate and compare the antimicrobial effects and surface alterations of GP cones when disinfected with 1% ALX, 2% CHX, 5.25% NaOCl and saline against *E.faecalis.*

Materials and methods

E.faecalis suspension

Pure E. faecalis (ATCC 29212) (Rockville, MD, USA) culture strain was cultivated in test tubes containing 5 ml of the Brain-Heart Infusion (BHI) broth (HiMedia, Mumbai, India) and incubated at 37°C for 24 hours. After bacterial growth, the concentration was agitated and regulated to 0.5 McFarland scale (1.5×10^8 CFU/ml) (BioMerieux, Densichek Plus).

Contamination of GP cones

A total of 80 GP cones (ISO size 80, Dentsply, USA) from freshly opened boxes under sterile conditions were selected. Damaged and bent cones were discarded. The GP cones were contaminated by immersion in 5 ml of bacterial suspension and incubated at 37°C for 72 hours.

After the incubation period, the cones were dried using sterile gauze.

The GP cones were divided into four experimental groups according to test irrigants used (n=20). Groups 1, 2, 3 and 4 were further subdivided into groups a (30 sec) and b (60 sec) according to the disinfecting time. In group 1, 1% solution of ALX was prepared by dissolving 10 mg ALX dihydrochloride powder in 1 ml of sterile distilled water. Other test irrigants were available commercially. Ten GP cones were immersed for 30 seconds and other ten were immersed for 60 seconds in each test irrigant individually. The same procedure was repeated for all the groups.

Group 1 - 1% ALX (M68182628; Gentaur, Kampenhout, Belgium) for 30 seconds (Group 1a) and 60 seconds (Group 1b).
Group 2 - 2% CHX (Drogsan, Cubuk, Ankara, Turkey) for 30 seconds (Group 2a) and 60 seconds (Group 2b).
Group 3 - 5.25% NaOCl (Sultan) for 30 seconds (Group 3a) and 60 seconds (Group 3b).
Group 4 - 0.9% saline (Control) for 30 seconds (Group 4a) and 60 seconds (Group 4b).

Verification of GP cone contamination

Each treated cone was placed on sterile gauze to remove excess test irrigant before immersing in a test tube with 20ml of sterile BHI broth. All the test tubes were incubated at 37°C for 72 hours. However, bacterial growth was recorded by the presence of turbidity in the broth at the end of 24 hours. After the incubation period, sub-culturing of the bacterial colonies was done. The colonies were spread over BHI agar plates with the help of sterile cotton swab. The plates were then incubated at 37°C for 48 hours and results were recorded in colony-forming units (CFU/mL) on each plate with the help of digital colony counter.

Scanning electron microscope (SEM) evaluation of GP cones

GP cones from freshly opened boxes were immersed in all test irrigants for 1 minute and allowed to air dry for 30 minutes (n=10). Scanning electron microscope (FEI Quanta 200 ESEM FEG) at 1000X magnification was used to evaluate surface alterations.

Statistical analysis was performed by using one-way analysis of variance (ANOVA) to measure the mean values. The intergroup comparison was done by Tukey HSD post hoc test using SPSS software. The criterion for statistical significance was set as $p < 0.05$.

Results

Disinfection of GP cones

Groups 1a, 2b and 3a showed absence of turbidity while groups 4a and 4b showed intense turbidity after 24 hours (**Table – 1, Figure - 1**). In group 1, the cones showed absence of turbidity immediately after 30 seconds. However group 2 showed bacterial growth after 30 seconds. The absence of turbidity was seen only after 60 seconds. In group 3, absence of the turbidity was seen after 30 seconds. Group 4 did not reveal any bactericidal action, resulting in intense turbidity in all samples at both time periods.

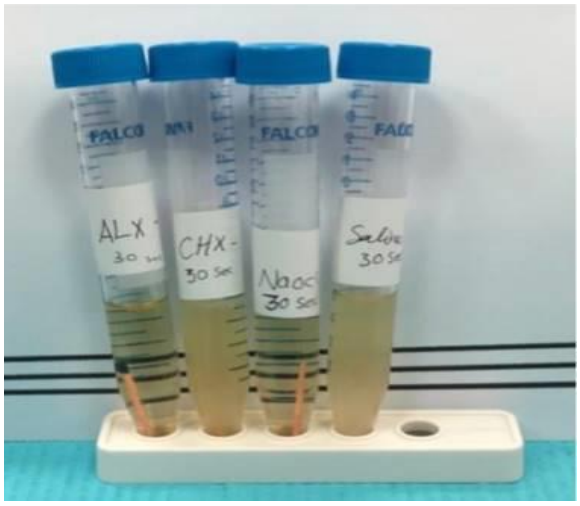
Table – 1: Bacterial growth after 24 hours by the presence (+) or absence (-) of turbidity in all the groups (n=10).

	30 seconds (a)	**60 seconds (b)**
Group 1	-	-
Group 2	+	-
Group 3	-	-
Group 4	+	+

Table – 2: Comparison of the antimicrobial activity of decontamination protocols expressed in log CFU/ml.

	30 seconds (a)	**60 seconds (b)**	**p value**
Group 1	0.00 ± 0.00	0.00 ± 0.00	p<0.05
Group 2	2.80 ± 0.14	0.00 ± 0.00	p<0.05
Group 3	0.00 ± 0.00	0.00 ± 0.00	p<0.05
Group 4	3.57 ± 0.14	3.13 ± 0.10	p<0.05

Figure - 1: The tubes showing turbidity after 24 hours of incubation of 30 seconds treated GP cones in group 1, 2, 3 and 4.

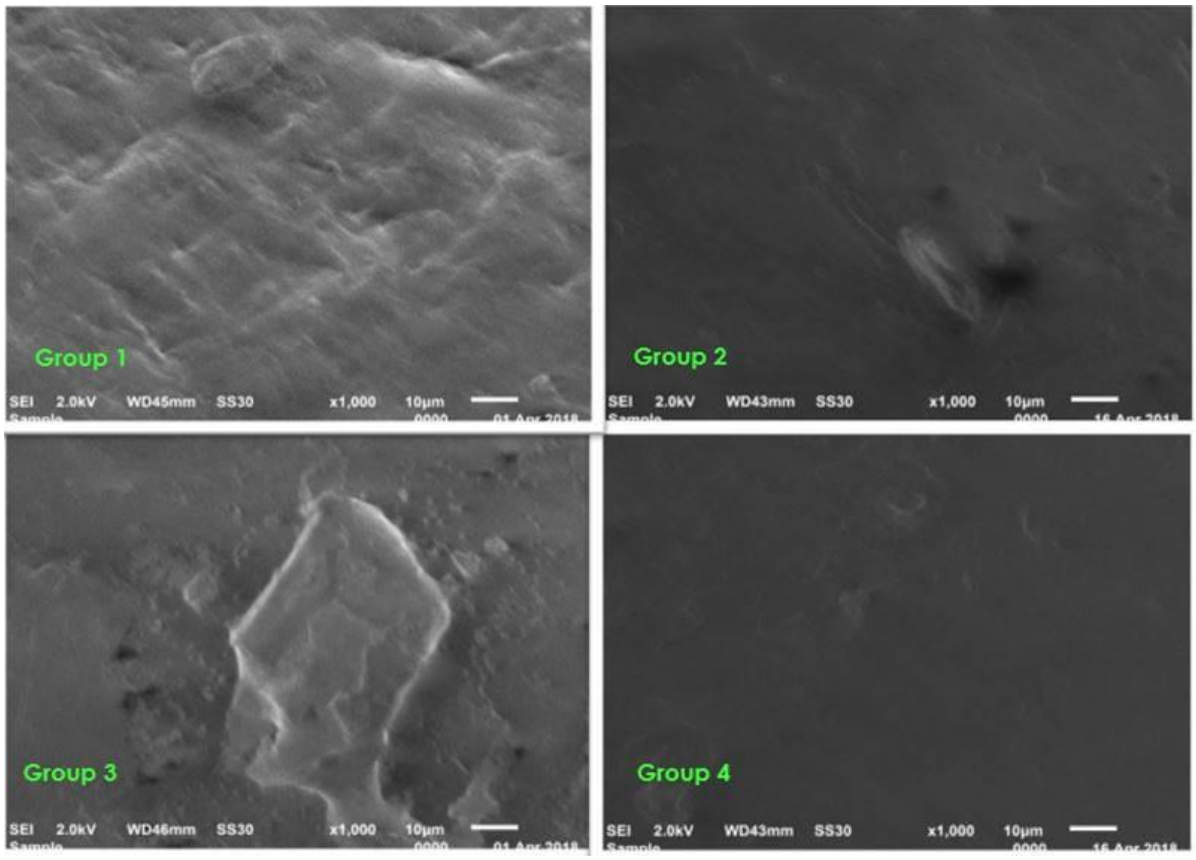


After Sub-culturing of the bacterial colonies and determination of the number of colony forming units (CFU), groups 1 and 3 did not demonstrate growth of E.faecalis till 60 seconds, with the results being statistically significant from all other groups ($p<0.05$). Group 2 showed growth of E.faecalis after 30 seconds treatment with statistically significant difference between the observation periods for same group ($p<0.05$). Group 4 showed no antimicrobial activity against the tested microorganism (**Table - 2**).

SEM analysis of GP cones

Topographical examination of gutta-percha cones (**Figure - 2**) showed the presence of some residues over the cones after treatment with NaOCl. ALX and CHX left little residue over the cones after 1 minute.

Figure - 2: SEM analysis of gutta-percha cones after immersing in all groups for 1 minute.



Discussion

The success of endodontic therapy is due to neutralization of microorganisms from the root canal system. Furthermore, the filling materials inhibit the infection by acting as a barrier to prevent any surviving bacteria within the root

canal system and stopping periapical tissue fluids from reaching bacteria in the root canal [8]. Despite GP cones being produced under aseptic conditions and stored in sealed packages [9], their sterilization is questionable, and they can be easily contaminated by handling [10, 11].

Handling of gutta-percha cones in incorrect way during the retreatment or during master cone fitting before final rinse may cause contamination of the cones with E. faecalis, which is known for its superior virulence factors and considered as a gold standard microorganism that may contaminate gutta-percha cones [4]. Therefore, we chose E. faecalis as the test organism in this study.

The importance of disinfection of gutta-percha points prior to root canal filling is widely recognized to prevent possible contamination of the root canal system. Thus, it is important to use an inexpensive, rapid, reliable, and effective disinfection solution.

Extreme topographical changes occur as a result of sterilization procedures with contact between the gutta-percha cones and the chemical agents. GP cones present irregular regions on their surfaces and root canal sealers are known to fill these irregularities. However, these gap areas could create a large interface with root canal walls, resulting in the leakage of molecules that will serve as nutrients for the microorganisms present in the root canal system [12]. SEM is the standard tool for investigation of the surface alternations of the GP cones [7]. For topographical examination, SEM at 1000× magnification was used since it allows a detailed examination of topography of any material at different magnifications [8].

CHX is a cationic bis-biguanide with broad antibacterial activity. The CHX molecule reacts with negatively charged groups on the bacterial cell surface, causing an irreversible loss of cytoplasmic constituents, membrane damage, and enzyme inhibition [13]. We chose 2% concentration of CHX in this study which has been shown to have a better antibacterial efficacy. Senia, et al. [14] found that 2% CHX was unable to eliminate E.faecalis in 30 seconds. Our results are in accordance with them. SEM examination revealed fewer crystal deposits on the GP cones. However, due its longer disinfection time of 60 seconds, it cannot be recommended to be an ideal disinfectant for GP cones.

NaOCl is widely used in root canal treatments as an irrigant and at different concentrations for sterilization of gutta-percha against possible contamination. The disinfecting efficiency of NaOCl depends on the concentration of undissociated hypochlorous acid (HClO) in solution. HClO exerts its germicidal effect by an oxidative action on sulphydryl groups of bacterial enzymes [15]. Several studies recommend the use of NaOCl for disinfecting GP cones [9]. However, at very high concentrations (5.25%), NaOCl produced a large quantity of chloride crystals on the GP cone surface. These crystals on GP prevent the ability of the filling material to provide an adequate sealing and might cause loss of elasticity which could impede the obturation [7]. But lower concentrations will take more time to inhibit bacterial growth than higher concentrations. Hence, it cannot be recommended for rapid chair-side disinfection of GP cones.

The antimicrobial activity of ALX has not been studied till now against contaminated GP cones. For this reason, ALX was selected as a test solution in this study, which is a disinfectant similar to CHX except that it contains ethylhexyl end groups. This component favours hydrophobic penetration into membrane lipids and electrostatic adhesion to the negative sites of cell membranes resulting in faster and more effective bactericidal activity [16, 17]. ALX showed fastest elimination of E.faecalis from GP cones in 30 seconds only. SEM examination revealed minimal crystal deposits on the surface of GP cones. Hence, we found it to be the best

disinfectant for rapid chair-side disinfection of GP cones. Our results cannot be corroborated with other researches as this is the first study of its kind to test ALX as a GP disinfectant.

Conclusion

Within the limitations of this study, it can be concluded that:

- ALX and NaOCl were found to be the fastest disinfecting solution eliminating E. faecalis in 30 seconds.
- CHX completely eliminated E. faecalis after 60 seconds.
- The topographical examination of gutta-percha cones found some deposits after 1minute disinfection procedure with all disinfecting solutions used. ALX and CHX were found to leave lesser deposits compared to NaOCl.
- ALX demonstrated to be the best disinfecting solution amongst all in terms of shortest time for disinfection and fewer crystal depositions on GP cones.

References

1. Chandrappa MM, Mundathodu N, Srinivasan R, Nasreen F, Kavitha P, Shetty A. Disinfection of gutta-percha cones using three reagents and their residual effects. J Conserv Dent., 2014; 17(6): 571-4.
2. Kayaoglu G, Gürel M, Omürlü H, Bek ZG, Sadik B. Examination of gutta percha cones for microbial contamination during chemical use. J Appl Oral Sci., 2009; 17: 244-7.
3. Klager P, Dupont AA. The significance of environmental contamination of sealer and gutta-percha before endodontic obturation. Oral Surg Oral Med Oral Pathol., 1987; 63(5): 606-9.
4. Gajan EB, Aghazadeh M, Abashov R, Salem Milani A, Moosavi Z. Microbial flora of root canals of Pulpally infected teeth: Enterococcus faecalis a prevalent species. J Dent Res Dent Clin Dent Prospects, 2009; 3: 24-7.
5. Zorko M, Jerala R. Alexidine and chlorhexidine bind to lipopolysaccharide and lipoteichoic acid and prevent cell activation by antibiotics. J Antimicrob Chemother., 2008; 62(4): 730-7.
6. Gomes BP, Berber VB, Montagner F, Sena NT, Zaia AA, Ferraz CC, Souza-Filho FJ. Residual effects and surface alterations in disinfected gutta-percha and resilon cones. J Endod., 2007; 33: 948-51.
7. Short RD, Dorn SO, Kuttler S. The crystallization of sodium hypochlorite on gutta-percha cones after the rapid-sterilization technique: an SEM study. J Endod., 2003; 29(10): 670-3.
8. Goldberg F, Massone EJ, Pruskin E, Zmener O. SEM study of surface architecture of gutta-percha cones. Endod Dent Traumatol., 1991; 7: 15-8.
9. Motta PG, Figueiredo CBO, Maltos SMM, et al. Efficacy of chemical sterilization and storage conditions of gutta percha cones. Int Endod J., 2001; 34: 435–9.
10. Cardoso CL, Redmerski R, Bittencourt NLR, Kotaka CR. Effectiveness of different chemical agents in rapid decontamination of gutta-percha cones. Braz J Microbiol., 2000; 31: 72-5.
11. Özalp N, Ökte Z, Özcelik B. The rapid sterilization of gutta-percha cones with sodium hypochlorite and glutaraldehyde. J Endod., 2006; 32: 1202-4.
12. Valois CR, Silva LP, Azevedo RB. Effects of 2% chlorhexidine and 5.25% sodium hypochlorite on gutta-percha cones studied by atomic force microscopy. Int Endod J., 2005; 38(7): 425-9.
13. Raveendran L. Chair side disinfection of Gutta percha points-an in vitro comparative study between a herbal alternative propolis extract with 3% sodium hypochlorite, 2% chlorhexidine

and 10% povidone iodine. Int J Bioassays, 2015; 4(10): 4414-7.

14. Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L. Rapid sterilization of gutta-percha cones with 5.25 ‘o sodium hypochlorite. J Endod., 1975; 1: 136-40.
15. Gomes BPFA, Vianna ME, Matsumoto CU, et al. Disinfections of gutta-percha cones with chlorhexidine and sodium hypoclorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod., 2005; 100(4): 512-7.
16. Chawner JA, Gilbert P. Interaction of the bisbiguanides chlorhexidine and alexidinewith phospholipid vesicles: evidence for separate modes of action. J Appl Bacteriol., 1989; 66(3): 253-8.
17. Chawner JA, Gilbert P. A comparative study of the bacteriocidal and growth inhibitoryactivities of the bisbiguanides alexidine and chlorhexidine. J Appl Bacteriol., 1989; 66(3): 243-52.