Assessment of Topographic Changes of Gutta-Percha Cones Treated with Three Different Reagents and a Final Rinse of Distilled Water- A Scanning Electron Microscope Study

Srivastava Swati[1]*

1. Department of Conservative Dental Sciences, College of Dentistry, Qassim University, Buraydah, Saudi Arabia.

Abstract

This study aimed to determine the topographic changes (TC) of gutta-percha cones (GPC) after disinfection with 5.25% sodium hypochlorite (NaOCl), 1% Alexidine (ALX) and MTAD with and without a final rinse with distilled water (DW). 140 GPC were divided into seven groups and treated with three types of disinfection reagent- 5.25% NaOCl, 1% ALX and MTAD with or without a final rinse with DW.

The TC changes of GPC were examined under scanning electron microscope (SEM). The obtained data was statistically analysed using SPSS software. 90% GPC were found to have deposit formation with 5.25% NaOCl which was statistically significant than as compared to groups treated with 1% ALX and MTAD ($p<0.05$). Moreover, significant difference in deposit formation were observed in the groups which received a final rinse with DW ($p<0.05$). ALX and MTAD were identified as the optimal disinfecting solution of GPC with a final rinse of DW.

The TC seen in GPC were maximum with 5.25%NaOCl and minimal with 1% ALX and MTAD within a clinically acceptable time of 1 minute. It is crucial to conduct a final rinse of GPC with DW to remove any surface deposits.

Experimental article (J Int Dent Med Res 2024; 17(1): 77-81)

Keywords: Alexidine, Biopure MTAD, Gutta-Percha, Microorganism, Scanning Electron Microscopy, Sodium Hypochlorite, Topography.

Received date: 14 December 2023 **Accept date:** 03 January 2024

Introduction

The effectiveness of endodontic therapy is primarily contingent on eradicating microorganisms (MO) from the canal and upholding a continuous state of aseptic conditions.[1] GPC stands out as a validated obturating material due to its dimensional stability, biocompatibility, radiopacity, thermoplastic nature, and inherent antimicrobial properties. It is proposed that disinfecting GPC before obturation may not be obligatory due to the presence of zinc.[2]

Despite being manufactured under aseptic conditions, GPC can still be susceptible to contamination through factors such as aerosols, improper storage, and physical handling of the cones. GPC retrieved directly from their packages pose an additional risk of contamination through glove handling or inadvertent storage. Research has demonstrated the existence of MO in freshly opened boxes. Although the initial count of these MO was found to be relatively low at the time of packaging, their numbers increased during clinical usage, contributing to the contamination of GPC.[3,4] Dentists need to be cautious of the endogenous MO and the presence of bacteria like cocci, rods, and yeasts in the air during chair-side procedures, as these can potentially compromise the sterility of GPC.[5,6]

The sterilization of GPC is pivotal in the process of root canal obturation.[5] The conventional sterilization methods like autoclaving or hot air ovens are not suitable due to the chemical and physical properties of GPC. Chemical sterilization emerges as a viable alternative, and the choice of disinfectant is crucial as it can impact the TC of GPC.[7] Senia et al[6] asserted that the most effective and cost-efficient method employed for GPC sterilization is the rapid sterilization technique. An ideal disinfectant should enable rapid chair-side

Corresponding author:
Associate Professor, Dr. Swati Srivastava,
Department of Conservative and Endodontics,
College of Dentistry, Qassim University,
Buraydah, Saudi Arabia.
E-mail: s.kumar@qu.edu.sa

disinfection without altering the GPC structure. NaOCl is a broad-spectrum antimicrobial agent. NaOCl (5.25%) is the preferred option for achieving efficient disinfection of GPC within a span of 1 minute. The antimicrobial effectiveness of NaOCl is directly linked to its concentration. According to Da Motta et al., 5.25% NaOCl significantly influences the TC of GPC.[8] Milton's solution (1%) and Dakin liquid (0.5%) have been employed for disinfection, with durations ranging from 3 to 25 minutes. Nevertheless, across all concentrations, crystal deposition has been noted to impede the bond between sealers and GPC, resulting in microleakage.[5]

ALX (Gentaur, Belgium), is a bisguanide disinfectant. It exhibits a higher affinity for major virulence factors such as bacterial lipopolysaccharide and E. faecalis lipoteichoic acid.[9] It is employed as a disinfectant in mouthwash solutions and contact lens solutions, exhibiting specific properties against Gram-negative bacteria.[10] It has quick bactericidal activity and significantly faster bacterial permeabilization.[11] Another endodontic irrigant is MTAD (Dentsply, Tulsa Dental, Tulsa, OK, USA). It is a solution composed of a blend of tetracycline, citric acid, and polysorbate 80 detergent. It is utilized to deliver an antibacterial effect during root canal treatment.[12]

The existing literature has not revealed any study to date that compares the effect of these reagents as disinfecting solutions for GPC on its TC. Therefore, the objective of the current study was to assess and compare the TC of GPC when disinfected with 5.25% NaOCl, 1% ALX and MTAD with and without a final rinse with DW under SEM. The first null hypothesis was that there was no effect of 5.25% NaOCl, 1% ALX and MTAD on the TC of GPC. The second null hypothesis was that there was no effect of final rinse with DW on the TC of GPC after disinfection with 5.25% NaOCl, 1% ALX and MTAD.

Materials and methods

A total of 140 GPC (ISO size 80, Dentsply, USA) were chosen from freshly opened boxes under sterile conditions. Cones that were damaged or bent were excluded from the selection. A 1% solution ALX was prepared by dissolving 10 mg of ALX dihydrochloride powder in 1 ml of sterile distilled water. The remaining test irrigants were commercially available. The GPC were divided into seven groups (n=20) based on the type of disinfection reagent used as follows:

Group I- GPC disinfected with 5.25% NaOCl (Sultan);
Group II- GPC disinfected with 5.25% NaOCl (Sultan) followed by a final rinse with DW (SPI);
Group III- GPC disinfected with 1% ALX (M68182628; Gentaur, Kampenhout, Belgium);
Group IV- GPC disinfected with 1% ALX (M68182628; Gentaur, Kampenhout, Belgium) followed by a final rinse with DW (SPI);
Group V- GPC treated with BioPure MTAD (Dentsply, Tulsa Dental, Tulsa, OK, USA);
Group VI- GPC treated with BioPure MTAD (Dentsply, Tulsa Dental, Tulsa, OK, USA) followed by a final rinse with DW (SPI);
Group VII (Control)- GPC taken straight from the box and examined.

In group I, III and V, the GPC were disinfected with their corresponding reagents for 1 minute and then shifted on 4 inch X 4 inch gauze pads where they were allowed to air dry for 30 minutes. Following this, they were examined under SEM (FEI Quanta 200 ESEM FEG) for TC. In group II, IV and VI, the GPC were disinfected with their corresponding reagents for 1 minute and given a final rinse with DW. Next, they were shifted on 4 inch X 4 inch gauze pads where they were allowed to air dry for 30 minutes. Following this, they were examined under SEM for TC. In group VII, GPC were taken straight from the box with the help of locking tweezers and subsequently examined under SEM.

Statistical Analysis

Data analysis was performed with the help of the Statistical Package for Social Sciences Version 22 (SPSS Inc., Chicago, IL, USA). Differences in the TC of GPC were compared using the Chi-square test. The criterion for statistical significance was set as $p < 0.05$.

Results

SEM analysis revealed that there were TC on the GPC after 1 minute disinfection procedure in Groups I, II, III and V (Figure 1). On comparing group I with group II, significant differences were found ($p < 0.05$) (Table 1).

Group I showed TC in 90% GPC and group II showed TC in 30% GPC. Intergroup comparison showed significant difference between Group I and group II, group I and group III, group I and group IV, group I and group V, group I and group VI and group I and group VII ($p < 0.05$) (Table 1). On comparing Group II with group I, group III, group IV, group V, group VI and group VII, significant differences were observed ($p < 0.05$) (Table 1). No significant differences were observed between group III, group IV, group V, group VI and group VII ($p > 0.05$) (Table 1) (Graph 1).

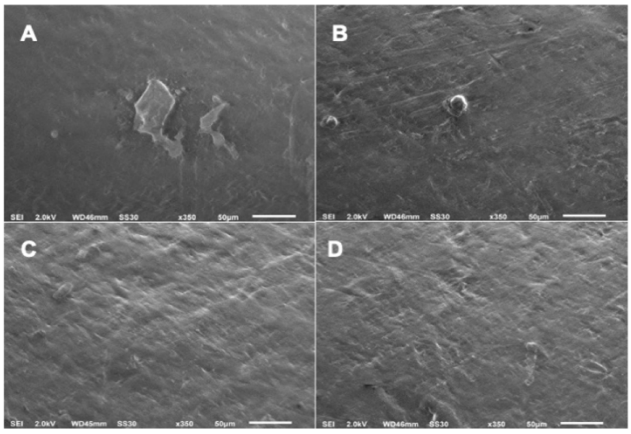
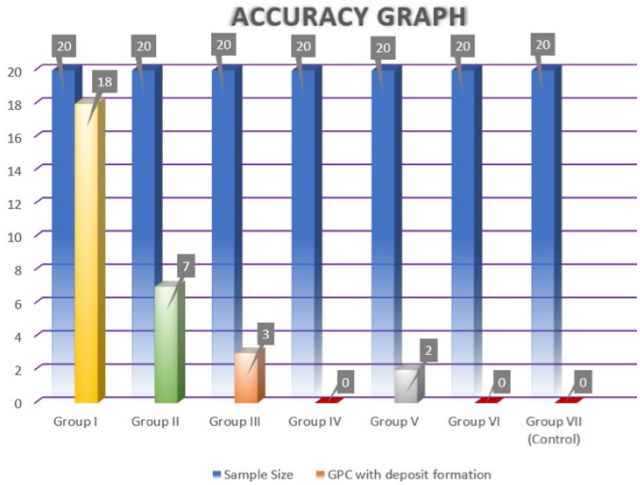


Figure 1. SEM images of (A) Group I- shows deposit formation on the surface of GPC disinfected with 5.25% NaOCl; (B) Group II- shows GPC disinfected with 5.25% NaOCl followed by a final rinse with distilled water; (C) Group III- GPC disinfected with 1% ALX and; (D) Group V- GPC treated with BioPure MTAD.

	Sample size	GPC with deposit formation n(%)
Group I	20	18 (90%)^{A}
Group II	20	7 (35%)^{B}
Group III	20	3 (15%)^{C}
Group IV	20	0 (0%)^{C}
Group V	20	2 (10%)^{C}
Group VI	20	0 (0%)^{C}
Group VII (Control)	20	0 (0%)^{C}

Table 1. Number (n) and percentage (%) of GPC showing deposit formation after different types of disinfection reagent used in each group. Different superscript uppercase in the same column indicate statistically significant difference ($p < 0.05$).



Graph 1. Accuracy graph of GPC showing deposit formation in all the groups after following disinfection protocol in the form of bar diagram.

Discussion

The primary objective of endodontic therapy is to maintain the health and functionality of the affected teeth within the oral cavity. Success rates for endodontic treatment range from 86% to 98%.[13] Ingle asserted that the wiping technique for decontamination has drawbacks as it primarily relies on physical contact to eliminate MO and does not allow sufficient time for the chemical solution to effectively interact with MO. Immersing a GPC in a potent germicidal solution for a proper duration proves to be an effective method for decontamination of GPC.[14]

In this study, SEM was employed to examine the TC of GPC. It revealed the presence of deposits following a 1-minute disinfection procedure by all the disinfecting solutions. ALX and MTAD were observed to leave fewer deposits compared to NaOCl. However, these deposits were effectively eliminated when the GPC were rinsed in DW after the disinfection procedure.

Chemical agents employed as root canal irrigants in endodontics encompass tissue-dissolving agents such as NaOCl and bacteriostatic agents like Mixture of Tetracycline, Acid, and Detergent (MTAD). MTAD is formulated with an antibiotic component (doxycycline), a chelator (citric acid), and a detergent (Tween-80).[15] 5.25% NaOCl is

recognized for its antibacterial activity attributed to hypochlorous acid in the solution. It serves as a potent disinfectant with the potential for significant TC and aggressive deterioration. It is regularly employed as a root canal irrigating solution, and is commonly used as a disinfectant for GPC with an application time of 60 seconds.[16] The GPC treated with 5.25% NaOCl exhibited the most significant TC compared to the other groups which showed minimal TC. It was observed that NaOCl induces deposits on the surface of GPC, potentially diminishing the bond strength or adhesion of GPC to the endodontic sealers. It has been observed to enhance the elasticity of GPC, leading to the breakage of resin bonds and a subsequent reduction in bonding capacity. Additionally, crystals of NaOCl may form, precipitate, and persist on the surface.[17,18] Therefore, these effects on sealing ability and reinforcement in the root canal are identified as potential contributors to endodontic failure. Our results are in corroboration with other studies who found similar TC of GPC with NaOCl.[16,19,20]

In contrast, MTAD and ALX seems to cause lesser TC than as compared to NaOCl. In this study, ALX was chosen as a test solution, whose antimicrobial activity is proven in literature. The ethylhexyl end group present in ALX promotes hydrophobic penetration into membrane lipids and electrostatic adhesion to the negatively charged sites of cell membranes, leading to a quicker and more efficient bactericidal activity.[21,22] SEM examination unveiled minimal TC like crustal deposition and surface irregularities on the surface of GPC when treated with ALX and MTAD. The deposits observed on the GPC were effectively eliminated when the GPC were rinsed in DW following the disinfection procedure. Consequently, they were identified as the optimal disinfectant for swift chair-side disinfection of GPC. It is essential to note that the results with ALX lack direct comparison with other research findings, as this study stands as the first of its kind to evaluate ALX for TC of GPC. However, numerous earlier studies have assessed the impact of MTAD on the TC of GPC and have consistently reported minimal alterations.[16]

The first and second null hypothesis were rejected. Alterations in the surface topography of GPC were noted, including crystal deposits and surface irregularities, when treated with all disinfectants. These changes were more pronounced when the GPC were treated with 5.25% NaOCl for 1-minute disinfection. The observed TC have the potential to hinder the contact between the GPC and the sealer, compromising the obturation seal and, subsequently, the success of endodontic therapy.

Conclusions

Ensuring the sterility of GPC is crucial for the success of endodontic therapy. Consequently, this study aimed to develop a clinically efficient and rapid disinfection method without compromising the topography of GPC. In this context, both 1% ALX and MTAD, followed by a final rinse with DW, demonstrated minimal impact on the TC of GPC within an acceptable clinical time of 1 minute. It is essential to perform a final rinse of GPC with DW to eliminate any surface deposits.

Acknowledgements

The author would like to thank the Electron Microscopy Unit, College of Applied Sciences, King Saud University, Kingdom of Saudi Arabia and Gentaur Molecular Products BVBA Kampenhout, Belgium.

Declaration of Interest

The authors report no conflict of interest.

References

1.Siqueira Jr JF, da Silva CP, Cerqueira MD, Lopes HP, De Uzeda M. Effectiveness of four chemical solutions in eliminating Bacillus subtilis spores on gutta-percha cones. Dent Traumatol 1998; 14(3): 124-6.
2.Moorer WR, Genet JM. Evidence for antibacterial activity of endodontic gutta-percha cones. Oral Surg Oral Med Oral Pathol 1982; 53(5): 503-7.
3.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.
4.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: 606-9.
5.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: 670-3.
6.Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L. Rapid sterilization of gutta-percha cones with 5.25% sodium hypochlorite. J Endod 1975; 1: 136-40.
7.Pradeep K, Pavithra J, Rao N. Chair side disinfection of gutta-percha points – An in vitro comparative study between five different agents at different concentrations. Int Endod J 2010; 4: 20-31.

8.da Motta PG, de Figueiredo CB, Maltos SM, Nicoli JR, Ribeiro Sobrinho AP, Maltos KL, et al. Efficacy of chemical sterilization and storage conditions of gutta-percha cones. Int Endod J 2001; 34: 435-9.
9.Zorko M, Jerala R. Alexidine and chlorhexidine bind to lipopolysaccharide and lipoteichoic acid and prevent cell activation by antibiotics. J Antimicrob Chemoth 2008; 62(4): 730-737.
10.Yip KW, Ito E, Mao XL et al. Potential use of alexidine dihydrochloride as an apoptosis-promoting anticancer agent. Mol Cancer Ther 2006; 5: 2234-40.
11.McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999; 12: 147–79.
12.Torabinejad M, Shabahang S, Aprecio R, Kettering JD. The antimicrobial effect of MTAD: An in vitro investigation. J Endod 2003; 29: 400-3.
13.Barazy R, Eshoa G. Influence of Root Canal Preparation with Different Tapers on Apical Leakage. J Int Dent Med Res 2020; 13(2): 486-90.
14.Ingle JI, Bakland LK. Text book of Endodontics. 6th ed. Japan: United Publishers Services Limited, Ajanta Offset and Packaging Limited; 2008.
15.Bajrami D, Stavileci M, Dragidella A, Kamberi B, Aliu N. In vitro Analysis of Minimal Inhibitory Concentrations of NaOCl, CHX, MTAD, and EDTA against Enterococcus faecalis. J Int Dent Med Res 2020; 13(2): 480-5.
16.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.
17. Bloomfield SF, Miles GA. The antibacterial properties of sodium dichloroisocyanurate and sodium hypochlorite formulations. J Appl Bacteriol 1979; 46(1): 65-73.
18. Pina-Vaz I, Espinar MJ, Noites R, de Carvalho MF. In vitro evaluation of the antimicrobial activity of different antiseptics on contaminated gutta-percha cones. Archives of Oral Research 2008; 29: 4(3) 153-159.
19.John BM, Purra A, Dutta A, Zargar AW. Topographical effects of Gutta Percha immersed in different concentration of Sodium Hypochlorite disinfection at different time interval: An atomic force microscopy study. Int J Oral Health Dent 2017; 3: 54-8.
20.Valois CR, Silva LP, Azevedo RB. Structural effects of sodium hypochlorite solutions on gutta-percha cones: atomic force microscopy study. J Endod 2005; 31(10): 749-51.
21.Chawner JA, Gilbert P. Interaction of the bisbiguanides chlorhexidine and alexidine with phospholipid vesicles: evidence for separate modes of action. J Appl Bacteriol 1989; 66(3): 253-8.
22.Chawner JA, Gilbert P. A comparative study of the bacteriocidal and growth inhibitory activities of the bisbiguanides alexidine and chlorhexidine. J Appl Bacteriol 1989; 66(3): 243-52.