



Evaluation of Residual Antimicrobial Effects and Surface Changes of Gutta-Percha disinfected with Different Solutions

G Şahinkesen, EA Oktay, Ö Er, MM Koçak, A Kiliç

ABSTRACT

Aim: To determine the possible residual antibacterial effects and possible surface changes caused by 5.25% NaOCl, 2.5% NaOCl, 2% CHX and 0.05% Octenisept on in vitro gutta-percha points after different disinfection protocols.

Materials and methods: A total of 121 standard # 40, 0.2% gutta-percha cones were cut 10 mm from the tip, sterilized with ethylene oxide. The samples were divided into five groups, and each group was divided into three subgroups according to immersion periods of gutta-percha cones into solutions. One gutta-percha cone from each group was selected for SEM evaluation.

Results: All of the tested solutions were effective on the selected microorganisms when compared with the control solution. No surface alterations were detected on the gutta-percha cones.

Conclusion: In the limits of this study, immersion of gutta-percha into 2% CHX for one minute was found the most effective method to eliminate the selected microorganisms.

Clinical significance: Although gutta-percha points are aseptic in their package, once opened and used, gutta-percha cones may be contaminated. In order to eliminate bacteria and not to cause surface changes on gutta-percha cones, disinfection solution must be selected carefully.

Keywords: Chlorhexidine, Gutta-percha disinfectants, Octenidine hydrochloride, Sodium hypochlorite.

How to cite this article: Şahinkesen G, Oktay EA, Er Ö, Koçak MM, Kiliç A. Evaluation of Residual Antimicrobial Effects and Surface Changes of Gutta-Percha disinfected with Different Solutions. J Contemp Dent Pract 2011;12(1):47-51.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

The goal of endodontic therapy can be achieved by an accurate diagnosis followed by successful entrance, cleaning and shaping of the root canal system, and filling. Among these procedures, the priority is to eliminate microbial contamination.¹

Since its introduction in endodontics over 100 years ago, gutta-percha cones have been the most commonly used material for filling root canals. They are biocompatible, dimensionally stable, radiopaque, and thermoplastic.² Although most endodontic instruments can be sterilized by autoclaving or by dry heat procedures, because of its thermoplastic behavior, gutta-percha cannot be sterilized with thermal-related conventional techniques. Even though the gutta-percha cones are manufactured under aseptic conditions, most manufacturers do not claim that their gutta-percha cones are sterile and gutta-percha cones can also be contaminated by handling, by aerosols and during the storage process.^{3,4} Therefore, studies about gutta-percha disinfection have evaluated several chemical cold disinfection solutions, such as polyvinylpyrrolidone-iodine, ethyl alcohol, sodium hypochlorite (NaOCl), hydrogen peroxide, glutaraldehyde, and chlorhexidine (CHX).⁵⁻⁸

Among the various methods, immersing gutta-percha into 5.25% of NaOCl for 1 min is a good standard for rapid decontamination of gutta-percha.^{7,9} However, it was reported that 5.25% NaOCl, a strong oxidizing agent, may cause topographic alterations to gutta-percha cones and failure of endodontic obturation.¹⁰ CHX also has an increasing popularity in endodontics by its antimicrobial efficiency.¹¹ CHX was also found as an effective gutta-percha disinfectant and showed no surface alterations.¹² Octenisept is an antiseptic for skin burns, wound disinfection and mouth rinses consisting of octenidine hydrochloride and phenoxyethanol.¹³ Octenidine hydrochloride demonstrates broad spectrum antimicrobial effects covering both gram-positive and gram-negative bacteria, fungi and several viral species.¹⁴

The aim of this study was to evaluate possible residual antibacterial effects of 5.25% NaOCl, 2.5% NaOCl, 2% CHX and 0.05% octenisept on *Staphylococcus aureus*,

Enterococcus faecalis when used as a gutta-percha disinfectant and to evaluate the possible surface alterations of solutions on gutta-percha points.

MATERIALS AND METHODS

A total of 121 standard # 40, 0.2% gutta-percha cones (VDW, GmbH, München, Germany) were cut 10 mm from the tip, sterilized with ethylene oxide and used in this study. The samples were divided into five groups, and each group was divided into three subgroups, as follows:

- *Group 1:* Seven cones per subgroup were immersed in 5 ml of 5.25% NaOCl for 1 minute (subgroup I), 5 minutes (subgroup II) or 10 minutes (subgroup III).
- *Group 2:* Seven cones per subgroup were immersed in 5 ml of 2.5% NaOCl for 1 minute (subgroup I), 5 minutes (subgroup II) or 10 minutes (subgroup III).
- *Group 3:* Seven cones per subgroup were immersed in 5 ml of 2% CHX (Drogsan, Cubuk, Ankara, Turkey) for 1 minute (subgroup I), for 5 minutes (subgroup II) or for 10 minutes (subgroup III).
- *Group 4:* Seven cones per subgroup were immersed in 5 ml of 0.05% octenisept (Schülke and Mayr, GmbH, Norderstedt, Germany) for 1 minute (subgroup I), for 5 minutes (subgroup II) or for 10 minutes (subgroup III).
- *Group 5 (positive control group):* Seven cones per subgroup were immersed in 5 ml of sterile saline solution (Vacoliter, Eczacıbaşı– Baxter, Ayazağa, İstanbul, Turkey) for 1 minute (subgroup I), for 5 minutes (subgroup II) or for 10 minutes (subgroup III).

All chemicals were of high-grade purity and had a minimum of 12 months before the expiration date. After removal from the test solutions, the cones were dried with sterile gauze. Each cone was immediately added to the surface of the media for microbiologic testing after removal from the test solutions and drying process.

AGAR DIFFUSION TEST

The species of microorganisms used in this study were *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212.

A modified Kirby Bauer disk diffusion method was used to evaluate zone size of the gutta-percha cones disinfected with different antimicrobial solutions.¹⁵ The isolates were grown overnight on a nonselective medium at 35°C. Bacterial inocula were prepared by suspending the freshly grown bacteria in sterile normal saline adjusted to a 0.5 McFarland Standard (approximately 1 to 2×10^8 CFU/mL). The suspensions were inoculated onto 9 cm. The gutta-percha cones were pressed lengthwise into the agar surface. Mueller-Hinton agar plates were incubated at 35°C in

ambient air at least for 16 to 18 hours. After incubation, zone size was assessed by measuring the diameter perpendicular to the long axis of the gutta-percha.

SCANNING ELECTRON MICROSCOPE (SEM)

A total number of 16 standard #40, gutta-percha cones were immersed into test solutions (except one for control) and analyzed by scanning electron microscope (JEOL, JSM 6360 LV, Tokyo, Japan) in order to evaluate surface alterations.

STATISTICAL ANALYSES

The difference between the initial stage and the removal times of cones from the test solutions was evaluated with a Wilcoxon test. The differences between solutions were evaluated with Kruskal-Wallis; additionally, Bonferoni-adjusted and Mann-Whitney-U tests were done to confirm the source of the difference. Significance level for all tests was set to $p < 0.05$.

RESULTS

The mean areas of the inhibition zone for the different microorganisms caused by four different solutions and a control solution for three different time periods are presented. Tables 1 and 2 show the differences between time periods.

Table 1: The median inhibition zones (in mm)

| Groups | Solutions | Initial | 1 min | 5 mins | 10 mins |
|--------------------|-------------|---------|-------|--------|---------|
| <i>S. aureus</i> | 5.25% NaOCl | 0 | 4 | 5 | 4 |
| | 2.5% NaOCl | 0 | 2 | 3 | 3 |
| | 2% CHX | 0 | 10 | 10 | 10 |
| | 0.05% Oct | 0 | 2 | 2 | 3 |
| | SSS | 0 | 0 | 0 | 0 |
| <i>E. faecalis</i> | 5.25% NaOCl | 0 | 3 | 5 | 4 |
| | 2.5% NaOCl | 0 | 3 | 2 | 2 |
| | 2% CHX | 0 | 8 | 8 | 8 |
| | 0.05% Oct | 0 | 3 | 2 | 2 |
| | SSS | 0 | 0 | 0 | 0 |

SSS: Sterile saline solution, Oct: Octenisept

Table 2: Comparison between time periods

| Micro-organisms | Solutions | Initial 1 min | Initial 5 mins | Initial 10 mins | 1-5 mins | 1-10 mins | 5-10 mins |
|--------------------|-------------|---------------|----------------|-----------------|----------|-----------|-----------|
| <i>S. aureus</i> | 5.25% NaOCl | * | * | * | ∅ | ∅ | * |
| | 2.5% NaOCl | * | * | * | ∅ | ∅ | ∅ |
| | 2% CHX | * | * | * | ∅ | ∅ | ∅ |
| | 0.05% Oct | * | * | * | ∅ | * | * |
| | SSS | ∅ | ∅ | ∅ | ∅ | ∅ | ∅ |
| <i>E. faecalis</i> | 5.25% NaOCl | * | * | * | * | ∅ | ∅ |
| | 2.5% NaOCl | * | * | * | ∅ | ∅ | ∅ |
| | 2% CHX | * | * | * | ∅ | ∅ | ∅ |
| | 0.05% Oct | * | * | * | * | ∅ | ∅ |
| | SSS | ∅ | ∅ | ∅ | ∅ | ∅ | ∅ |

∗: $p < 0.05$, ∅: no significance, Oct: Octenisept, SSS: Sterile saline solution

When the solutions are compared with each other in time intervals:

NaOCl (5.25%) is significantly more effective than 2.5% NaOCl or 0.05% octenisept against *S. aureus* when immersed for five minutes, and against *E. faecalis* for five or ten minutes of immersion ($p < 0.05$). CHX (2%) is significantly more effective than 5.25% NaOCl or 2.5% NaOCl against *S. aureus* after five and ten minutes of immersion and against *E. faecalis* at all time periods ($p < 0.05$). CHX (2%) is more effective than 0.05% octenisept against both microorganisms at all time periods ($p < 0.05$). Finally, there is no difference between 2.5% NaOCl and 0.5% octenisept when compared in all situations ($p > 0.05$).

None of the tested solutions showed any alteration on surface of gutta-percha points during selected time intervals (Figs 1A to D).

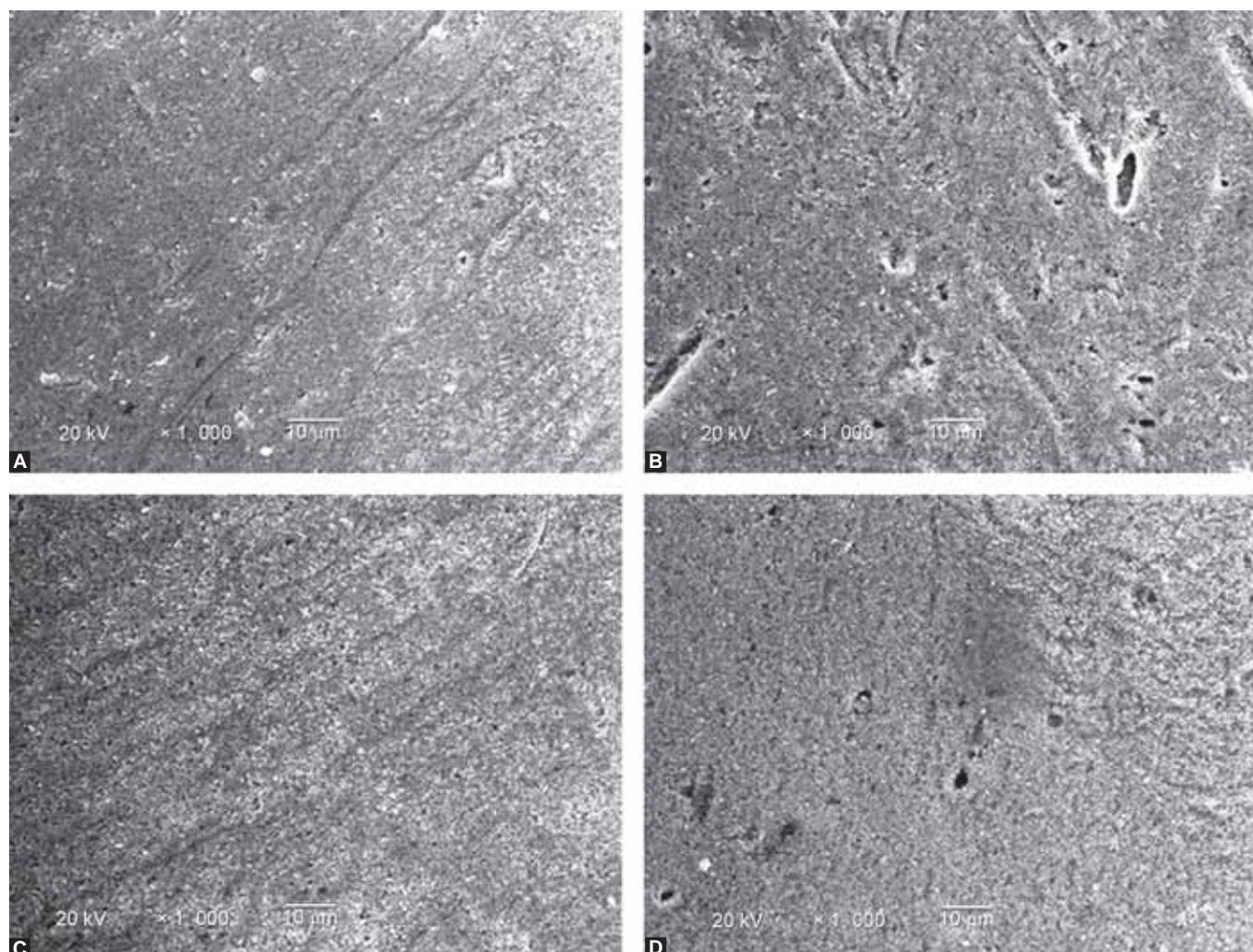
DISCUSSION

In this study, the residual antibacterial effect of different gutta-percha disinfectant solutions was evaluated against

two bacteria. Since it was reported that *S. aureus* and *E. faecalis* were found to be the most resistant microorganisms to instrumentation and irrigation procedures,^{16,17} these bacteria were chosen for this study. Although facultative anaerobic cocci as *S. aureus* are seldom found in infected root canals despite the fact that they are normal inhabitants of saliva and the human skin, gutta-percha cones can be easily contaminated during storage or if incorrectly manipulated.^{7,18}

NaOCl is widely used in root canal treatments as an irrigant and at different concentrations for sterilization of gutta-percha against possible contamination. Using the direct contact method, it was shown that 5.25% NaOCl eliminates most organisms within 15 seconds.^{19,20} In addition, 2.5% NaOCl was found to be effective against all microorganisms except *E. faecalis* biofilm after five minutes.²⁰

CHX (2%) was also reported to be used either as an irrigant solution or as an intracanal medication.²¹ A 2% CHX solution was found effective at 15 seconds to 2 hours in direct contact with infected gutta-percha cones.⁷ Although



Figs 1A to D: Surface images with SEM, (A) control group, (B) 10 mins of 0.05% octenisept (C) 10 mins of 5.25% NaOCl, (D) 10 mins of 2% CHX

several articles have reported that the residual microbial activity of CHX lasted from 48 hours to 28 days in a root canal when used as an irrigant,²²⁻²⁴ in this study, CHX was used as a gutta-percha disinfectant and was evaluated for residual activity on gutta-percha cones. There were also several studies about the use of CHX for sterilization of root canal filling materials, in which CHX was compared with NaOCl for activity against different microorganisms.^{7,11}

Octenisept was demonstrated to have broad-spectrum antimicrobial effects against both Gram-positive and Gram-negative bacteria, fungi and several viral species,¹⁴ and the efficacy of octenidine was reported against dental plaque-associated bacteria when compared with CHX.^{25,26} Octenisept was chosen as a test solution in this study after the publication of reports that it was used as a root canal dressing material and had antibacterial activity against *E. faecalis* in root canal and dentine after one minute.¹³

There are a few studies that evaluate the residual antimicrobial activity of the immersion of gutta-percha into different solutions.¹² In this study, we showed that, for all time periods of immersion, NaOCl at all concentrations was effective in all time periods, but only 5.25% NaOCl continued to affect *E. faecalis* after five or ten minutes of immersion and *S. aureus* only after five minutes. This may be because gauze does not provide a totally dry surface and because the efficiency of high concentrations of NaOCl may continue on moist surfaces of gutta-percha. The present study showed that 2% CHX had the best residual antimicrobial effects against all tested microorganisms at all time periods. This continuing effect may be due to CHX coating gutta-percha with a thin film as it does when used as a mouth rinse, or it may be due to the remnants of CHX on gutta-percha. Although octenisept also had a residual effect against bacteria when compared with the initial effect, 5.25% NaOCl and 2% CHX were found to be more effective than octenisept, and there was no difference between 2.5% NaOCl and octenisept. Slow release of CHX and a high concentration of NaOCl may be the reasons for these results.

In the literature, there are some contradictory results related to the surface alterations. In a study Valois et al²⁷ reported the surface alterations on gutta-percha cones even immersed in NaOCl in short time periods but Gomes et al¹² did not mention any surface alteration of gutta-percha cones. The results of our study also supported the findings of Gomes et al.

Although we took clinical situations into consideration with respect to the time needed for immersion of gutta-percha into solutions and in the selection of solutions, clinically, the effects of these solutions may change

according to the root canal environment and root canal filling procedures.

CONCLUSIONS AND CLINICAL SIGNIFICANCES

- Although gutta-percha points are aseptic in their package, once opened and used, gutta-percha cones may be contaminated.
- In the limits of this study, immersion of gutta-percha into 2% CHX for one minute was found the most effective method to eliminate the selected microorganisms.

ACKNOWLEDGMENTS

The authors thank to Gazi University, Faculty of Engineering, for assistance with using the SEM which was supported by Turkish Prime Ministry, State Planning Organisation Project 2001K120590.

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