Antimicrobial efficacy of mixture tetracycline citric acid and detergent, sodium hypochlorite and chlorhexidine on rapid disinfection of gutta-percha cones

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ABSTRACT

Objective: The aim of this study was to compare the disinfection efficacy of various disinfectants on gutta-percha root canal filling materials. Materials and Methods: Totally, 370 standard #80 gutta-percha cones were used for the study. The gutta-percha cones were sterilized with ethylene oxide. Ten cones were separated for negative control group. Then the gutta-percha cones were randomly divided into four groups containing 90 points each and contaminated with Escherichia coli, Enterococcus faecalis, Candida albicans and Streptococcus mutans. Specimens were incubated in 10 ml brain heart infusion broth at 37°C for 72 h. After incubation, cones were dried with sterile gauze; ten of the cones from each group were separated for positive control groups and rest of the cones were divided into four subgroups according to the chemical disinfectant used (n = 80 each): About 5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate (CHX); 10% povidone iodine; and mixture tetracycline citric acid and detergent (MTAD) groups. For each subgroup, the gutta-percha cones were immersed into the disinfectant for 1 and 10 min individually. In plates showing growth, the number of colony forming units were counted. Results: NaOCl, CHX and MTAD, were equally effective on tested microorganisms. Conclusion: About 5.25% NaOCl, 2% CHX, and MTAD are effective agents for a rapid high disinfection level of gutta-percha cones.

KEY WORDS: Antimicrobial activity, disinfection, endodontics, gutta-percha cones, mixture tetracycline citric acid and detergent

INTRODUCTION

The preservation of the aseptic chain is one of the most important factors affecting the success of root canal treatment. During endodontic treatment, the removal of bacteria, bacterial metabolites and prevention of re-infection of root canals are essential to prevent apical pathology [1]; this condition is valid for materials that are used for root canal filling. A breakdown in the aseptic chain may cause the contamination of gutta-percha points and contaminated root canal filling materials may lead to post-treatment disease or a delay in healing [2].

Gutta-percha points are sterilized during the manufacturing process however once their packages are open they are shown to be contaminated with microorganisms such as bacteria and fungi [3,4]. Contamination of root canal filling material might lead to chronic and persistent endodontic infections, which further leads to the failure of root canal treatments.

Gutta-percha is a widely accepted root canal filling material and has been used routinely since its introduction to endodontics. Gutta-percha is biocompatible, radio-opaque, thermoplastic, and dimensionally stable [5]. Root canal obturation is commonly achieved with gutta-percha points without checking its sterility [6]. Contamination of root canal filling material might compromise the success of endodontic treatment, so the disinfection of gutta-percha points is recommended [7]. Gutta-percha points can easily be contaminated with oral and mucosal bacteria from aerosols and incorrect manipulation [7,8].

Gutta-percha points cannot be sterilized with conventional methods, such as moist and dry air, due to their thermoplastic properties. Consequently, rapid chemical disinfection methods are required [8]. The disinfection agent must be effective against various species of bacteria and also prevent communication between different microorganisms. For this purpose, the most preferred disinfectants are sodium hypochlorite (NaOCl),...
glutaraldehyde, alcohol, chlorhexidine gluconate (CHX), hydrogen peroxide and povidone iodine (PI).

NaOCl is widely used due to the antiseptic properties of active chlorine and its strong oxidizing ability [6]. CHX eliminates vegetative bacteria by destroying membrane integrity and inducing the cytoplasmic aggregation [9,10]. CHX adheres to the cell walls of bacteria, leaks intracellular components and CHX shows substantivity from 48 h to 28 days [11]. PI is a strong and rapid antiseptic with bactericidal, fungicidal and sporicidal properties, and has been used for the disinfection of surfaces, skin, and operation sites for many years [12]. PI works by causing protein denaturation and harming enzymatic systems. Mixture tetracycline citric acid and detergent MTAD, a mixture of the tetracycline isomer, acid, and detergent, is used for the final irrigation of root canals [13].

The aim of this study was to compare the antimicrobial effects of various disinfectants, i.e., 5.25% NaOCl, 2% CHX, 10% PI and MTAD, on contaminated gutta-percha points at 1 and 10 min intervals.

**MATERIALS AND METHODS**

Three hundred and seventy standard #80 gutta-percha cones (DiaDent Dent Plus; Seoul, Korea) were used for the study. Initially, the gutta-percha cones were sterilized with ethylene oxide. Ten cones were separated randomly as negative control group; then the gutta-percha cones were divided into four groups containing 90 points each. *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10231) and *Streptococcus mutans* (ATCC 25175) were produced by making fresh passage of bacteria in 5% blood agar. After that, all bacteria were set at a concentration of 0.5 McFarland scale in saline solution; this means that there are 10^8 colony forming units (CFU) per milliliter bacteria in this solution. Then this solution was diluted at a ratio of 1:10 with brain heart infusion (BHI) broth (BHI agar; Lab M Ltd, Bury, UK) in order to obtain 10^7 CFU/ml bacteria in the solution according to standards. Gutta-percha cones were contaminated with this solution of bacteria and specimens were incubated in 10 ml BHI Broth at 37°C for 72 h.

Following incubation, cones were dried with sterile gauze, ten cones from each group were separated for positive control [Figure 1] and rest was divided into four subgroups according to the chemical disinfectant used (n = 80 for each group): About 5.25% NaOCl (Caglayan Kimya; Konya, Turkey); 2% CHX gluconate (Klorhex, Drogsan; Ankara, Turkey); 10% PI (POVIOD, Saba; Istanbul, Turkey); and Bio-Pure MTAD (Dentsply Tulsa Dental, Johnson City, TN, USA) [Table 1].

For each subgroup, the gutta-percha cones were immersed into the disinfectant for 1 and 10 min individually. Following immersion into the disinfectant, the cones were vortexed in 2 ml BHI. Then, the cones were dropped onto 100 µl 5% sheep blood agar and eosin methylene blue (EMB) agar medium using a 10 µl loop. Specimens were incubated at 37°C for 72 h, and then the results were evaluated. In plates showing growth, the number of CFUs were counted and expressed as CFU/ml. The data were evaluated with two-way ANOVA using SPSS software (PASW Statistics 20; SPSS Inc, Chicago, IL, USA). The significant effects and interactions were further investigated with post-hoc Bonferroni test. The level of statistical significance was set at P < 0.05.

**RESULTS**

The mean numbers of CFUs are presented in Table 2. MTAD, 5.25% NaOCl and 2% CHX were equally effective for disinfection of gutta-percha points contaminated with *E. coli*, *E. faecalis*, *C. albicans* and *S. mutans* at all-time intervals (P > 0.05 among all). In 10 min, no growth was detected in groups disinfected with MTAD, 5.25% NaOCl or 2% CHX. 10% PI exhibited significantly superior antimicrobial effect on tested microorganisms after 10 min interval compared to 1 min (P < 0.05). All solutions showed a superior disinfecting effect when compared with PI (P < 0.05) at all times intervals. The positive control groups showed growth at all-time intervals and the negative control group showed no growth.

**DISCUSSION**

Gutta-percha points have a high risk of being contaminated due to incorrect storage and manipulation leading to contamination with oral and mucosal bacteria. They cannot be sterilized by
thermal methods because of their plastic properties. Therefore, during endodontic treatment the master and accessory cones must be disinfected with routine chemical disinfectants [14,15]. In addition, due to the unpredictability of the number of accessory cones required, a rapid and simple disinfection procedure is required [7,16]. In this study, *E. faecalis* and *S. mutans*, which regularly cause primary endodontic infections, *C. albicans*, which is related with persistent infections, and *E. coli*, which represents Gram-negative bacteria are chosen in order to represent bacterial groups common in endodontic infections.

The antibacterial properties of NaOCl increase with concentration, so higher concentrations show faster effect than low ones. Many studies used NaOCl at various concentrations for gutta-percha disinfection [5,7,8,12,13,16]. Cardoso et al. [16] evaluated effects of different concentrations of NaOCl on *E. coli* and *S. aureus* and reported a bactericidal effect of 1% NaOCl on *E. coli* in after 1 min. However, in the present study, after application of 5.25% NaOCl for 1 min *E. coli* growth was detected and NaOCl exhibited bactericidal effects after 10 min. Nabeshima et al. [17] reported that 1% NaOCl was ineffective against *E. faecalis* after 1 min but showed bactericidal effects after 10 min. The different results of these studies might be due to the different concentrations of NaOCl that were used.

The disinfection of gutta-percha points with CHX was found to be effective, and various studies recommend it [8,12,13,17]. Similarly, the present study showed that 2% CHX is effective against all bacteria after 10 min. Cardoso et al. [12] compared the efficacy of 2% CHX, 1% NaOCl and 10% PI and reported that 2% CHX is the most effective disinfectant. They report a bactericidal effect on *E. faecalis* and *E. coli* after 1 min, which is contrary to the present study. Gomes et al. [8] reports a rapid disinfection effect of CHX on *E. faecalis* and *C. albicans* after just 15 s.

In our work, PI disinfection was better after 10 min than after 1 min, which indicates that its effectiveness increases with time. Similarly, Subha et al. [2] reported that disinfection after 5 min was better than after 1 min. In the same study, 2% CHX is reported to be superior compared with 3% NaOCl, and both of these disinfectants are superior to PI. These results are consistent with the result of the present study, and the ineffectiveness of PI might be attributed to the relative low concentration of the solution.

In this study, MTAD showed disinfactive effects on *E. faecalis*, *E. coli* and *C. albicans* after 10 min, whereas Royal et al. [13] report that MTAD was effective in rapid disinfection of gutta-percha cones in 1 min. In addition, Shabahang et al. [18] report that MTAD is superior to 5.25% NaOCl in disinfecting root canals contaminated with saliva and suggest that clinicians who use MTAD as a routine irrigation solution should also disinfect gutta-percha points with MTAD.

**CONCLUSION**

In conclusion, 5.25% sodium hypochlorite, 2% chlorhexidine, and MTAD, when used for disinfection of gutta-percha points, eradicate both Gram-negative and Gram-positive bacteria residing in endodontic infections in 10 min. On the other hand, 10% PI is ineffective for this purpose.

**REFERENCES**


