Effectiveness of different chemical agents for disinfection of gutta-percha cones

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Abstract
This aim of this study was to evaluate and compare the efficacy of different chemical methods to disinfect gutta-percha cones (GP). Eighty-six size 80 GP cones were used. The cones were contaminated by immersion in saliva and Enterococcus faecalis. Four chemical agents were used: 1% sodium hypochlorite (G1), 2% chlorhexidine gluconate (G2), 10% povidone iodine (G3) and 0.9% saline solution (G4). GP cones were immersed in the solutions for periods of 1 and 10 min. After the disinfection procedure, the cones were incubated in blood heart infusion and the presence of bacterial growth was analysed by turbidity of the medium. In G4, bacterial growth was observed in all specimens; G3 showed growth after immersion for 1 min when contaminated with E. faecalis; G1 showed diverse results after the immersion for 1 min. Meanwhile, G1 and G3 after 10 min, and G2 at both times evaluated did not show bacterial growth. The immersion of GP cones in 2% chlorhexidine gluconate for 1 min was an effective method for GP disinfection, while 10% povidone iodine and 1% sodium hypochlorite needed 10 min of immersion to disinfect the GP.

Introduction

The purpose of endodontic treatment is the cleaning, shaping and disinfection of the root canal, followed by the obturation of the endodontic system, so that the tooth can be restored to function. The presence of microbes inside the canal is the main reason for post-treatment infection (1). Therefore, the maintenance of the disinfection obtained during the treatment is imperative (2–6).

Obturation is the final stage of endodontic treatment, eliminating the root canal space. This obturation is achieved by introduction a root-filling material combined with a sealer. Gutta-percha cones (GP) are the most widely used material for this purpose.

GP cones are usually purchased in sterile, sealed packages, but once exposed to the dental office environment or even by handling, they can be contaminated by any number of microorganisms (7). Supplementary decontamination of GP cones is critical, because they cannot be sterilised by moist or dry heat. Thus, cold sterilisation, using disinfectants should be used. Various chemical agents have been proposed as GP disinfectants, including sodium hypochlorite (NaOCl) (8–14), glutaraldehyde (8–10,13), alcohol, iodine compounds and hydrogen peroxide (9). The appropriate disinfectant should be the one that can be used routinely in dental clinics, providing a fast disinfection without modifying the structure of the cone.

In order to accomplish the appropriate decontamination of the cones, the disinfectant agent ought to be effective in killing different bacterial species and should also create difficulties for the establishment of interrelations between the different microorganisms (15–17). Studies have reported that Enterococcus faecalis is the most common bacteria associated with post-treatment infection of the root canal system (2–5,18,19); also, it is known...
that this strain can survive in dentinal tubules for long periods (3–6,18,19).

The aim of this study was to compare the effectiveness of 1% NaOCl, 2% chlorhexidine gluconate (CHX), 10% polyvinylpyrrolidone-iodine (povidone iodine, PVPI) and 0.9% saline solution to disinfect GP cones contaminated by either a polymicrobial infection or *E. faecalis* alone, after immersion periods of 1 min or 10 min.

**Materials and methods**

In this study, 86 size 80 standardised GP cones (Dentsply, Petrópolis, RJ, Brazil) were used. Prior to the experiment, the cones were sterilised by ethylene oxide, and 84 cones were randomly divided into two groups (*n* = 42 per group). In group A, GP cones were contaminated by immersion in 20 mL of a pure culture of *E. faecalis* (ATCC 29212; Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil), that was inoculated in a brain heart infusion (BHI) broth in a suspension that contained approximately 10^7 CFU mL^{-1}; likewise, in group B, GP cones were contaminated in 20 mL of human saliva obtained of patients scheduled for endodontic treatment.

All samples were incubated at 37°C for 72 h. After the incubation period, the cones were dried using sterile gauze and divided into four groups of 10 samples according to the chemical agent used:

- **Group 1 – 1% NaOCl**
- **Group 2 – 2% CHX**
- **Group 3 – 10% PVPI**
- **Group 4 – 0.9% saline solution**

Five GP cones were immersed for 1 min in one of the agents and other five were immersed for 10 min. The same procedure was repeated for all the groups and for both contaminant groups – *E. faecalis* and saliva.

The positive control group comprised two cones contaminated by *E. faecalis* and two cones contaminated by saliva without contacting any disinfection agents. On the other hand, the negative control was two cones that were kept sterile after the initial sterilisation by ethylene oxide.

The cones were once again dried and inserted individually into test tubes containing 20 mL of sterile BHI broth and incubated at 37°C for 72 h. Bacterial growth was evaluated by the presence of turbidity in the broth. The presence or absence of turbidity resulted in quantitative data and the results were statistically analysed by Kruskal–Wallis test. Statistical significance level was established at *P* < 0.05.

**Results**

The comparison between the bactericidal activities of the chemical agents in disinfecting GP cones in this study is shown in Table 1. It was observed that the saline solution did not demonstrate any bactericidal action, resulting in intense turbidity in all samples and in both time periods. In G3, all cones contaminated by *E. faecalis* showed bacterial growth after 1 min in povidone iodine and in 20% of the cones after the immersion for 10 min; while, 20% of the cones contaminated by saliva after 1 min demonstrated turbidity of the medium.

The immersion of the cones contaminated by *E. faecalis* and by saliva in 1% NaOCl showed absence of turbidity after 10 min. In addition, 40% of the samples contaminated by *E. faecalis* and 20% of the samples contaminated by saliva showed bacterial growth after 1 min. In all samples for both time periods 2% CHX demonstrated absence of the turbidity in the test tubes; indicating no bacterial growth. The Kruskal–Wallis test revealed significant difference between the groups (*P* = 0.01).

**Discussion**

The presence and persistence of microorganisms in root canals is the main cause of failure in the endodontic treatment. Poor permanent restoration, inadequate cleaning and shaping, unsatisfactory filling of the canal as well as the use of contaminated materials for these procedures could be a possible explanation for this problem (2,14).

Root canals are usually filled with GP cones. They have been the material of choice because of properties such as biocompatibility, dimensional stability, radiopacity and thermoplasticity (11). Despite GP cones being produced under aseptic conditions and sold in sealed packages (10),

| Table 1 Bacterial growth (turbidity) between samples |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Time (min) | Saline solution | PVPI (povidone iodine) | 1% NaOCl | 2% CHX | EF | Saliva |
| 1 | + | + | + | + | + | + | + | + | + | - | - | + | + | + | - | - | - | - | - | - | - | - |
| 10 | + | + | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | + | + | + | + | - | - | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| 10 | + | + | + | + | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - |

CHX, chlorhexidine gluconate; NaOCl, sodium hypochlorite.
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their sterilisation is questionable, and they can be easily contaminated by handling (9,11,13). The presence of zinc oxide in their composition might provide antimicrobial properties (8,11,15,16), but this action is uncertain (17). Storage conditions have also been discussed, but they do not seem to create serious problems (10,13). The inability to sterilise at high temperatures leaves the need to use chemical agents for disinfection. Furthermore this should be an efficient, inexpensive and quick method.

In this study, the cones were intentionally contaminated with microorganisms that are associated with endodontic failure. Saliva represented an initial polymicrobial contamination of the root canal system, because of the presence of a great number and variety of bacterial species. On the other hand, E. faecalis was chosen because of its great capacity to live for long periods without nutrients and its great adaptation to the endodontic system. Also, E. faecalis is the bacteria most frequently isolated in chronic and persistent post-treatment infections (2–6).

NaOCl is a strong oxidising agent that is widely used during root canal preparation, where it shows excellent antiseptic properties. Several studies recommend the use of NaOCl to disinfect GP cones (8–11,13,14). However, at very high concentrations (5.25%), NaOCl produces a large quantity of chloride crystals on the cone surface (7), and might causes the deterioration of GP points, including increased depth of surface irregularities and loss of elasticity, which could make it difficult to achieve appropriate obturation sealing (12). The present results shows that 1% NaOCl can be effective (8–10,13), as long as a 10 min immersion period is used.

Iodine compounds have been used for decades for the disinfection of surfaces, skin and operating fields; they are known as fast-acting and efficient bactericidal, fungicidal and sporicidal agents (9), where the molecular iodine is responsible for the antimicrobial activity (1). It has been used as an endodontic irrigant against E. faecalis (18), because of its efficient action in the presence of hydroxyapatite (19). Furthermore, the results of this study showed that the use of a solution of PVPI promoted an adequate disinfection of the GP cones contaminated by E. faecalis after 10 min of immersion. This means that the action of iodine compounds on this strain depends on factors other than just the specific antimicrobial activity. Ten minute immersion was necessary for the antimicrobial effect.

Nowadays, there is increasing interest in the antimicrobial activity of CHX that is widely used in periodontics, and is known to kill vegetative bacteria by disrupting the membrane integrity and inducing the precipitation of the cytoplasm. In endodontics, CHX is used as irrigant because of antibacterial and sporicidal activity and substantivity (1,11). Other investigations found that this agent acts on a large number of microorganisms in a short period of time (9,11,14). This agrees with the results of this study. Unlike NaOCl, chlorhexidine does not have the aggressive potential to cause the deterioration of GP points (12).

The results show that while saliva is a mixed bacterial culture, E. faecalis was more resistant than the microorganisms present in the oral microflora. Chlorhexidine was the fastest-acting chemical agent to eliminate all bacteria. Saline solution produced the worst results, showing that cleaning surfaces with just gauze soaked in saline results in a lack of antibacterial activity. This demonstrates the need of an antimicrobial treatment to eliminate bacteria over the cone surface.

Conclusion

According to the results, it can be concluded that the immersion of GP cones in a solution of 2% CHX for 1 min is an efficient method to promote their disinfection. The use of 1% NaOCl and 10% povidone iodine required 10 min to provide an effective action, and the use of sterile gauze soaked with 0.9% saline solution produced no action to disinfect cones.

References


