Australian Endodontic Journal
The Journal of the Australian Society of Endodontology Inc. and
the Australian and New Zealand Academy of Endodontists

Aust Endod J 2011; 37: 118–121

ORIGINAL RESEARCH

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

Cleber K. Nabeshima, DDS[1,2]; Manoel Eduardo de Lima Machado, PhD[1,3]; Maria Leticia Borges Britto, PhD[2]; and Raul Capp Pallotta, PhD[2,3]

1 Department of Restorative Dentistry, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil
2 Post Graduation Course in Endodontics, University Cruzeiro do Sul, São Paulo, SP, Brazil
3 Post Graduation Course in Endodontics, São Paulo General Hospital (HGeSP), São Paulo, SP, Brazil

Keywords
disinfection, endodontics, gutta-percha.

Correspondence
Dr Cleber K. Nabeshima, Avenue Amador Bueno da Veiga, 1340, 03636-100, São Paulo, SP, Brazil. Email: cleberkn@hotmail.com

doi:10.1111/j.1747-4477.2010.00256.x

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 sterilise 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 sterilise 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					
1	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	–	–	–	–	*EF*
10	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	
1	+	+	+	+	+	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	Saliva
10	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

CHX, chlorhexidine gluconate; NaOCl, sodium hypochlorite.

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 leads to 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 hydroxylapatite (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

1. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endod Top 2005; 10: 77–102.
2. Haapasalo M, Udnæs T, Endal U. Persistent, recurrent, and acquired infection of the root canal system post-treatment. Endod Top 2003; 6: 29–56.
3. Portenier I, Waltimo TMT, Haapasalo M. *Enterococcus faecalis* – the root canal survivor and 'star' in post-treatment disease. Endod Top 2003; 6: 135–59.
4. Rôças IN, Siqueira JF Jr, Santos KRN. Association of *Enterococcus faecalis* with different forms of periradicular diseases. J Endod 2004; 30: 315–20.
5. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006; 32: 93–8.
6. Schirrmeister JF, Liebenow AL, Braun G, Wittmer A, Hellwig E, Al-Ahmad A. Detection and eradication of microorganisms in root-filled teeth associated with periradicular lesions: an *in vivo* study. J Endod 2007; 33: 536–40.
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: 670–4.

8. Cardoso CL, Kotaka CR, Redmerski R, Guilhermetti M, Queiroz AF. Rapid descontamination of gutta-percha cones with sodium hypochlorite. J Endod 1999; 25: 498–501.
9. 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.
10. 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.
11. Gomes BPFA, Vianna ME, Matsumoto CU *et al*. Desinfection of gutta-percha cones with chlorhexidine and sodium hypoclorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: 512–7.
12. Valois CRA, 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: 425–9.
13. Özalp N, Ökte Z, Özcelik B. The rapid sterilization of gutta-percha cones with sodium hypochlorite and glutaraldehyde. J Endod 2006; 32: 1202–4.
14. Royal MJ, Williamson AE, Drake DR. Comparison of 5,25% sodium hypochlorite, MTAD, and 2% chlorhexidine in the rapid disinfection of polycaprolactone-based root canal filling material. J Endod 2007; 33: 42–4.
15. Attin T, Zirkel C, Pelz K. Antibacterial properties of electron bean-sterilized gutta percha cones. J Endod 2001; 27: 172–4.
16. Lui JN, Sae-Lim V, Song KP, Chen NN. *In vitro* antimicrobial effect of chlorhexidine-impregnated gutta-percha points on *Enterococcus faecalis*. Int Endod J 2004; 37: 105–13.
17. Tanomaru JMG, Pappen FG, Tanomaru Filho M, Spolidorio DMP, Ito IY. *In vitro* antimicrobial activity of different gutta-percha points and calcium hydroxide pastes. Braz Oral Res 2007; 21: 35–9.
18. Sirén EK, Haapasalo MPP, Waltimo TMT, Ørstavik D. *In vitro* antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on *Enterococcus faecalis*. Eur J Oral Sci 2004; 112: 326–31.
19. Portenier I, Haapasalo H, Rye A, Waltimo T, Ørstavik D, Haapasalo M. Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin. Int Endod J 2001; 34: 184–8.