Effectiveness of peracetic acid in rapid disinfection of gutta-percha and Resilon cones exposed to *Enterococcus faecalis*

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ABSTRACT

Objective: The aim of this study was to assess effectiveness of 2% peracetic acid in rapid disinfection of gutta-percha and Resilon cones compared to sodium hypochlorite and chlorhexidine. **Methods:** Gutta-percha and Resilon cones were immersed during five minutes for contamination with *Enterococcus faecalis* suspension. Subsequently, they were divided into groups (n = 10) to test the disinfection protocol: 2% peracetic acid (one and three minutes); 5.25% NaOCl (one and three minutes); 2% chlorhexidine (one and three minutes). After the tested protocols were carried out, the cones were transferred to test tubes containing Enterococcosel™ agar, and then kept in an oven at 37°C for 48 hours. After the observation period, tubes were assessed and those presenting turbidity of the medium were considered positive. **Results:** Results demonstrated that 2% peracetic acid seems to be effective in decontaminating both types of cone, similarly to 5.25% NaOCl. Conversely, 2% chlorhexidine was less effective ($p < 0.05$). **Conclusion:** Both solutions, 2% peracetic acid and 5.25% NaOCl, were effective in decontaminating gutta-percha and Resilon cones at the tested times.

Keywords: Decontamination. *Enterococcus faecalis*. Gutta-percha.

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Introduction

Elimination or reduction of microorganisms in the root canal by means of chemical-mechanical preparation is one of the main factors responsible for therapy success.[1] Treatment completion is achieved with three-dimensional canal filling, which aims at properly sealing the endodontic cavity in the apical, lateral and crown regions.[1,2]

In Endodontics, gutta-percha is the used solid material most commonly for root canal filling due to being biocompatible and not interfering in the repair process.[1-3] An alternative to gutta-percha cones are Resilon cones, which are thermoplastic synthetic polymer-based. The latter contains radiopaque charge and bioactive glass particles. They are similar to gutta-percha cones in shape, size, radiopacity, and commercial presentation.[4,5]

Gutta-percha and Resilon cones, despite being sold in sealed packages, may be contaminated as from factory or be susceptible to contamination as a result of clinician's handling processes.[1,3,6,7] Thus, cones asepsis before the filling procedure, as well as precaution taken in order to prevent contamination of instruments and filling material during treatment iare of paramount importance to prevent cross-infection.[1,6,8] Studies have demonstrated that secondary bacteria inoculation into the canals, as it is the case of Pseudomonas aeruginosa and some species of Staphylococcus, may result in persistent and difficult-to-treat endodontic infections.[1,2]

Another microorganism related to persistent infections is Enterococcus, which includes several commensal species resident in the gastrointestinal tract, vagina, and oral cavity. On the other hand, some species, such as *Enterococcus faecalis* and *Enterococcus faecium*, can cause diseases, including urinary tract infections and endocarditis. Enterococcus comprise a group of Gram-positive bacteria that are associated with endodontic infections in Dentistry.[9,10] The prevalence of *Enterococcus faecalis* in the root canal is higher in patients previously subjected to endodontic treatment or in cases of retreatment, which can be explained by low sensitivity to antimicrobial agents used as irrigating solution or intracanal medication.[9,10,11] Additionally, it has been demonstrated that the microorganism presents easily offers resistance to antimicrobial drugs,[11] thus making it highly relevant to search for alternative antimicrobial agents.

Therefore, the aim of this study was to assess the effectiveness of peracetic acid as an alternative antimicrobial agent in the chemical disinfection of gutta-percha and Resilon cones contaminated with Enterococcus faecalis, in comparison to two agents commonly used in Dentistry.

Material and methods

Inside a laminar flow cabinet, 60 gutta-percha cones (Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil) and 60 Resilon Real Seal cones (SybronEndo Corp., Orange, USA) were immersed in *Enterococcus faecalis* ATCC29212 suspension during five minutes for contamination (Fig 1). Subsequently, they were divided into 12 groups (n = 10) for assessment of the following decontamination protocols: immersion in 5.25% NaOCl; 2% chlorhexidine; and 2% peracetic acid, all of which had been prepared in a compouding pharmacy(Mil Fórmulas, Rio de Janeiro, RJ, Brazil) at previously determined times (Chart 1, Fig. 2).

By the end of each disinfection protocol, the cones were transferred to test tubes containing EnterococcoselTM agar (BD, Sparks, MD 21152, USA), selective for Enterococcus, and kept in an oven at 37°C for 48 hours. By the end of this period, results were recorded based on the darkening or not of the culture medium. For positive control, three cones contaminated with *Enterococcus faecalis* selected from each group were immersed in distilled water before being transferred to the tubes with culture medium. On the other hand, negative control used three decontaminated cones of each type, immersed in 5.25% NaOCl for one minute.

Group	Cone	Solution	Concentration (%)	Time
1	Gutta-percha	NaOCl	5,25	1
2	Resilon	NaOCl	5,25	1
3	Gutta-percha	NaOCl	5,25	3
4	Resilon	NaOCl	5,25	3
5	Gutta-percha	Chlorexidine	2	1
6	Resilon	Chlorexidine	2	1
7	Gutta-percha	Chlorexidine	2	3
8	Resilon	Chlorexidine	2	3
9	Gutta-percha	Peracetic acid	2	1
10	Resilon	Peracetic acid	2	1
11	Gutta-percha	Peracetic acid	2	3
12	Resilon	Peracetic acid	2	3

Chart 1. Protocol of gutta-percha or Resilon cone disinfection, according to solution (%) and time (minutes) of immersion.

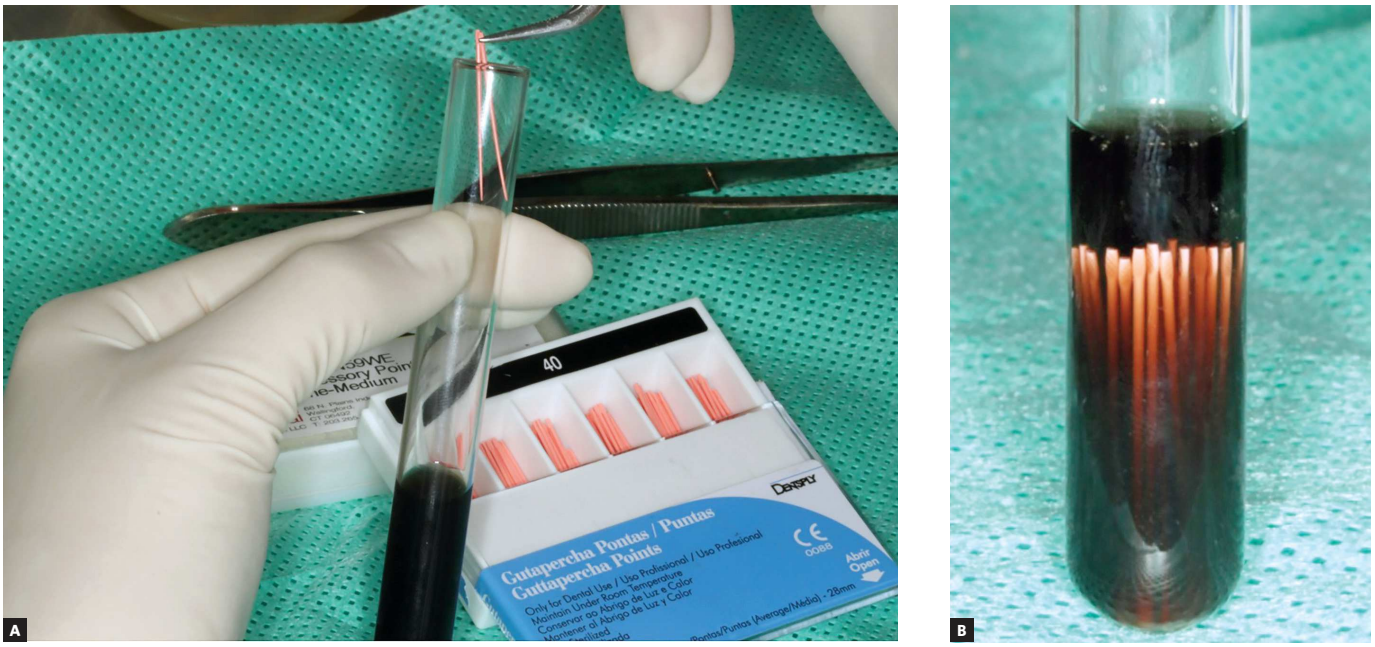

Figure 1. Cones kept in test tubes with Enterococcus faecalis ATCC29212 suspension.

Figure 2. Cones divided into groups for assessment of decontamination protocol.

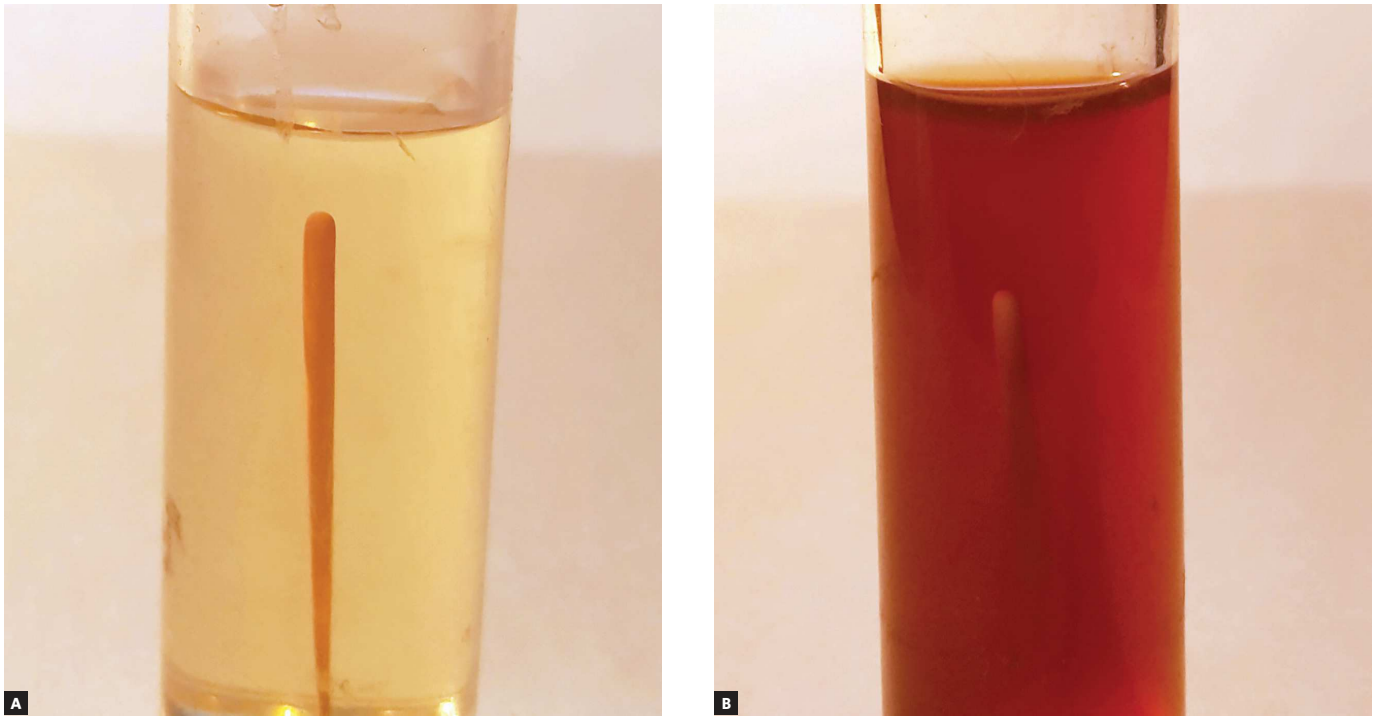

Figure 3. Cones kept in test tubes with Enterococcosel™ agar after disinfection protocol. **A**) Unchanged agar. **B**) Turbid agar (contaminated).

Table 1. Percentage of effectiveness of cone decontamination promoted by NaOCl, chlorhexidine, and peracetic acid.

Group	Cone	Solution	Concentration (%)	Time	%
1	Gutta-percha	NaOCl	5,25%	1	100[b]
2	Resilon	NaOCl	5,25%	1	100[b]
3	Gutta-percha	NaOCl	5,25%	3	100[b]
4	Resilon	NaOCl	5,25%	3	100[b]
5	Gutta-percha	Chlorexidine	2%	1	100[b]
6	Resilon	Chlorexidine	2%	1	10[a]
7	Gutta-percha	Chlorexidine	2%	3	90[b]
8	Resilon	Chlorexidine	2%	3	30[a]
9	Gutta-percha	Peracetic acid	2%	1	20[a]
10	Resilon	Peracetic acid	2%	1	100[b]
11	Gutta-percha	Peracetic acid	2%	3	100[b]
12	Resilon	Peracetic acid	2%	3	100[b]

* Different superscript letters represent significant difference (ANOVA, $p > 0.05$).

Results

Bacterial growth was absent in groups disinfected by NaOCl and peracetic acid for one and three minutes. As for the chlorhexidine group, except for group 7 (gutta-percha immersed for three minutes), the medium became turbid, thus revealing growth in groups 5, 6 and 8 (Table 1). Data were displayed in tables, the percentage of effectiveness of protocols was established, and statistical analysis carried out. No significant difference was found between chlorhexidine groups(ANOVA, $p > 0.05$).

Discussion

Endodontic treatment success is based on correct achievement of several stages of therapy, which must be carried out by means of adequate techniques and material, taking biological principles into consideration in order to stimulate periapical tissue repair.[1,2]

In cases of persistent or secondary infections, with the latter being caused by microorganisms introduced during or between sessions of endodontic treatment, treatment prognosis may be unfavorable, as some facultative anaerobic species can be found in root canals, namely: *Streptococcus*, *species of Enterococcus faecalis* and *Pseudomonas aeruginosa*.[9,10] Thus, efforts should be made to prevent secondary infections. To this end, it is essential to maintain aseptic chain during therapy, thereby preventing cross-infection.[1,3,6]

Gutta-percha and Resilon cones usually touch periapical tissues during root canal filling. For this reason, it is imperative that they are free of contamination, once that could represent a potential locus of secondary infection or persistence of lesion. Therefore, some authors have assessed contamination of gutta-percha cones removed from sealed packages. In 1971, Montgomery[10] concluded that 8% of cone samples were contaminated, and highlighted the need for decontamination before filling, thus corroborating the findings of other studies.[1,3,6,8] Nevertheless, other authors concluded cone disinfection is unnecessary, once bacterial growth was absent when cones taken out of their original sealed packages were assessed.[13,14] That revealed cone composition is not favorable to bacterial development . Other authors have pointed out that cones are not means of infection and that breaking the aseptic chain is the most critical factor.[1,8,15]

Thus, since there is a chance of cone contamination even when they are directly taken out of their original sealed packages,[1,3,6,7] in addition to the fact that the same box is used in several patients, the need for cone disinfection is emphasized.[3,8] The disinfectant solution used for decontamination should be described in detail, and so should the protocol. In 1966, Buchbinder[14] proved the effectiveness of paraformaldehyde as a method for cone sterilization. In another study, the authors found cone sterilization was reached by exposure to formocresol vapor for 16 hours, thus eliminating Gram-positive, Gram-negative, and spore-forming microorganisms.[17]

NaOCl, in several concentrations, has been shown to be effective, but its activity is directly related to concentration and immersion time in solution. Some authors[18-21] recommend chemical treatment of gutta-percha cones with immersion in 1% sodium hypochlorite for one minute, or for five minutes when immersed in 0.5% with sodium hypochlorite. Other authors considered that 2.5% NaOCl was effective in decontaminating the cones within minute.[1,2,11,22,23]

Chlorhexidine has been used as antimicrobial solution in endodontic therapy, being recommended for patients allergic to NaOCl or in cases of refractory treatment, in which the use of intracanal medication has not been successful.[22,24,25] Some studies found 2% chlorhexidine gel was able to eliminate *Enterococcus faecalis* from dentinal tubules after 15 days.[26,25]

In 1998, Siqueira et al[17] concluded that 5.25% NaOCl was effective after one minute, and that 2% chlorhexidine was not effective even after ten minutes. The data are in agreement with those of the present study, which demonstrated that chlorhexidine was not effective in cone disinfection in both tested times ($p < 0.05$).[22,26] Another study found different results and concluded 4% chlorhexidine used for one minute was effective in cone decontamination, thus corroborating previous studies.[25,26] Other authors consider the required time for exposure to chlorhexidine aimed at disinfection is ten minutes regardless of concentration.[3]

Peracetic acid is a combination of acetic acid and hydrogen peroxide. It was launched in the market worldwide in the second half of the last century, when it was included as a disinfectant/sterilizing active principle by Decree No. 15 of August 23rd, 1988, sub-section 1, item I issued by the Brazilian Health Surveillance Agency (ANVISA). The active principle of peracetic acid was included in the aforementioned decree and further recognized as an active principle authorized by the Brazilian Ministry of Health, as stated in Decree No. 122 of November 29th, 1993. It has a broad spectrum of microbial action, acting even in the presence of organic matter.[27]

The antimicrobial effect of the solution was tested by Guerreiro-Tanomaru et al.[28] The authors con-

ducted an in vitro study to assess the antibacterial activity of conventional irrigating solutions acting against *Enterecoccus faecalis*. Assessed was carried out by means of direct contact test: 2.5% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), and 1% peracetic acid. Both 2.5% NaOCl and 2% CHX eliminated *E. faecalis* after 30 seconds of contact. Peracetic acid reduced bacterial count by 86% after three minuted and eliminated *E. faecalis* after 10 minutes. Those results allowed us to conclude that 1% peracetic acid is effective against cultures of Enterecoccus faecalis, despite its slower action when compared to 2.5% NaOCl and 2% CHX.

A similar study was conducted by Chandrappa et al[29] assessing the same solutions (5.25% NaOCl, 2% CHX, and 2% peracetic acid) used for disinfection of Resilon cones contaminated with *Enterecoccus faecalis*. However, turbidity was assessed for one, five or ten minutes only. The authors found the best results for NaOCl, followed by peracetic acid. When confronted with the results achieved by the present work, the aforementioned outcomes can be considered similar, once peracetic acid was effective in disinfecting gutta-percha and Resilon cones in the time periods of one and three minutes, thus revealing to be a great alternative to hypochlorite use.[24-26,28,29]

Conclusion

According to the methods employed and the results achieved herein, it can be concluded that 2% peracetic acid, as well as 5.25% NaOCl, were effective in decontaminating both gutta-percha and Resilon cones. Additionally, 2% chlorhexidine was not able to promote decontamination at the tested times.

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