
IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF DIFFERENT ANTISEPTICS ON CONTAMINATED GUTTA-PERCHA CONES

Estudo in vitro da atividade antimicrobiana de diferentes soluções antissépticas em cones de gutta-percha contaminados

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Abstract

OBJECTIVES: The purpose of this *in vitro* study was to evaluate the effectiveness of different antiseptics for decontamination of gutta-percha cones. **MATERIALS AND METHODS:** Thirty-six cones were contaminated with standardized pure cultures of six different microorganisms: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*. The cones were treated for five minutes with different antiseptic solutions: 2% iodine alcohol, 3% sodium hypochlorite, 0.2% chlorhexidine and 5% citric acid. **RESULTS:** Iodine alcohol was the most effective for all the microorganisms, except for *Candida albicans*. For this microorganism, the best results were obtained with 3% sodium hypochlorite and 5% citric acid; these two solutions showed moderate activity on other microorganisms. Chlorhexidine was not effective on any of the microorganisms.

Keywords: Gutta-percha cones; Antiseptics; Antimicrobial activity; Endodontics.

Resumo

OBJETIVOS: Neste trabalho avaliou-se a atividade antimicrobiana *in vitro* de diferentes soluções antissépticas na desinfecção de cones de gutta-percha. **MATERIAIS E MÉTODOS:** Trinta e seis cones de gutta-percha foram contaminados com culturas puras de diferentes microrganismos: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* e *Candida albicans*. Os cones foram imersos em diferentes soluções antissépticas: álcool iodado a 2%, hipoclorito de sódio a 3%, clorhexidina a 0,2% e ácido cítrico a 5%,

durante 5 minutos. **RESULTADOS:** Os resultados indicam que o álcool iodado é o mais eficaz sobre todos os microrganismos, exceto sobre a *C. albicans*. Sobre esta, o mais eficaz foi o hipoclorito de sódio a 3 %, mostrando moderada eficácia sobre os restantes microrganismos, tal como o ácido cítrico. A clorhexidina não mostrou eficácia sobre qualquer dos microrganismos, nestas condições experimentais.

Palavras-chave: Cones de gutta-percha; Antissépticos; Atividade antimicrobiana; Endodontia.

INTRODUCTION

Microorganisms play a fundamental role in the aetiology of pulp and periapical diseases (1, 2). One of the major objectives of root canal treatment is the reduction or elimination of microorganisms from the root canal system. This treatment has two crucial initial phases: the mechanical phase, which involves instrumentation, and the chemical phase, which involves using antimicrobial solutions as irrigation agents. The third phase of endodontic treatment is the filling of the root canal system, which should be as tightly closed as possible, three-dimensional, and effectively sealed both apically and coronally. The definitive filling of the root canal system should eliminate empty spaces, preserving the status of disinfection after the biomechanical preparation and reducing potential risks of re-infection.

In endodontic treatment, considerable effort should be made to remove existing microorganisms from the root canal; however, it is equally important to prevent other microorganisms from entering during or after the treatment.

Because filling a root canal is an important step in endodontic treatment to avoid infection or re-infection, maintaining an aseptic environment is one of the key concerns of the endodontist, and it is essential in the success of root canal therapy.

Gutta-percha cones, widely used in the filling of the root canals, can be contaminated by microorganisms when exposed to the environment. Caution must be taken during this procedure to prevent contamination of filling materials and to avoid root canal contamination (3, 4, 5, 6).

Gutta-percha cones are composed of organic (gutta-percha polymers and resins) and inorganic (zinc oxide and barium sulphate) components (7).

There is no consensus on the need for decontamination of gutta-percha cones; some believe that it may be unnecessary due to the antimicrobial

properties of the material of the cones (8) and/or the antimicrobial properties of the sealer, which is generally in contact with the cones in the filling. However, sterilization or disinfection of the cones before root filling is recommended by most authors (5, 6, 9-11).

Several methods have been proposed for the rapid decontamination of gutta-percha cones. Such methods include decontamination with the following chemical agents: iodine-alcohol solutions, sodium hypochlorite, chlorhexidine, hydrogen peroxide and glutaraldehyde (5, 9-13). However, there is no agreement among authors on which is the best agent. In Portugal, an iodine-alcohol solution is commonly used for rapid decontamination of gutta-percha cones before insertion into root canals.

The aim of this *in vitro* study was to evaluate the antimicrobial effect of several chemical agents on gutta-percha cones contaminated with different microorganisms.

MATERIAL AND METHODS

Gutta-percha cones

The gutta-percha cones were cones n°40 (Zipperer®) sterilized by ethylene oxide.

Contamination

Four types of bacteria from the American Type Culture Collection (ATCC) (*Staphylococcus aureus* 25923, *Enterococcus faecalis* 29212, *Escherichia coli* 35218, *Klebsiella pneumoniae* 12657) and yeast (*Candida albicans* 10231) were cultivated in 5 ml of Brain Heart Infusion (BHI) (Difco®) and incubated aerobically at 35±1°C for 24 hours.

To assure purity, subcultures in blood-agar medium (Difco®) were created. From these subcultures, microbial suspensions were prepared

in BHI, with the density of 0.5 McFarland corresponding to 1.5×10^8 cfu/ml (cfu = colony forming units) for bacteria and 1.5×10^6 cfu/ml for yeast.

Viability was tested by inoculating each suspension in solid medium at $35 \pm 1^\circ\text{C}$ for 24 hours. For the contamination of gutta-percha cones, two cones were immersed in each suspension for 10 minutes and incubated at $35 \pm 1^\circ\text{C}$.

Four antiseptic solutions were evaluated: 2% iodine alcohol (in 80 vol. of alcohol), 3% sodium hypochlorite, 0.2% chlorohexidine and 5% citric acid.

After contamination of the gutta-percha cones, one cone was immersed for five minutes in each of the four antiseptic solutions, while the other cone was transferred to a sterile gauze, which was used as the positive control (cone with contamination without antiseptic treatment).

Measurement of colony forming units

The gutta-percha cones were cultured in blood-agar plates (Maki technique) and incubated at 35°C for 24 hours. The number of colony forming units (cfu) of the gutta-percha control cones were compared with those obtained from the treated cones immersed in the different antiseptics. All experiments were repeated at least twice.

Statistics

Paired-t tests were used to evaluate for the difference between the numbers of colony forming units in the antiseptic-treated cones and in the positive control.

RESULTS

The contaminated cones showed steady microbial growth, and these were used as positive controls for the comparison of microbial growth inhibition of the different test solutions.

After incubation with iodine alcohol, none of the microorganisms grew, except for *C. albicans* (Table 1).

TABLE 1 - Percentage of growth inhibition regarding the different antiseptics used on gutta-percha cones decontamination in relation to the different microorganisms tested (medium values of two experiments)

Micro-organisms	2% Iodine alcohol	3% Sodium hypochlorite	0,2% Chlorohexidine	5% Citric acid
<i>S. aureus</i>	100%	0%	0%	0%
<i>E. faecalis</i>	100%	1,7%	0%	10%
<i>K. pneumoniae</i>	100%	10%	0%	1,3%
<i>E. coli</i>	100%	3,3%	0%	6.7%
<i>C. albicans</i>	0%	100%	0%	0%

Sodium hypochlorite at 3% was the only agent that was effective on *C. albicans*, but it did not show any activity on *S. aureus*. In relation to the other bacteria, significant growth reductions were observed ($p < 0.05$) (Table 1), and the reductions were greater for *E. faecalis* (Figure 1) and *E. coli* than for *K. Pneumoniae*.

Chlorohexidine at 0.2% was not effective on any of the microorganisms studied (Table 1 and Figure 1).

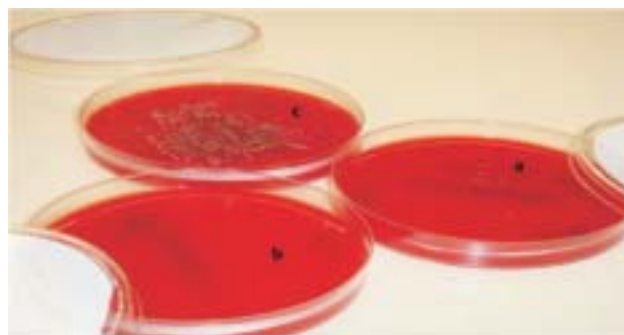


FIGURE 1 - Blood-agar cultures of gutta-percha cones contaminated with *E. faecalis* after incubation with a) 3% sodium hypochlorite; b) 2% iodine alcohol and c) 0.2% chlorohexidine

Citric acid did not show any efficacy on *C. albicans* and *S. aureus*. It significantly reduced the growth ($p < 0.05$) of other microorganisms, but it did so to a greater extent in relation to gram negative bacilli and to a lesser extent in relation to *E. faecalis* (Table 1).

DISCUSSION

Gutta-percha cones were easily contaminated by the different microorganisms and needed disinfection, despite the fact that some authors have said that there was no need to use disinfectant solutions on cones (8). Cones contaminated with well-defined microbial populations were sterilized in different antimicrobial solutions for comparison. The thermoplastic characteristic of gutta-percha cones prohibits sterilization by standard autoclave, which may cause deformation of cones. Therefore, the cones were sterilized with ethylene oxide. Several other chemical solutions have been proposed for a rapid decontamination of gutta-percha cones. The time window to apply these solutions for disinfection ranges from a few seconds to several minutes.

Our results with iodine alcohol are in accordance with those of the study by Montgomery (14), who observed a rapid bactericidal activity with an iodine solution (10% PVP-I) after 1 minute. Our study showed that the iodine solution was not sporicidal even after 15 minutes. Using a 2% alcohol iodine solution in 80 vol. of alcohol, we were able to eliminate all the microorganisms tested, after five minutes of immersion with the antiseptic, except for *C. albicans*.

Because the iodine solution did not have any effect on cones contaminated with *C. albicans*, we tested other antiseptics commonly used in endodontics as irrigant solutions and for cone decontamination.

One of the products tested was sodium hypochlorite (NaOCl). Sodium hypochlorite solution, at varying concentrations, is one of the most widely used endodontic solutions (either as an irrigant or for the decontamination of rubber dams and cones). Its antimicrobial activity is related to the concentration used and the exposure time (6, 10, 12). Gomes et al. (6) found that the time required for sodium hypochlorite to eliminate the microorganisms was inversely proportional to its concentration. The sodium hypochlorite solution at 5.25%, for example, eliminated *C. albicans* and *E. faecalis* after 45 seconds of contact, while at 2.5%, ten minutes of contact was needed to eliminate these microorganisms. Cardoso et al. (13) found that a 5-minute immersion in sodium hypochlorite at 1% was bactericidal and sporicidal (the microorganisms tested were *S. aureus*, *E. coli*, *E. faecalis*, and spores of *B. subtilis*).

In the present study, 3% sodium hypochlorite solution was able to eliminate *C. albicans* but not *S. aureus* after five minutes of exposure. It also showed decreased effectiveness towards *E. faecalis*, *E. coli* and *K. pneumoniae*. However, Souza et al. (15) reported that sodium hypochlorite at 5.25% can decontaminate cones after 15 and 45 seconds of exposure for the microorganisms such as *E. faecalis*, *S. aureus*, *C. albicans*, *B. subtilis* spores and *S. mutans*. Ozalp et al. (11) found that NaOCl at 2.5% was effective in destroying spores of *B. subtilis* on contaminated cones after five minutes of immersion, while Gomes et al. (6) were able to eliminate these spores after ten minutes with hypochlorite at 2.5% and after one minute with NaOCl at 5.25%.

Cardoso et al. (12) reported that the solutions of hypochlorite at 0.25% and 1% had bactericidal and sporicidal activity (against *S. aureus*, *E. coli*, and spores of *B. subtilis*) on contaminated cones after one minute of exposure. At the concentration of 0.25%, the solution of NaOCl was effective in destroying spores on contaminated cones after they were exposed for 5 minutes.

Using "Resilon" cones contaminated with *E. faecalis* and *C. albicans*, Dumani et al. (16) found that sodium hypochlorite at 1% and 5% were effective on both microorganisms, after one and five minutes for each concentration. Nevertheless, it might be difficult to compare the results with gutta-percha and "Resilon" cones. Although these two cones may have similar properties (5, 17), there are differences in their surface texture. In addition, gutta-percha cones have some antimicrobial properties, while Resilon cones do not (18).

According to Gomes et al. (6), the use of lower concentrations of sodium hypochlorite is not recommended for gutta-percha cone disinfection, because they require longer times to kill microorganisms. There are agents that are effective only after a long period of exposure, and this property may make them not practical to use in the endodontic clinic (5, 6, 9, 12).

However, there is evidence that exposure for 1 minute to a 5.25% sodium hypochlorite solution may deteriorate the gutta-percha cones by increasing their elasticity. Also, sodium hypochlorite at concentrations of 2.5% and 5.25% may cause topographic changes in the gutta-percha after five minutes of exposure (19). In this research, only NaOCl at 0.5% proved to be a safe alternative for the decontamination of gutta-percha cones. These

results showed that the chemicals, depending on their composition, concentration or the time of exposure, can alter the physical properties of gutta-percha or other materials (19, 20), and this may influence the quality of the filling of the root canal.

It is also reported that after immersing the gutta-percha cones for one minute in sodium hypochlorite at 2.5% and 5%, crystals of sodium hypochlorite may precipitate and remain on the surface. Unless properly removed, these crystals can impact the quality of the filling eventually (21, 22).

Nowadays, there is increasing interest in the antimicrobial activity of chlorhexidine (CHX) in endodontic practice. It has been used in endodontics either as an irrigant solution or as an intracanal medication (23, 24). This agent has inhibitory effects on bacteria and yeasts that are commonly found in endodontic infections (25). Thus, it is possible that it could also be effective in the decontamination of the gutta-percha cones. In a study by Dumani et al. (16) evaluating liquid chlorhexidine at 2%, only after 5 minutes were all the contaminated cones disinfected (after one minute of treatment with chlorhexidine at 2%, three of the seven cones contaminated with *E. faecalis* and one of the seven cones contaminated with *C. albicans* were not disinfected). On the other hand, Gomes et al. (6) showed that liquid chlorhexidine at 0.2%, 1% and 2% eliminated *C. albicans* in 15 seconds. They also showed that it eliminated *E. faecalis* after 30 seconds with CHX at 0.2% and after 15 seconds with CHX at 1% and 2%. In the study by Cardoso et al. (13), CHX at 2%, after one minute of exposure, was the most effective in terms of bactericidal and sporicidal action. Also, Pang et al. (22) found that CHX at 2% was a quick and effective disinfectant for the cones contaminated with *Staphylococcus spp.* In our study, a solution of chlorhexidine at 0.2% was not effective on any of the microorganisms after five minutes. Regarding chlorhexidine at 2%, topographical changes have been described in "Resilon" cones after a five-minute exposure (20).

Studies have shown that there are many disinfectants that can quickly sterilize gutta-percha cones (22), but more research is needed to determine the clinical relevance of the changes induced in their physical properties.

Citric acid at 5% is a chemical agent used in endodontics to remove the smear layer produced on the surface of root canals by endodontic instruments (26). The anti-bacterial action of citric acid has been indirect, as it helps to remove a possibly contaminated smear layer and it allows the

dental tubules to become more permeable to irrigation solutions or disinfectants products placed between sessions (27). Some studies (10) have shown some direct antimicrobial activity of citric acid on some of the most common microorganisms involved in the endodontic pathology, and they have suggested that it might be useful for use in the clinic for these direct effects. In our study, a 5-minutes exposure to citric acid at 5% did show some direct antimicrobial activity on all microorganisms, except for *C. albicans* and *S. aureus*.

Although gutta-percha cones are usually sterile during storage, they can be easily contaminated if incorrectly handled. Gomes et al. (6) showed that 100% of the cones handled with gloves had microbial growth. The microorganisms most frequently isolated were normal inhabitants of the human skin, such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Propionibacterium acnes*, *Lactobacillus spp.* and *Micrococcus spp.*, and mouth microorganisms such as *Streptococcus salivarius*. Other studies confirmed these findings (22). In endodontic practice, the microorganisms that contaminate gutta-percha cones during handling consist mainly of vegetative bacterial cells rather than resistant bacterial spores. Thus, the antimicrobial activity of these agents will likely be greater in practice than in the experimental settings of these studies.

Further studies with various microorganisms can be helpful for the study of recurrent infections. In this study, we included *Enterococcus faecalis* and *Candida albicans*, which are both highly resistant to the standard local root canal medicaments and frequently associated with therapy-resistant apical periodontitis (28-32).

Recent electron microscopy studies (33) on the topography of the apical portion of the gutta-percha cones showed morphological irregularities. The frequent presence of these irregularities may represent an increased risk of failure of the filling. Moreover, the sizes of these irregularities are similar to the sizes of the protein and other bacterial products, and thus, this stresses the importance of disinfection of gutta-percha cones before their introduction into the root canal.

CONCLUSION

Under the experimental conditions of the study, none of the solutions studied showed antimicrobial efficacy on all of the tested

microorganisms. Although the 2% iodine alcohol showed excellent antibacterial activity, it was not active against *C. albicans*. The solution of 0.2% chlorohexidine did not show antimicrobial activity on any of the tested microorganisms. The solution of sodium hypochlorite at 3% and citric acid at 5% revealed moderate antibacterial activity, and the 3% sodium hypochlorite solution was the only solution effective against *C. albicans*.

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