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ARQUIVOS EM ODONTOLOGIA

Effectiveness of disinfecting substances of gutta-percha cones

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Aim: To demonstrate the effectiveness of disinfecting substances with 2% and 5% Sodium Hypochlorite and 2% Chlorhexidine Gluconate at each of the pre-established times of 0:15 and 0:30 seconds, and 1, 2, 5, and 10 minutes.

Methods: This study selected 96 gutta-percha cones that were contaminated with Enterococcus Faecalis, dried and treated with the aforementioned substances and applied at pre-established times. Subsequently, these were transferred to sterile Brain Heart Infusion broth and placed in a bacteriological incubator at 37°C for 24 hours to evaluate microbial growth, as well as in a nutrient agar medium in Petri dishes. Half of the cone was transferred to individual filter paper packages and exposed to the environment in a dental clinic at Universidade José do Rosário Velano, for 7 days, with subsequent evaluation for microbial growth. The bacterial phenotype test was performed using Gram stain and growth in 6.5% saline solution. The results were submitted to statistical analysis using the Kruskal Wallis H test, with a significance level of 5%.

Results: The substances were effective at all times tested and individual storage supported disinfection. In the statistics test, the p-value was greater than 0.05, as there was no variability in the data configurations.

Conclusion: The disinfection of gutta-percha cones and individual storage was an effective protocol to be adopted with 2% and 5% Sodium Hypochlorite and 2% Chlorhexidine.

Unitems: Disinfection. Endodontics. Root canal obturation. Gutta-percha.

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INTRODUCTION

One of the goals of the endodontic treatment is the elimination or reduction of microorganisms from the root canal and periapical tissues. For this, the chemical mechanical preparation needs to be effective and the filling of the canal systems promotes adequate sealing in root canals[1-5].

Gutta-percha cones are solid materials used to fill the root canal system. They are biocompatible and do not cause dental alterations and interference in the repair process. They are also dimensionally stable, radiopaque, thermoplasticizable, and easily removed when necessary[6]. However, a gutta-percha cone has thermolabile characteristics and, as a result, cannot be sterilized by conventional methods. Despite being produced under aseptic conditions and having antimicrobial activity due to Zinc Oxide, which is present as one of its components, these materials can be contaminated by aerosols and physical aspects during storage and handling[7-9]. Therefore, disinfection of these cones is extremely important and may be carried out by biomechanical preparation with

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chemical substances. Such a fact prioritizes the maintenance of the aseptic chain during endodontic filling – a step that concludes the treatment in root canal systems[10,11].

The present study aimed to demonstrate the effectiveness of the disinfecting substances with 2% and 5% Sodium Hypochlorite and 2% Chlorhexidine gluconate, both at pre-established times of 0:15 and 0:30 seconds, and 1, 2, 5, and 10 minutes.

MATERIAL AND METHODS

The experimental part of this study was conducted at the Laboratory of Clinical Analysis at José do Rosário Vellano University, Unifenas campus in Varginha, MG, Brazil. To perform the study, the researchers used gloves, masks, and sterile instruments in accordance with biosafety standards.

As for the laboratory experiment, a BHI (Brain Heart Infusion) enrichment broth was produced by its contamination with Enterococcus faecalis ATCC 0012 (Newprov®, Pinhais, PR, Brazil), and a solution was produced using a sterile bacteriological loop, according to the McFarland 0.5 turbidity standard, in which the cones were immersed. This study used 96 units of the gutta-percha cone (Dentsply Maillefer®, Pirassununga, SP, Brazil). The experiment was performed in duplicate. The cone boxes were sealed and underwent asepsis with 96% alcohol before being opened. The cones were removed from the packaging using sterile tweezers, close to the Bunsen burner, and dipped for one minute in part of the E. faecalis ATCC 0012 solution produced by the contamination of the BHI broth. After the indicated time, each cone was transferred to a sterile Petri dish and was allowed to dry for 1 minute and was then transferred to a sterile beaker containing some of the disinfecting substances used for different times, as shown in Table 1.

Table 1. Proposal of disinfectant action treatment strategies categorized into groups (disinfectant versus established time).

	Group 1	Group 2	Group 3	Group 4
	Distilled Water	2% NaOCl	5% NaOCl	2% Chlorhexidine
A	15 sec.	15 sec.	15 sec.	15 sec.
B	30 sec.	30 sec.	30 sec.	30 sec.
C	1 min.	1 min.	1 min.	1 min.
D	2 min.	2 min.	2 min.	2 min.
E	5 min.	5 min.	5 min.	5 min.
F	10 min.	10 min.	10 min.	10 min.

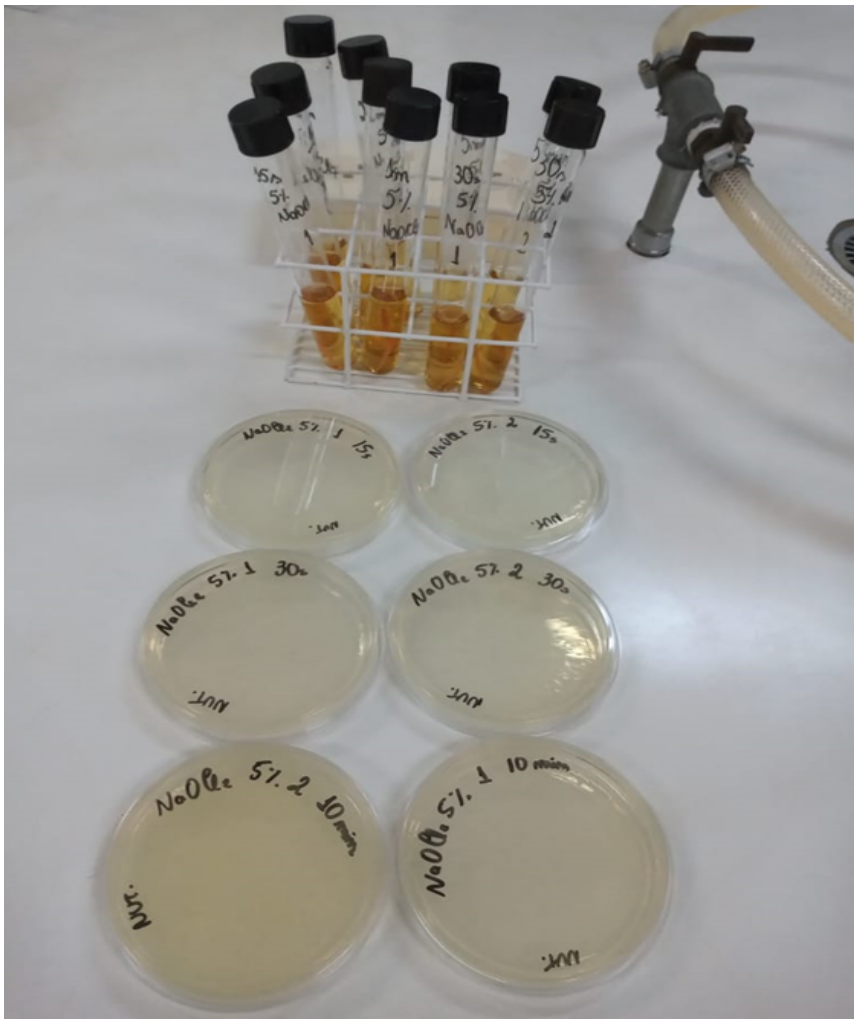
Source: the author.

After the treatment, the cones were placed on filter paper, identified with the proposed treatment for it. Afterwards, the cones were sealed with the filter paper and kept at room temperature for drying. In the next step, the treated cones were divided into two groups: Group A – cones evaluated for possible immediate disinfection – and Group B – cones exposed at the dental clinic for one week. Group B was later evaluated regarding the maintenance of probability of disinfection. The cones evaluated for the possible presence of E. faecalis ATCC 0012 after treatment/storage had the filter paper seal removed and were submerged in sterile BHI enrichment broth in a glass tube with a lid. These were then incubated in a bacteriological incubator at 37°C for 48 hours. The cones that were contaminated with E. faecalis ATCC 0012 and not submitted to the proposed treatments served as a positive control of post-incubation microbial growth. After the incubation period, the turbidity of the enrichment broth indicated microbial growth. The growth estimate was prepared by counting the colonies grown (CFU/mL) from the sowing of a 10µL aliquot part of the broth on nutrient agar medium in a dish, followed by incubation at 37°C for 24 hours. Confirmation of Enterococcus faecalis ATCC 0012 as a growth agent was made by staining, using the Gram method, analyzing a single drop of the broth in which each cone was kept and then the phenotype growth test in 6.5% NaCl broth was performed.

Figure 1. Cones deposited on sterile filter paper identified with the suggested treatment.

Source: the author.

Figure 2. Cones immersed in test tubes containing sterile BHI broth and Petri dishes containing nutrient agar medium to verify microbial growth.



Source: the author.

The statistical Kruskal-Wallis H test was applied from the data collected using the SPSS computer package (SPSS, Chicago, IL, USA) and considering the 5% significance level.

RESULTS

After incubation in a bacteriological incubator, groups 2, 3, and 4 showed no evidence of bacterial growth – represented by the turbidity in the test tubes. However, in group 1, due to the lack of disinfectant potential of the distilled water, turbidity occurred at all established times.

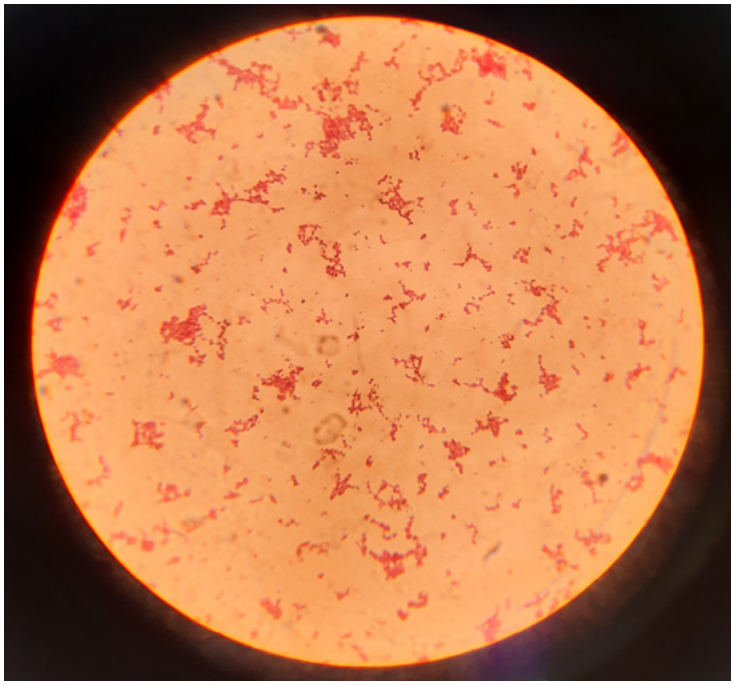
Inoculation of the broth from the evaluated tubes in Petri dishes containing nutrient agar revealed microbial growth only for group 1. Groups 2, 3, and 4 showed no proliferation of microorganisms, confirming the result achieved through the test tubes and the effectiveness of the tested substances. Therefore, the null hypothesis proposed by this study was upheld.

The positive control group revealed microbial growth in both the tubes and Petri dishes, verifying the presence of contaminating microorganisms in the broth. In the analysis of the negative control group, microbial growth was absent, displaying that the cones were neither contaminated in the factory nor during experimental handling.

In the evaluated Petri dishes, the presence of isolated colony growth was not demonstrated, which made it impossible to perform a quantitative analysis of the microbial growth.

In the phenotype test, using the Gram method with subsequent microscopic analysis, it was possible to verify the presence of Gram-positive cocci, a characteristic of Enterococcus faecalis. Hence, after the analysis of growth in a 6.5% saline solution, the presence of the microorganism was demonstrated, complementing the Gram method. A proliferation of the microorganism was observed in all of the tubes, which suggests that the cones were not secondarily contaminated during the experiment.

Figure 3. Microscopic image depicting gram-positive cocci.



Source: the author.

The evaluation of the maintenance of disinfection demonstrated no presence of microbial growth in all evaluated cones, which supports the effectiveness of the proposed storage protocol.

In the Kruskal-Wallis H test, p-values were greater than 0.05 ($p > 0.05$). Due to the lack of data variability, the alternative hypothesis was ruled out, and the null hypothesis was accepted.

DISCUSSION

Enterococcus faecalis is the main pathogen responsible for endodontic failure and is highly resistant to disinfectant solutions. These microorganisms can adapt to unfavorable conditions when exposed to stressful situations[12,13]. As the gutta-percha cones are the main filling materials and are in constant

contact with the periapex, it is crucial that the cones be free of microorganisms in order for the endodontic treatment to be satisfactory[14,15].

Some authors[10,16], when studying gutta-percha cones immediately after they were removed, with sterile clinical tweezers from their respective sealed packages, observed that these materials may be contaminated from the factory. However, in the present study, no microbial growth was observed during the analysis of cones that were immediately removed from the sealed package. Such results are compatible with those noticed in similar studies[8,15].

When analyzing the disinfection potential of sodium hypochlorite, the author[7] concluded that sodium hypochlorite at a concentration of 5.25% was extremely effective in disinfecting the cones from the experiment with a 30-second immersion. Such findings meet the results found in the present work, since the time of 15 seconds was enough to effectively decontaminate all the tested cones, but at a concentration of 5%. In other works, researchers[18,19] also obtained differing results for the same concentration, since the time of one minute was not enough for an effective disinfection. The same results were found in the present study, during the experiment using 2% sodium hypochlorite. Nevertheless, a previous work[20] showed that decontamination only took place within one minute.

In the present work, the tests performed with 2% chlorhexidine also indicated satisfactory decontamination within 15 seconds in all tested cones. Such findings are contrary to those found in the literature, whose results showed disinfection only within one minute[18,20]. However, some authors have shown that the time of 30 seconds is sufficient[21], while other studies have shown that 2% chlorhexidine is only effective within 10 minutes[14].

In the practice of dental care, pathogenic microorganisms from saliva and other fluids may be suspended in the air for a long period of time through the aerosols produced during the procedures[22-24]. Therefore, the maintenance of decontamination was evaluated by storing the cones treated with the above-mentioned substances, wrapped in individual packages in an environment with a high potential for contamination. Nonetheless, the subsequent analysis showed no microbial growth in any treated cone, showing the effectiveness of disinfecting substances and storage of cones in individual packages, which supports findings in the literature stating that this type of storage could prevent the contamination of these materials by handling or other features, thereby ensuring the safety of the procedure[15].

The bacterial phenotype test, carried out through the analysis of microbial growth in a 6.5% saline solution as a complement to the Gram method used to identify Enterococcus faecalis, demonstrates conclusions which had already been reported in the literature, stating that this microorganism has advantages over other species, as it is able to tolerate and adapt to harsh environmental conditions[12,13].

CONCLUSION

The Gutta-percha cones must be disinfected routinely, and 2% and 5% sodium hypochlorite and 2% chlorhexidine are effective substances for this purpose after 15 seconds.

The analyzed cones were industrially produced and packaged in aseptic conditions. However, there are different brands of gutta-percha cones that have different manufacturing methods and that can have a strong impact on the control of the manufacturing environment until reaching the final consumer (e.g.: cones are rolled by hand and this can contaminate them with bacteria when they arrive from the factory).

The storage of cones in individual sealed packages guarantees the maintenance of decontamination even in environments where there is a large generation of aerosols, and can thus be considered an effective protocol for the maintenance and storage of these materials in the dental office environment.

CONFLICTS OF INTEREST

None.

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Efetividade de agentes desinfectantes de cones de guta percha

Objetivo: Demonstrar a eficácia de substâncias desinfetantes, Hipoclorito de Sódio a 2% e 5% e Gluconato de Clorexidina 2% em cada um dos tempos pré-estabelecidos de 15 e 30 segundos, e 1,2, 5 e 10 minutos.

Métodos: Este estudo selecionou 96 cones de guta-percha,contaminados com Enterococcus Faecalis, secos e tratados com as substâncias citadas e aplicadas em tempos pré-estabelecidos. Posteriormente, estes foram transferidos para tubos contendo caldo Infusão Cérebro Coração estéril e colocados estufa bacteriológica a 37°C por 24 horas para avaliar o crescimento microbiano, também verificado em meio ágar nutriente em Placas de Petri. Metade dos cones foram transferidos para embalagens individuais de papel de filtro, e expostas ao ambiente da clínica odontológica da Universidade José do Rosário Velano por 7 dias, com posterior avaliação do crescimento microbiano. O teste do fenótipo bacteriano foi realizado pela coloração de Gram e crescimento em solução salina a 6,5%. Os resultados foram submetidos à análise estatística por meio do Teste H de Kruskal Wallis, com nível de significância de 5%.

Resultados: As substâncias foram eficazes em todos os tempos testados e o armazenamento individual favoreceu a desinfecção. No teste estatístico, o valor de p foi maior que 0,05, pois não houve variabilidade nas configurações dos dados.

Conclusão: A desinfecção dos cones com Hipoclorito de Sódio 2% e 5% e Clorexidina 2% a partir de 15 segundos, e o armazenamento individual foram protocolos eficazes para serem adotados.

Palavras-chave: Desinfecção. Endodontia. Obturação do canal radicular. Guta-percha.