

# A Preliminary Study of the Antibacterial Potential of Cetylpyridinium Chloride in Root Canals Infected by *E. faecalis*

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The aim of this preliminary study was to verify the antibacterial potential of cetylpyridinium chloride (CPC) in root canals infected by *Enterococcus faecalis*. Forty human maxillary anterior teeth were prepared and inoculated with *E. faecalis* for 60 days. The teeth were randomly assigned to the following groups: 1: Root canal preparation (RCP) + 0.1% CPC with positive-pressure irrigation (PPI, Conventional, NaviTip®); 2: RCP + 0.2% CPC PPI; 3: RCP + 2.5% NaOCl PPI; 4: RCP + 2.5% NaOCl with negative-pressure irrigation system (NPI, EndoVac®); 5: Positive control; and 6: Negative control. Four teeth of each experimental group were evaluated by culture and 4 by scanning electron microscopy (SEM). In all teeth, the root canals were dried and filled with 17% EDTA (pH 7.2) for 3 min for smear layer removal. Samples from the infected root canals were collected and immersed in 7 mL of Letheen Broth (LB), followed by incubation at 37°C for 48 h. Bacterial growth was analyzed by turbidity of culture medium and then observed with a UV spectrophotometer. The irrigating solutions were further evaluated for antimicrobial effect by an agar diffusion test. The statistical data were treated by means, standard deviation, Kruskal-Wallis test and analysis of variance. Significance level was set at 5%. The results showed the presence of *E. faecalis* after root canal sanitization. The number of bacteria decreased after the use of CPC. In the agar diffusion test, CPC induced large microbial inhibition zones, similar to 2% chlorhexidine and large than 2.5% NaOCl. In conclusion, cetylpyridinium chloride showed antibacterial potential in endodontic infection with *E. faecalis*.

Key Words: cetylpyridinium chloride, sodium hypochlorite, chlorhexidine, quaternary ammonium, *E. faecalis*.

## INTRODUCTION

Infected root canals are a continuous challenge for different antibacterial strategies. The areas inaccessible to endodontic instruments and irrigants that remain untouched after canal sanitization may be responsible for persistence of the infection (1,2).

The endodontic literature describes several root canal preparation techniques and chemical substances used for endodontic infection control (3-5). In addition, irrigation techniques have also been suggested to allow better action of antibacterial substances in inaccessible regions of the root canal (6-8). Irrigant efficiency is related to the direct contact with the microorganisms

present in the anatomic complex, and may be increased by the frequency and volume of the irrigating solution, the penetration depth of the irrigating cannula, the time of exposure of irrigant and the irrigation protocol (9). Different irrigating solutions have been considered to decrease endodontic infection and contribute to canal sanitization, including: halogenated compounds (sodium hypochlorite - NaOCl), chlorhexidine (CHX), detergents (anionic, cationic), chelating agents (EDTA, citric acid), MTAD, ozonated water, apple vinegar (9). However, NaOCl and CHX are the most often indicated antimicrobial agent for treatment protocols against endodontic and periodontal infections (1,3-5,9).

The quaternary ammonium compounds are cationic

substances with antimicrobial ability, stability and solubility in water. Their cleansing and antibacterial activity is related to positively charged part of the molecule (the cation). The structures of quaternary ammonium compounds are related to ammonium chloride (NH<sub>4</sub>Cl) (10). The cationic environment of the molecule encourages linking with anionic compound at the bacterial surface and is able of altering the cytoplasmic membrane integrity. Once the cytoplasmic membrane is damaged, alteration of the functions involving cytoplasmic membrane permeability may be observed. Inactivation of the enzymes of cytoplasmic membrane brings serious consequences such as protein denaturation (9-11). The ionic detergents (in particular quaternary ammonium compounds) are widely used as surface active agents. Two of these detergents are well known - benzalkonium chloride and cetylpyridinium chloride (CPC) (11).

There is a continuous demand for antimicrobial agents to overcome the deficiencies of mechanical action of endodontic instruments in inaccessible areas. The inhibition of bacterial plaque encouraged several studies concerning CPC. Considering the results of this substance which is part of the formulation of mouthwashes and toothpastes (12-18) and the lack of studies associated with application in endodontic infections, it seems appropriate and necessary to conduct more studies involving its antibacterial properties. Thus, the aim of this preliminary study was to verify the antibacterial potential of CPC in root canals infected by *E. faecalis*.

## MATERIAL AND METHODS

### *Bacteria*

A reference strain of *E. faecalis* (ATCC 29212) obtained from the American Type Culture Collection was inoculated in 7 mL of Brain Heart Infusion broth (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 24 h. The experimental suspension was prepared by cultivating the biological marker on the surface of Brain Heart Infusion agar (BHIA; Difco Laboratories), following the same incubation conditions. The bacterial cells were resuspended in saline to reach a final concentration of about  $3 \times 10^8$  cells/mL adjusted to No. 1 McFarland turbidity standard. The bacterial concentrations before and after the sanitization process were interpreted using a UV spectrophotometer (Model Nova 1600 UV, Piracicaba, SP, Brazil) regulated to read

at  $\lambda=600$  nm, adopting as pattern the No. 1 McFarland scale, which corresponded to the absorbance of 0.137 nm after the zero reading of sterile saline solution.

### *Teeth Preparation*

Forty extracted maxillary central incisors with intact cementum were selected for this study, after approval by the institutional Ethics Committee (Protocol #2012/0303). The teeth were removed from storage in 0.2% thymol and immersed in 5% NaOCl for 30 min to remove organic tissues. Radiographs were taken in buccolingual and proximal directions, using periapical films (Eastman Kodak Co., Rochester, NY, USA) to confirm the presence of a single canal and absence of anatomical variations. Standard access cavities were made. The anatomical diameter was standardized from the initial preparation with BioRace instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland) BR0 #25/0.08, BR1 #15/0.05, BR2 #25/0.04, BR3 #25/0.06, BR4 #35/0.04, BR5 #40/0.04 and BR5C #40/0.02. During root canal preparation (RCP), the canals were irrigated with 3 mL of 2.5% NaOCl at each change of instrument, using Ultradent syringe and a 0.30 mm diameter Navitip<sup>®</sup> needle (Ultradent Products Inc., South Jordan, UT, USA). NaOCl solution was prepared shortly before use (Terapêutica, Goiânia, GO, Brazil). Next, the crowns were removed under continuous air/water spray with a fissure bur (EndoZ; Maillefer, Ballaigues, Switzerland) in a high-speed handpiece at 90° to the long axis of tooth. Tooth length was standardized to 16 mm (from root apex to coronal border). Root canals were dried and filled with 17% EDTA (pH 7.2) for 3 min for smear layer removal. After completion of RCP, the teeth were autoclaved at 120°C for 30 min.

### *Experimental Design*

In the experimental model, a split platform was used during the period of inoculation with the biological marker. The coronal portion of the root canal of each tooth was connected to the cut end of a 1.5 mL polypropylene Eppendorf tube (Cral, São Paulo, SP, Brazil) using a cyanoacrylate adhesive (Super Bonder, Itapevi, SP, Brazil) to prevent leakage at the connection. The tooth-tube connections were entirely coated with two layers of nail polish (Max Factor, Cosmetics and Fragrances, London, UK). The specimens (teeth coupled to the polypropylene tubes) were sterilized in

5% NaOCl for 30 min and then were placed into the culture medium (BHI) and, to ensure disinfection, the test apparatus was incubated at 37°C for 24 h. No growth was observed after this period. Five milliliters of sterile BHI were mixed with 5 mL of the bacterial inoculum, and the experimental and positive control groups were inoculated with *E. faecalis* for 60 days, using sterilized syringes of sufficient volume to fill the root canal. This procedure was repeated every 72 h, always using 24 h pure cultures prepared and adjusted to #1 McFarland turbidity standard. The teeth were maintained in a humid environment at 37°C. After the period of contamination, the root canals were dried and refilled with sterile distilled water. Each sample was collected using three #40 paper points maintained in the canal for 1 min. The points were then transported individually and immersed in 7 mL of Lethen Broth (LB; Difco Laboratories), a medium added with neutralizers [Lecithin, Tween 80 and sodium thiosulfate (P.A.; Art Laboratories, Campinas, SP, Brazil)] in appropriate concentrations, followed by incubation at 37°C for 48 h in a reduced oxygen atmosphere. After confirming bacterial growth, the experimental groups were prepared.

The teeth (n=32) were randomly assigned to 4 experimental and two control groups (n=8), according to the tested irrigating solutions, as follows: 1: RCP + 0.1% CPC (Terapêutica) with conventional positive-pressure irrigation (PPI, NaviTip®); 2: RCP + 0.2% CPC with PPI (NaviTip®); 3: RCP + 2.5% NaOCl with PPI (NaviTip®); 4: RCP + 2.5% NaOCl with negative-pressure irrigation system (NPI, EndoVac®; Discus Dental, Culier City, CA, USA); 5: Positive control; and 6: Negative control (Table 1).

The teeth were prepared with BioRace system (FKG Dentaire, Switzerland), following the sequence BR5C #40/0.02, BR6 #50/0.04, BR7 #60/0.02. Each instrument was used in only 5 canals. The total volume of irrigating solution was calculated for the use of the same volume during the whole experiment. In Groups 1 to 3, the conventional irrigation technique was performed with an Ultradent 5 mL syringe and Navitip® irrigation needle (Ultradent Products Inc.) 0.30 mm gauge placed at 12 mm. The initial irrigation was performed with 5 mL of test solution, using short up-down movements. At every instrument change, the irrigating protocols were repeated with 5 mL of solution. In Group 4, the EndoVac® system was used according to the manufacturer's recommendations.

After RCP, the root canals were dried with sterile

absorbent #60 paper points and filled with 3 mL of 17% EDTA, kept under agitation with a hand instrument for 3 min. A final rinse was made with 5 mL of the irrigating solutions. The negative control group was used to verify the samples' sterility and the positive control group was used to ascertain the bacterial viability during the experiment. Thereby, during 60 days of contamination of the root canals, 4 non-inoculated teeth were kept incubated at 37°C, as an aseptic control and 4 teeth were inoculated with *E. faecalis* under similar atmospheric conditions.

Four teeth of each experimental group were evaluated by culture and 4 by scanning electron microscopy (SEM). Four root canals of each experimental group were dried and then filled with sterile distilled water. All samples were collected using three #40 paper points, kept for 1 min in the root canal. The points were individually transported and immersed in 7 mL of LB (Difco Laboratories), followed by incubation at 37°C for 48 h in a reduced oxygen atmosphere. After 72 h, a new collection was done as described above. Bacterial growth was analyzed by turbidity of the culture medium and then observed using a UV spectrophotometer after 20 min and 72 h. The measurement of culture medium optical density was proportional to the number of present bacteria. Samples were taken at random and cultivated to check the purity of *E. faecalis*, according to an earlier study (19). After the evaluation of changes in the culture medium, an inoculum of 0.1 mL obtained from the medium was transferred to 7 mL of BHI and incubated at 37°C for 48 h. The Gram staining of the BHI culture was used to verify the *E. faecalis* contamination. All collections were carried out under aseptic conditions.

Data were analyzed statistically using the SPSS for Windows 2.0 statistical software (SPSS Inc., Chicago, IL, USA) by means, standard deviation, Kruskal-Wallis test and analysis of variance. Significance level was set at 5%.

### SEM Analysis

After 72 h of the sanitization process, 4 teeth of each group were analyzed by SEM. The teeth were fixed in a buffered formalin solution for 1 week, dehydrated by immersion in ethanol solutions of increasing concentrations (70%, 95% and 100%), with two solution changes each 30 min. In 4 teeth of each group, longitudinal grooves were made along the entire length of each root with a metallic water-refrigerated disk (KG

Sorensen Ind. Com., São Paulo, SP, Brazil) carefully and using a surgical chisel to create a buccolingual split along the long axis to expose the entire extent of the root canal. The teeth were submitted to metallographic preparation for analysis in a scanning electron microscope (JEOL, JSM-6360LV, Tokyo, Japan). Initially, the specimens were analyzed by navigation on the images to observe the bacterial contamination in different magnifications. For the comparative analysis between groups, two SEM micrographs were obtained from each third. The root canal was measured and the central part of each middle third was evaluated. The SEM images were obtained at 1,600 and 5,000 magnifications. The images were then analyzed to identify of presence or absence of contamination and debris on root canal surface. Considering this study as a preliminary essay, SEM analysis aimed to determine an initial parameter for sanitization with the tested substances.

### Agar Diffusion Test

For the agar diffusion test, 5 Petri plates with 20 mL of BHIA were inoculated with 0.1 mL of the bacterial suspension (*E. faecalis*, ATCC 29212), using sterile swabs that were spread on the medium, obtaining growth in junction. Twenty paper disks (9 mm diameter) were immersed in the experimental solutions [0.1% CPC, 0.2% CPC, 2.5% NaOCl, 2.0% CHX (F.G.M., Joinville, SC, Brazil), and sterile distilled water] for 1 min and then 4 paper disks were placed over the BHIA surface in each agar plate. The plates were maintained for 1 h at room temperature and then incubated at 37°C for 48 h. The diameter of microbial inhibition was measured around the paper disks containing the substances. Positive and negative controls were done, maintaining the plates inoculated and without inoculum, for the same periods and under identical incubation conditions. All assays were carried out under aseptic conditions.

## RESULTS

The results showed presence of *E. faecalis* after the sanitization process, irrespective of the irrigating solution. The analyzed mean optical densities in both assessment periods showed significant bacterial reduction and significant

differences when comparing CPC and 2.5% NaOCl ( $p < 0.05$ ) (Table 2). The number of bacteria was reduced after use of CPC. There was no statistically significant differences among 0.1% and 0.2% CPC ( $p > 0.05$ ). SEM images after 60 days of contamination showed root canal colonized with *E. faecalis* (Figs. 1A and 1B). Considering the root canal thirds after RCP plus CPC, it was observed that the coronal and middle thirds were cleaner than the apical third, where there was also some residues of this irrigating substance (Figs. 1C-H and Figs. 2A-F). The results of the agar diffusion test are shown in Table 3. In this experimental test, 2.5% NaOCl and 2% CHX were used because they are reference solutions in studies using irrigating solutions. The results showed similar antibacterial effect to 2% CHX, 0.1% CPC and

Table 1. Distribution of experimental groups.

Groups	Antibacterial strategies	Culture (n=20)	SEM (n=20)
1	RCP + 0.1% CPC (PPI, Convencional, NaviTip®)	4	4
2	RCP + 0.2% CPC (PPI, Convencional, NaviTip®)	4	4
3	RCP + 2.5%NaOCl (PPI, Convencional, NaviTip®)	4	4
4	RCP + 2.5%NaOCl (NPI, EndoVac®)	4	4
5	Positive control	2	2
6	Negative control	2	2

RCP: root canal preparation. NPI: negative-pressure irrigation. PPI: positive-pressure irrigation.

Table 2. Mean optical densities associated with the number of bacteria present.

Groups	20 min	Mean/SD optical density of medium ( $p=0.012$ )	72 h	Mean/SD optical density of medium ( $p=0.013$ )
1	+++	0.009 (0.009) <sup>b</sup>	+++	0.0051 (0.0048) <sup>b</sup>
2	+++	0.002 (0.003) <sup>b</sup>	+++	0.0054 (0.0035) <sup>b</sup>
3	+++	0.030 (0.011) <sup>a,b</sup>	+++	0.098 (0.070) <sup>a</sup>
4	+++	0.071 (0.067) <sup>a</sup>	+++	0.095 (0.023) <sup>a</sup>
5	+++	0.208 (0.064)	+++	0.245 (0.072)
6	---	0.000	---	0.000

+++ : presence of bacteria. --- : absence of bacteria. Different letters indicate statistically significant difference at  $p < 0.05$  (Kruskal-Wallis test).

0.2% CPC and greater than 2.5% NaOCl.

**DISCUSSION**

CPC used in the sanitization process decreased

the number of bacteria in endodontic infection by *E. faecalis*, using both culture and SEM images. In the agar diffusion test, the outcomes indicated CPC had similar antibacterial activity to CHX and greater than

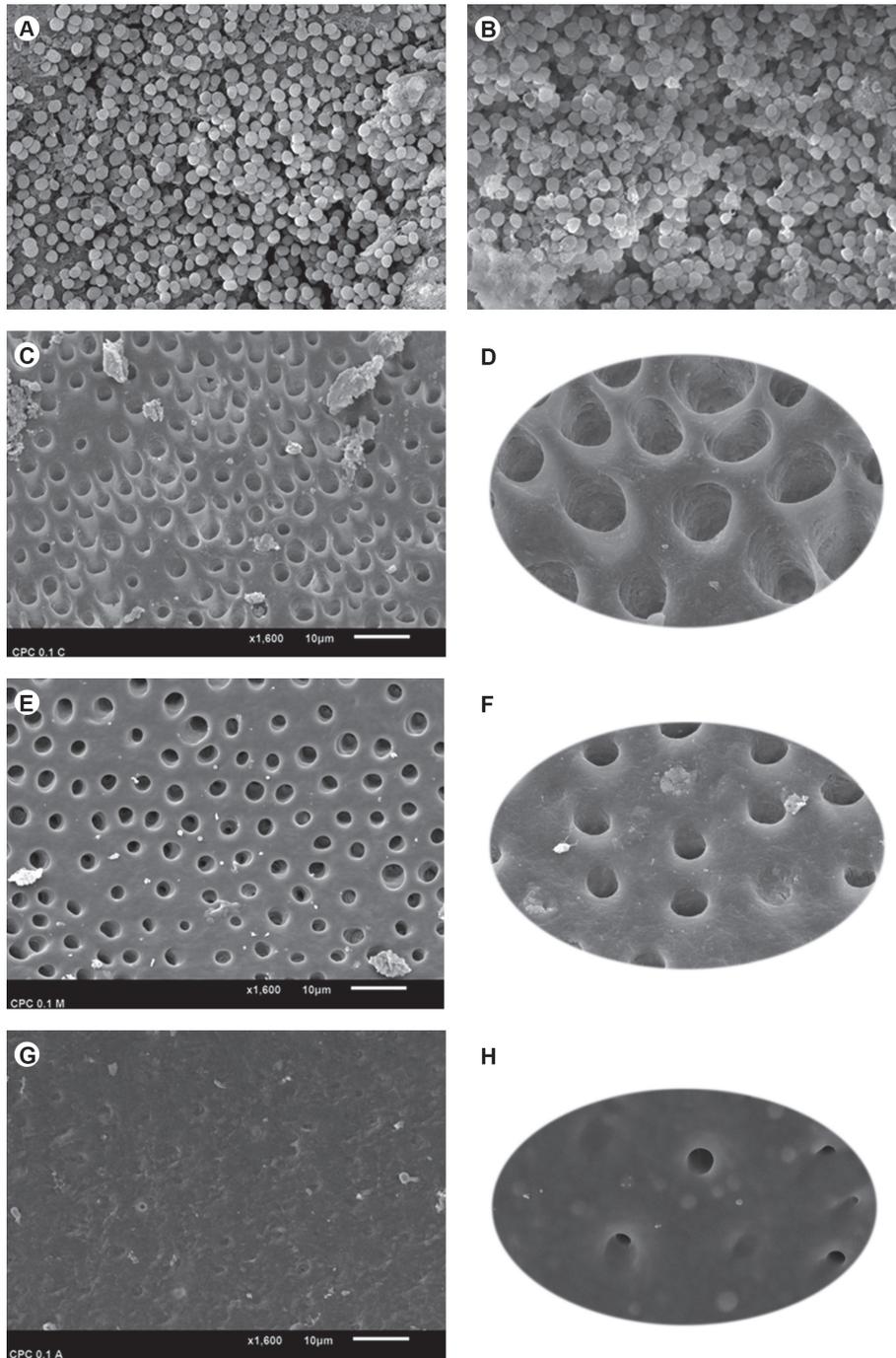


Figure 1. A, B: Positive control, coronal (A) and apical (B) thirds (SEM  $\times 1,600$ ). C, D: 0.1% CPC, coronal third, SEM  $\times 1,600$  (C) and  $\times 5,000$  (D). E, F: 0.1% CPC, middle third, SEM  $\times 1,600$  (E) and  $\times 5,000$  (F). G, H: 0.1% CPC, apical third, SEM  $\times 1,600$  (G) and  $\times 5,000$  (H). Coronal and middle thirds were cleaner than the apical third, where there was also presence of residue of this irrigating substance.

## 2.5% NaOCl.

The endodontic infection and the host response has directed researches to various therapeutic tendencies (1-9). Chemical and mechanical cleaning and shaping significantly reduce the number of microorganisms, but do not eliminate them (9). Although many of them have shown varied degrees of antimicrobial effectiveness, it is difficult to choose the ideal irrigating solution and its concentration. Thus, exterminating the bacteria from infected root canals remains an endodontic challenge. This is the reason for a balance between the aggressive agents (bacteria) and the defense (host cells). The use of irrigating solutions has several purposes

that must always be evaluated before choosing the ideal one: neutralize the components of endodontic infection (inactivate endotoxins), facilitate the action of endodontic instrumentation, promote tissue dissolution, and present good tissue tolerance. However, there are two essential clinical conditions to be considered: the presence or absence of microorganisms (in the inflamed or infected pulp).

NaOCl and CHX have been indicated and demonstrated antibacterial effectiveness (4,5). Some care is required whenever they are used, especially due to the different adverse effects to the oral tissues. The ideal concentration and ideal substance for endodontic

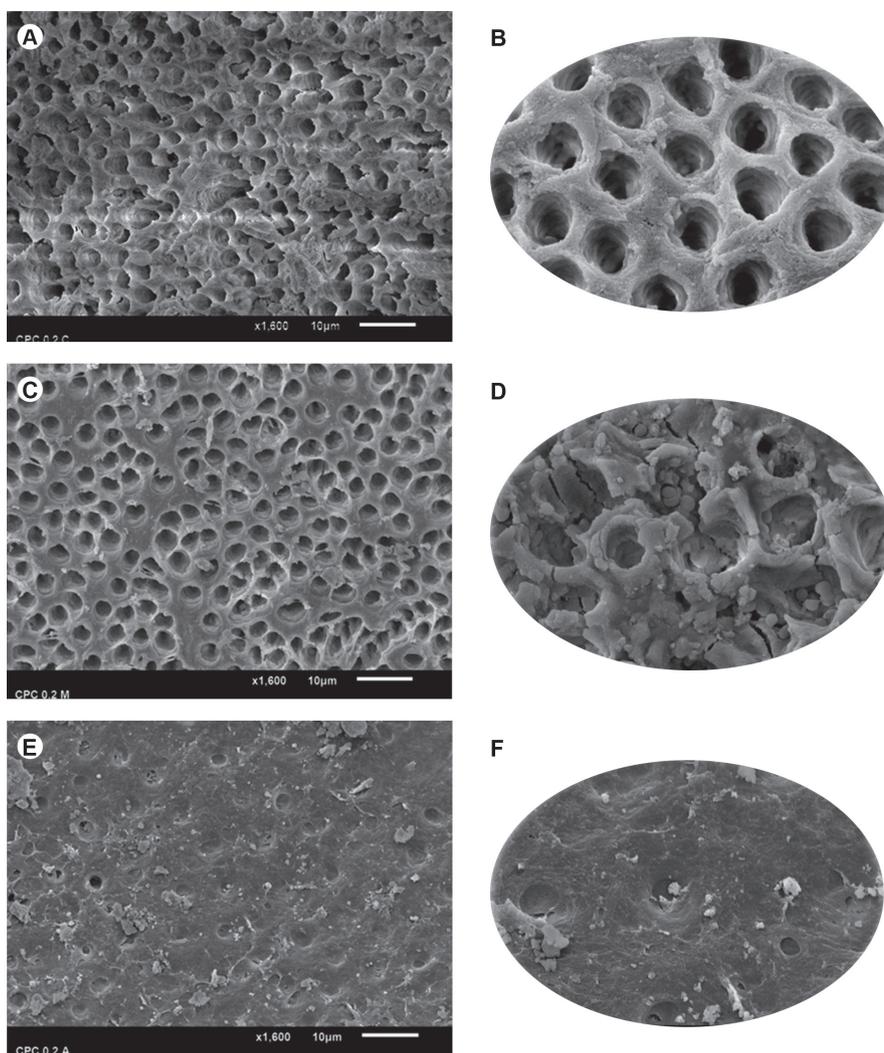


Figure 2. A, B: 0.2% CPC, coronal third, SEM  $\times 1,600$  (A) and  $\times 5,000$  (B). C, D: 0.2% CPC, middle third, SEM  $\times 1,600$  (C) and  $\times 5,000$  (D). E, F: 0.2% CPC, apical third, SEM  $\times 1,600$  (E) and  $\times 5,000$  (F). Coronal and middle thirds were cleaner than the apical third, where there was also presence of residue of this irrigating substance.

infections still require consensus (9). The structure of the CHX molecule poses a systemic risk because it is likely to decompose into reactive byproducts, such as *para*-Chloroaniline (pCA) (20,21). CHX may release pCA and reactive oxygen species (ROS) as function of time, alkaline environment (high pH) and heat. Barbin et al. (22) detected pCA in solutions with low concentrations of CHX (0.2% aqueous solution) 14 days after preparation, but no traces of pCA were found in the paste produced by mixing 0.2% CHX and calcium hydroxide at different time points, although ROS were generated by 0.2% CHX alone and mixed with calcium hydroxide at all time points. In another study (23), pCA and ROS were detected in 2% CHX and in the combination of 2% CHX and calcium hydroxide at all time points, but 1-Chloro-4-nitrobenzene (pCNO) was not found. NaOCl also presents serious inconvenience if it overflows into the periapical or oral tissues (9), apart from its instability. Thus, it is important to study other alternatives to use in endodontic infections. In the present study, specific care has been taken in the use of NaOCl. The solution was prepared shortly before use and in concentration of 2.5%.

Due the concern about the need for alternative irrigating solutions, the study involved one of the quaternary ammonium compounds (CPC, monocationic). The effectiveness of the CPC on plaque was discussed several years ago (10-14,18). Gjermo et al. (14) and Bonesvoll and Gjermo (15) found promising results of CPC on salivary bacteria and plaque inhibition. Busscher et al. (12) compared the effects of three CPC formulations with and without alcohol and Tween 80 on physicochemical properties of salivary pellicles, bacterial detachment *in vitro* and bacterial killing *in vivo*. CPC bioavailability in a formulation without alcohol and Tween80 could be demonstrated by the measures of pellicle surface properties and bacterial interactions *in vitro* as well as bactericidal actions on oral biofilms *in vivo*. Quirynen et al. (17) examined the antibacterial capacity and side effects of a new CHX solution with a lower concentration of 0.05% CHX in combination with 0.05% CPC without ethanol, over a 6-month period.

The data showed that the two CHX-containing mouth rinses improved the supragingival plaque control and had an additional beneficial effect on both the degree of gingival inflammation and the microbial load within the oral cavity. The new CHX 0.05% + CPC 0.05% solution has an antiplaque effect comparable with that of a 0.2% CHX + alcohol solution, but with less side effects. Alves et al. (18) made a review to understand what has been studied and published in the last 10 years about CPC and if this compound is effective in reducing plaque and controlling the degree of gingival inflammation. A search was done to find meta-analyses and systematic reviews in Pubmed primary basis and Cochrane secondary basis, supplemented with randomized clinical trials published after the last systematic review. The results showed that despite the scientific evidence being still scarce, the use of CPC-containing mouthwash in addition to the mechanical oral hygiene appears to provide a small but significant benefit in reducing plaque and gingival inflammation when compared to brushing or brushing followed by rinsing with placebo.

The method to determine the contamination conditions before and after the antibacterial strategies by turbidity of the culture medium using a UV spectrophotometer suggested approximate information of bacteria recovered after irrigation protocols (19). This was observed by SEM images with high magnification of the root canal colonized with *E. faecalis* (Figs. 1A and 1B). Considering the root canal thirds after RCP plus CPC, it could be observed that the coronal and middle thirds were cleaner than the apical third, and there was also some residue of this irrigating solution (Figs. 1C-1H and Figs. 2A-2F). Microbiological analysis by culture suggested the presence of viable bacteria, which might be in the dentinal tubules and/or ramifications. The period of root canal contamination was 60 days, which is sufficient for the *E. faecalis* to infect the root canal surface and invade the dentinal tubules (9). *E. faecalis* was well discussed in previous studies and showed to be susceptible to intracanal antimicrobials (9,24,25). In the present study, none of the irrigants was able to eradicate the root canal bacteria. These results agree with previous studies (1,3).

Different CPC concentrations have been tested, such as 0.05%, 0.75% and 0.1% (13-18). The concentrations used in the present study were of 0.1%

Table 3. Means of the diameters (in mm) of the inhibition zones by the agar diffusion test.

Bacteria	0.1% CPC	0.2% CPC	2.5% NaOCl	2% CHX	Distilled water
<i>E. faecalis</i>	18	18	14	18	0

CPC: cetylpyridinium chloride. CHX: chlorhexidine.

and 0.2%, with no difference between them with respect to the antibacterial effects. In the agar diffusion test, the results showed similar antibacterial efficacy among 0.1%, 0.2% CPC and 2% CHX and greater than 2.5% NaOCl.

The technique with negative-pressure irrigation uses a high-power suction system that allows irrigation with great volume of irrigant solution. The ability of this system to inject a deeper irrigant flow in the root canal was demonstrated, which favors a better cleaning compared to the conventional irrigation protocol (8). In the present study was used the technique of negative-pressure irrigation, and the results based on the present experimental model, when compared with positive-pressure irrigation, were similar. These results agree with those of a previous study (6). Heilborn et al. (7) compared the efficacy of root canal cleaning and measured the volume of irrigation in the apical third of negative and positive pressure systems in two different exposure moments. The negative pressure system promoted better debris cleaning in the apical third in a shorter exposure period.

Root canal preparation was performed with the instrument BR7 #60/0.02, which allowed better mechanical removal of infected dentin and consequently better chemical action of the irrigant. It is recognized that the mechanical action of instruments is indispensable for biofilm disruption (1,2). Presence of irrigant in canal does not mean that it is sufficient to inactivate all microorganisms. The antimicrobial potential of any chemical substance cannot be reached in plenitude when a medicament does not reach the target microorganism.

However, considering that this experiment represents a preliminary test with a view to offer a new alternative of endodontic irrigant for application in endodontic infections, several investigations should be performed before its clinical recommendations. Some questions involving the physical and chemical characteristics, the ideal concentration for effective antibacterial power, adverse effects (possibility of interactions, degradation and formations of byproducts), and tissue tolerance must first be answered. The extrapolation of *in vitro* data to clinical outcome must always be made with caution. Thus, further researches are essential to offer new guidelines to the irrigation protocol of endodontic infections.

Under the tested laboratory conditions, it may be concluded that CPC showed antibacterial potential in endodontic infection with *E. faecalis*.

## RESUMO

O objetivo deste estudo preliminar foi verificar o potencial antibacteriano de cloreto de cetilpiridínio (CCP) em canais radiculares infectados por *E. faecalis*. Quarenta dentes anteriores de humanos foram preparados e inoculados com *E. faecalis* por 60 dias. Os dentes foram aleatoriamente distribuídos como se segue: 1. Preparo do canal radicular (PCR) + CCP 0,1% com sistema de pressão positiva de irrigação (PPI, convencional, Navitip®); 2. PCR + CPC 0,2% PPI; 3. PCR + NaOCl 2,5% PPI; 4. PCR + NaOCl 2,5% com sistema de pressão negativa de irrigação (PNI, EndoVac®); 5 e 6. Controles positivos e negativos. Quatro dentes de cada grupo experimental foram avaliados por cultura e quatro por microscopia eletrônica de varredura (MEV). Em todos os dentes, os canais foram secos e preenchidos com EDTA 17% (pH 7,2) durante 3 min. As amostras dos canais radiculares infectados foram coletadas e imersas em 7 mL Lethen Broth (LB), seguido de incubação a 37° C durante 48 h. O crescimento bacteriano foi analisado pela turvação do meio de cultura, e mensurados por meio de um espectrofotômetro (UV). As soluções irrigantes foram ainda avaliadas em teste de difusão em ágar. A análise estatística utilizou de média, desvio padrão, teste de Kruskal-Wallis e análise de variância. O nível de significância foi de 5%. Os resultados mostraram a presença de *E. faecalis* posterior ao processo de desinfecção do canal radicular. O cloreto de cetilpiridínio mostrou reduzir o número de bactérias. No teste de difusão em ágar, o CPC determinou inibição microbiana, com resultados semelhantes à CHX a 2% e maiores do que o hipoclorito de sódio a 2,5%. O cloreto de cetilpiridínio demonstrou potencial antibacteriano em infecção endodôntica por *E. faecalis*.

## ACKNOWLEDGEMENTS

This study was supported in part by grants from the National Council for Scientific and Technological Development (CNPq) - grant #306394/2011-1 to CE. The authors deny conflicts of interest related to this study.

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Received August 17, 2012  
Accepted December 4, 2012