In vitro evaluation of the effectiveness of irrigants and intracanal medicaments on microorganisms within root canals

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Abstract

Menezes MM, Valera MC, Jorge AOC, Koga-Ito CY, Camargo CHR, Mancini MNG. *In vitro* evaluation of the effectiveness of irrigants and intracanal medicaments on microorganisms within root canals. *International Endodontic Journal,* **37**, 311–319, 2004.

Aim To evaluate *in vitro* the effectiveness of sodium hypochlorite (NaOCl), chlorhexidine (CHX) and five intracanal medicaments on microorganisms within root canals.

Methodology Ninety-six human single-rooted extracted teeth were used. After removing the crowns, canal preparation was completed and the external root surfaces were coated with epoxy resin. Following sterilization, the teeth were contaminated with *Candida albicans* and *Enterococcus faecalis*, and were incubated at 37 ± 1 °C for 7 days. The teeth were divided according to the irrigant solution or intracanal medicament: group 1, sterile physiologic solution (SPS) and calcium hydroxide ($Ca(OH)_2$) paste; group 2, SPS and camphorated paramonochlorophenol (CPMC); group 3, SPS and tricresol formalin; group 4, SPS and $CaOH_2$ + CPMC paste; group 5, SPS and PMC furacin; group 6, 2.5% NaOCl without intracanal medication; group 7, 2.0% CHX without intracanal medication and group 8, SPS without intracanal medication (control group). Microbiological samples were collected with sterile paper points, and bacterial growth was determined. The data were submitted to the analysis of variance (ANOVA, $P = 0.05$).

Results For *C. albicans*, groups 3 and 8 were statistically less effective than groups 1, 2, 4 and 5 (Kruskal–Wallis (K–W) = 65.241; gl = 7; $P = 0.001$). For *E. faecalis*, groups 6 and 8 were statistically less effective than groups 1–4 and 7 (K–W = 61.048; gl = 7; $P = 0.001$).

Conclusions $Ca(OH)_2$ + CPMC paste was the most effective intracanal medicament for the elimination of the two microorganisms; 2.0% CHX solution was more effective than 2.5% NaOCl against *E. faecalis.*

Keywords: calcium hydroxide, *Candida albicans*, chlorhexidine, *Enterococcus faecalis*, root canal irrigants, sodium hypochlorite.

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Introduction

Microorganisms play a fundamental role in the aetiology of pulp and periapical diseases (Kakehashi *et al.* 1965, Sundqvist 1992), and their control and elimination are important during endodontic treatment (Stevens & Grossman 1983, Gomes *et al.* 1996, Leonardo & Leal 1998, Valera *et al.* 2001).

Studies of the dynamics of root canal infections show that the relative proportions of anaerobic microorganisms and other bacterial cells increase with time, and that the number of facultative anaerobic bacteria increases when root canals remain infected for long periods (Sundqvist 1992, Le Goff *et al.* 1997, Peters *et al.* 2001). The proportional decrease of the facultative bacteria and the concomitant increase of strict anaerobic bacteria with time is because of oxygen consumption and a low oxidation–reduction potential, which

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collaborate to sustain the growth of these bacteria (Sundqvist 1992).

Waltimo *et al.* (1997) analysed the microorganisms within root canals of teeth with periapical lesions, and isolated 48 species of yeasts. The most common species was *Candida albicans*; it was associated with other bacteria in 87% of cases. Peciuliene *et al.* (2001) determined the occurrence and role of yeasts, Gram-negative enteric rods and *Enterococcus* species in filled root canals with chronic apical periodontitis. The authors found *C. albicans* in 18% of the cases, always associated with other bacteria, and in 50% of the cases, associated with *Enterococcus faecalis.* Kubo *et al.* (1997) isolated *C. albicans* in 11.3% of teeth with pulp necrosis.

The elimination of microorganisms from root canals is attempted using irrigating solutions during instrumentation and intracanal medicaments. It is hoped that these treatments will reach canal ramifications and other inaccessible areas (Byström & Sundqvist 1981, Stevens & Grossman 1983, Sundqvist 1992, Gomes *et al.* 1996, Leonardo & Leal 1998). Irrigant solutions in different concentrations with antimicrobial activity have been used during biomechanical instrumentation, particulary sodium hypochlorite (NaOCl). More recently, chlorhexidine (CHX) has been used (Byström & Sundqvist 1983, 1985, Valera *et al.* 2001). Calcium hydroxide ($Ca(OH)_2$) has been used in teeth with pulp necrosis and periapical lesions because of its antibacterial action, mainly against Gram-negative anaerobes (Stevens & Grossman 1983, Byström *et al.* 1985, Sjögren *et al.* 1991, Siqueira Junior *et al.* 1997, Valera *et al.* 2001). However, some microorganisms, especially *E. faecalis*, are resistant to $Ca(OH)_2$ therapy (Han *et al.* 2001, Evans *et al.* 2002, Sukawat & Srisuwan 2002), justifying the combination of this medicament with other antimicrobial medicaments, such as camphorated paramonochlorophenol (CPMC), which complements $Ca(OH)_2$ activity (Stevens & Grossman 1983, Behnen *et al.* 2001, Han *et al.* 2001, Sukawat & Srisuwan 2002).

This study aimed to evaluate the *in vitro* antimicrobial effectiveness of intracanal irrigants and medicaments used in endodontic therapy on *C. albicans* and *E. faecalis*.

Materials and methods

Selection and standardization of teeth

Ninety-six freshly extracted single-rooted human teeth, which were stored in 10% formalin for 24 h for disinfection and organic tissue fixation, were used. After this procedure, the teeth were cleaned, the crowns were sectioned with a diamond disk (Carbodent-Gysi S.A., Buenos Aires, Argentina) and the root lengths were standardized to approximately 16 mm. They were then stored in physiologic saline solution until use.

Initial preparation of root canals

Root canals were instrumented 0.5 mm beyond the apical foramen up to size 25 endodontic instruments (Dentsply Maillefer, Ballaigues, Switzerland); roots having apical diameters greater than a size 25 K-file were discarded.

Root canals were then instrumented 1 mm short of the root apex up to size 30 or size 50 K-files, depending on the experimental group. Irrigation was performed with 2 mL physiologic saline solution. The root surfaces were then coated with an epoxy resin (Araldite; Brascola LTDA, Taboão da Serra, SP, Brazil), except the cervical access and apical foramen.

After setting of the epoxy resin, the root canals were filled with 17% EDTA (Byofórmula Drugstore, São José dos Campos, SP, Brazil) for 3 min in order to remove the smear layer. This was followed by a 5 mL of physiologic saline solution. Subsequently, the roots were sterilized on autoclave at 121 °C for 20 min.

Cultivation of the microorganisms

The following procedures were performed inside a laminar flux chamber (Veco, Piracicaba, Brazil) using sterilized instruments and materials.

Candida albicans (F-72) and *E. faecalis* (ATCC 29212) were previously cultivated in Sabouraud Dextrose (SDA) broth (Difco, Detroit, USA) or Tryptic Soy broth (Difco), respectively, for 48 h, and then cultured in SDA or *Mitis salivarius* (MS) agar plates. SDA plates with *C. albicans* were incubated at 37 ± 1 °C, and MS plates with *E. faecalis* were incubated in an anaerobic chamber at 37 ± 1 °C for 48 h.

Suspensions of *C. albicans* and *E. faecalis* had the optical density adjusted spectrophotometrically to approximately 1.5×10^8 colony-forming units (cfu) mL^{-1}. Then, 1 mL of *C. albicans* suspension and 1 mL of *E. faecalis* suspension were collected in a single tube containing approximately 1.5×10^8 of each microorganism.

Root apices were sealed with a temporary cement (Cimpat, Septodont, Saint-Maur-des-Fossés, France) and the canals were contaminated with 30 μL of the suspension containing *C. albicans* (F-72) and *E. faecalis* (ATCC 29212), using an automatic micropipette (Gilson, Villiers-le-Bel, France). A sterile cotton ball was soaked

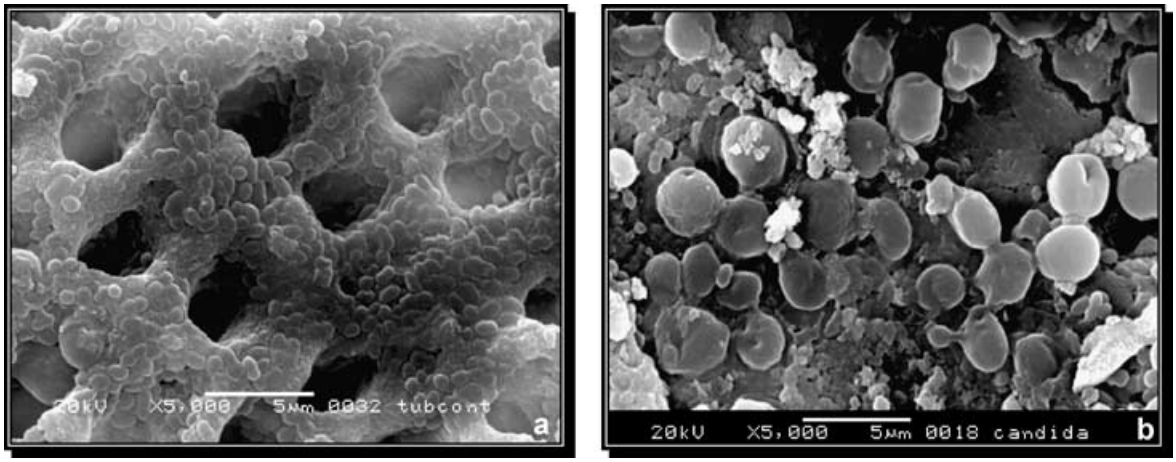


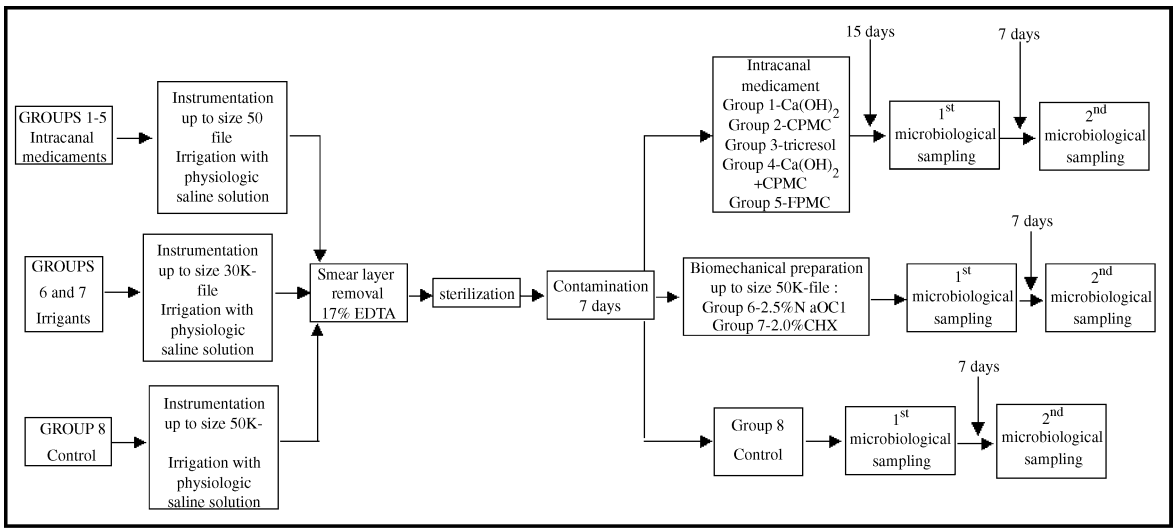
Figure 1 Photomicrograph of roots canals contaminated with *E. faecalis* (a) and *C. albicans* (b).

in the suspension of microorganisms and placed in the cervical-third of the canals. Afterwards, the cervical access was sealed with Cimpat temporary cement. The roots were placed on a gauze pad in a sterile Petri plate and were incubated at 37 ± 1 °C for 7 days. On the fourth day, the Cimpat temporary cement was removed, and SDA and Trypic Soy broths were added to the canals with an insulin 0.5-mL syringe (Becton Dickinson, Curitiba, Brazil); then the roots were again sealed with Cimpat temporary cement until 7 days were completed.

To confirm root-canal infection, two more specimens were included in each group submitted to the same initial instrumentation and contamination procedures, and were then observed under scanning electron microscopy (SEM, ×5.000; JSM 5510, JEOL, Peabody, USA; Fig. 1).

Placement of irrigants and intracanal medicaments

After contamination, the cervical seal was removed and the roots were randomly divided into eight experimental groups, with 12 roots in each group, according to irrigant solution or intracanal medicament. The procedures carried out in each group can be summarized as follows (Fig. 2):



Ca(OH)$_2$-calcium hydroxide paste; **FPMC**- camphorated paramonochlorophenol; **Ca(OH)$_2$+CPMC**- calcium hydroxide paste plus camphorated paramonochlorophenol; **FPMC**-furacin paramonochlorophenol; **2.5%NaOCl**-2.5% sodium hypochlorite

Figure 2 Flowchart of the methodology.

• Groups 1–5 (intracanal medicaments): instrumentation and irrigation with physiologic saline solution (0.85% NaCl) from size 25 up to size 50 K-files, smear layer removal with 17% EDTA for 3 min, irrigation with physiologic saline solution, sterilization and contamination for 7 days. Then, the canals were reinstrumented with a size 50 K-file and irrigated with saline solution. Root canals were dried with sterile paper points and filled with intracanal medicaments, according to the experimental group with group 1 (G1): $Ca(OH)_2$ paste (Calen, S.S.White, Rio de Janeiro, RJ, Brazil); group 2 (G2): CPMC 2.5 : 7.5 (Byofórmula Drugstore, São José dos Campos, SP, Brazil.); group 3 (G3): tricresol formalin (Iodontec, Industry and Trade, Porto Alegre, RS, Brazil); group 4 (G4): $Ca(OH)_2$ paste + CPMC (Calen CPMC; S.S. White, Rio de Janeiro, RJ, Brazil) and group 5 (G5): furacin paramonochlorophenol FPMC- (5 g of PMC with 28 mL of furacin; Byofórmula Drugstore, São José dos Campos, SP, Brazil).
• Groups 6 and 7 (irrigant solutions): instrumentation and irrigation with physiologic saline solution up to a size 30 K-file, smear layer removal with 17% EDTA for 3 min, irrigation with physiologic saline solution, sterilization, contamination for 7 days, instrumentation up to a size 50 K-file and irrigation, according to the experimental group with group 6 (G6): 2.5% NaOCl (Byofórmula Drugstore, São José dos Campos, SP, Brazil) without intracanal medication and group 7 (G7): 2.0% CHX solution (Byofórmula Drugstore, São José dos Campos, SP, Brazil) without intracanal medication.
• Group 8 (G8, control group): instrumentation and irrigation with physiologic saline solution up to a size 50 K-file, smear layer removal with 17% EDTA for 3 min, irrigation with physiologic saline solution, sterilization, contamination for 7 days, reinstrumentation with a size 50 K-file and irrigation with saline solution without intracanal medication.

$Ca(OH)_2$ pastes (Calen and Calen CPMC) were placed inside the canals, according to the manufacturer's recommendations, with a ML endodontic syringe (S.S. White, Rio de Janeiro, RJ, Brazil), filling all extensions of the root canal. Afterwards, a sterile cotton ball was adapted in the cervical-third of the root canal. CPMC and FPMC were applied on the root canal walls with a sterile paper point, and then a sterile cotton ball moistened in the solution was placed in the cervical-third of the root canal. For tricresol formalin application, a sterile cotton ball was moistened in the solution and then adapted to the cervical-third of the root canal. The cervical access cavities of the roots were sealed with Cimpat temporary cement.

Incubation and microbiological sampling

After placement of the intracanal medicaments in groups 1–5, the roots were incubated at 37 ± 1 °C for 15 days with daily verification of a humid environment and temperature conditions.

After 15 days, the cervical and apical seals were removed, and the canals were instrumented with a size 50 K-file and irrigated with 5 mL of sterile saline solution to remove the intracanal medication. With the canals filled with the saline, the first bacteriological sampling was taken using a sterile paper point size 50 (Tanariman, Manacapurú, AM, Brazil) that was placed inside the root canal for 1 min. The fluid in the canal was absorbed by the paper point and transferred to a test tube (Eppendorf) containing 0.5 mL of sterile saline solution, shaken for 30 s (Vortex-AP 56-Phoenix, Araraquara, SP, Brazil) and 0.1 mL aliquot was plated on SDA or MS agar plates. The SDA plates were incubated at 37 ± 1 °C for 48 h, and MS plates were incubated in an anaerobic chamber for the same period. Microbial growth was verified and the number of cfu of *C. albicans* and *E. faecalis* were counted and confirmed by Gram stain in a light microscope.

Groups 6 and 7 (irrigant solutions) were not incubated after the use of 2.5% NaOCl and 2.0% CHX. After instrumentation, irrigation with 0.6% sodium thiosulphate was ?twb› used to neutralize NaOCl, or with 0.5% Tween 80 + 0.07% lecithin to neutralize CHX. Then, the first microbiological sampling was carried out as described previously.

After the first microbial sampling, roots were filled with SDA broth for *C. albicans* and Trypic Soy Broth for *E. faecalis*, followed by placement of a sterile cotton ball in the cervical-third of the root canal. The cervical access and apical foramen were sealed with temporary cement, and the roots were incubated for a further 7 days. After this period, the second microbial sampling was performed, following the same procedures as the first bacteriological sampling.

Sampling of the control group occurred after the contamination period (first sampling) and after 7 days (second sampling).

Statistical analysis

The results of cfu mL^{-1} were submitted to the analysis of variance (ANOVA, one-factor Kruskal–Wallis (K–W) nonparametric test) with *P*-value of 0.05. The computer programs used were: STATISTIX for Windows (version 7.0, Analytical Software, 2000), MINITAB for Windows

Table 1 Mean (cfu mL^{-1} of *C. albicans* and *E. faecalis*) in the first and second microbiological sampling of the experimental groups

	Groups (cfu mL^{-1})															
	1 ($Ca(OH)_2$)		2 (CPMC)		3 (Tricresol formalin)		4 ($Ca(OH)_2$ + CPMC)		5 (FPMC)		6 (2.5% NaOCl)		7 (2.0% CHX)		8 (Control)	
Sampling	*C.a.*	*E.f.*	*C.a.*	*E.f.*	*C.a.*	*E.f.*	*C.a.*	*E.f.*	*C.a.*	*E.f.*	*C.a.*	*E.f.*	*C.a.*	*E.f.*	*C.a.*	*E.f.*
1st	0.0	0.0	0.0	9.16	151.0	0.83	2.5	1.66	4.16	21.6	0.0	0.0	0.0	0.0	380.0[a]	331.8
2nd	5.8	15.0	5.0	16.6	425.8[b]	29.1	0.0	0.0	0.0	182.5	380.0	564.1[c]	110.8	0.0	567.5[b]	448.33[c]

$Ca(OH)_2$: calcium hydroxide paste; CPMC: camphorated paramonochlorophenol; $Ca(OH)_2$ + CPMC: calcium hydroxide paste plus camphorated paramonochlorophenol; FPMC: furacin paramonochlorophenol; 2.5% NaOCl: 2.5% sodium hypochlorite; cfu mL^{-1}: colony-forming units per millilitre; *C.a.*: *Candida albicans*; *E.f.*: *Enterococcus faecalis*.

[a]Statistically different in relation to group 3 ($P = 0.014$).

[b]Statistically different in relation to groups 1, 2, 4 and 5 ($P = 0.001$).

[c]Statistically different in relation to groups 1–4 and 7 ($P = 0.001$).

(version 13.1, Minitab Inc., 2001) and STATISTICA for Windows (version 5.0, Statsoft Inc., 1995).

Results

Table 1 shows the mean growth of *E. faecalis* and *C. albicans* after treatment with irrigant solutions or intracanal medications at the first and second sampling.

At the first microbial sampling, groups 3 (tricresol formalin) and 8 (control group) had significant differences for *C. albicans* counts (Mann–Whitney, $U = 107.00$; $P = 0.014$).

Comparing the eight experimental groups at the second sampling for *C. albicans*, there were significant differences between groups 3 and 8 (tricresol formalin and control groups, respectively) and groups 1 (Calen), 2 (CPMC), 4 (Calen + CPMC) and 5 (FPMC; K–W = 65.241; gl = 7; $P = 0.001$; Fig. 3).

When the groups of intracanal medicaments were compared separately, group 3 (tricresol formalin) had lower antimicrobial effectiveness than the other groups (ANOVA of K–W = 44.412; gl = 4; $P = 0.001$). There was no significant difference when the groups of irrigants (groups 6 and 7) were compared for their effectiveness on *C. albicans* growth at the second sampling (Mann–Whitney, $U = 69.00$; $P = 0.882$).

When the eight experimental groups were compared for growth of *E. faecalis* at the second sampling, groups 6 and 8 (2.5% NaOCl and control groups, respectively) were statistically different from groups 1 (Calen),

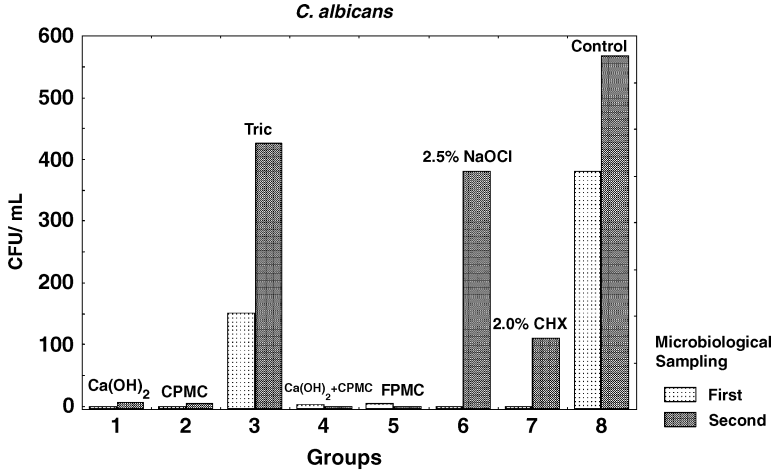
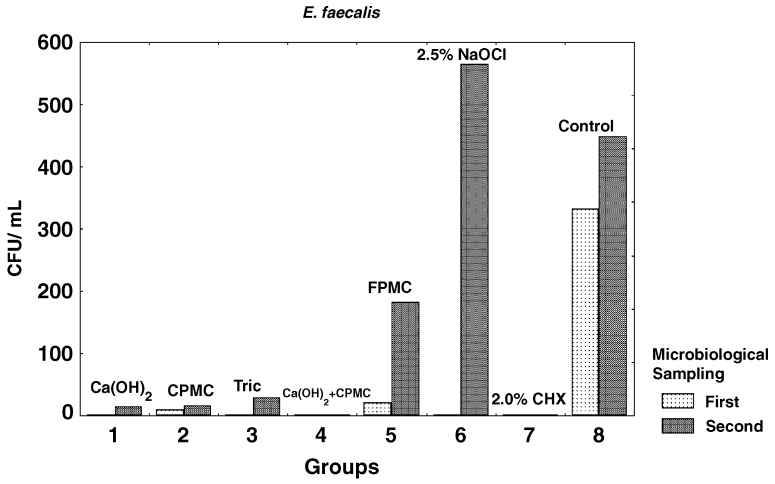


Figure 3 Mean of cfu mL^{-1} in the first and second microbiological sampling of the experimental groups for *C. albicans*.

$Ca(OH)_2$-calcium hydroxide paste; **CPMC-** camphorated paramonochlorophenol; **$Ca(OH)_2$+CPMC-** calcium hydroxide paste plus camphorated paramonochlorophenol; **FPMC-** furacin paramonochlorophenol; **2.5%NaOCl –** 2.5% sodium hypochlorite; **cfu/mL –** colony forming unit/mL.



Ca(OH)$_2$-calcium hydroxide paste; **CPMC**- camphorated paramonochlorophenol; **Ca(OH)$_2$+CPMC**- calcium hydroxide paste plus camphorated paramonochlorophenol; **FPMC**- furacin paramonochlorophenol; **2.5%NaOCl** – 2.5% sodium hypochlorite; **cfu/mL** – colony forming unit/mL.

Figure 4 Mean of cfu mL^{-1} in the first and second microbiological sampling of the experimental groups for *E. faecalis*.

2 (CPMC), 3 (tricresol formalin), 4 (Calen + CPMC) and 7 (2.0% CHX; K–W = 61.048; gl = 7; $P = 0.001$; Fig. 4).

Comparing only the groups of intracanal medicaments, group 5 (FPMC) had lower antimicrobial effectiveness than group 4 (Calen + CPMC), with a statistically significant difference (ANOVA of K–W = 12.142; gl = 4; $P = 0.016$); groups 1, 2 and 3 were significantly not different. Considering only the groups of irrigants, group 7 (2.0% CHX) was statistically more effective than group 6 (2.5% NaOCl; Mann–Whitney, $U = 0.00$; $P = 0.001$) on the growth of *E. faecalis* at the second bacteriological sampling.

Discussion

Various methodologies can be used to assess the antimicrobial activity of endodontic medicaments. Indeed, the methodology can be a possible explanation for the differences found in the results between studies. Some methodologies allow direct contact of the substances with the microorganisms (as in the agar diffusion test). In others, as in this study, microorganisms located inside the dentinal tubules did not necessarily have direct contact with the antimicrobial substances.

Bacteriological sampling is another important step that varies among the different methodologies (Siqueira *et al.* 1997). In this study, the bacteriological sampling was accomplished with a sterile paper point that absorbed the root-canal contents. The paper point was then transferred to tubes containing saline solution that were plated on SDA or MS agar plates. The use of paper points has the advantage that it can be performed *in vitro* and *in vivo*. On the other hand, bacteriological sampling with paper points is limited because only the microorganisms that are in the root canal can be sampled, while the ones that are located inside the dentine tubules are not.

Byström & Sundqvist (1981, 1983, 1985) verified that bacteria that survived instrumentation and irrigation quickly proliferated and recolonized root canals that remained empty between treatment sessions. In the present study, to verify root canal recolonization, a second bacteriological sampling after 7 days was completed, as in other studies (Jeansonne & White 1994, Leonardo *et al.* 1999, Dametto *et al.* 2002). This second sampling aimed to collect microorganisms that were not collected during the first bacteriological sampling, possibly because they were in the dentinal tubules. At the same time, two or more bacteriological samples allowed the verification of the residual effect of medicaments (Jeansonne & White 1994, Leonardo *et al.* 1999, Dametto *et al.* 2002).

In the present study, groups 6 and 7 (2.5% NaOCl and 2.0% chlorhexidine, respectively) were sampled just after biomechanical preparation in order to evaluate their antimicrobial action immediately after the instrumentation (first microbiological sampling) and the residual effect of these solutions after 7 days (second microbiological sampling). For groups 1–5 (intracanal medicaments), the first microbiological sampling was accomplished after 15 days as this is the period commonly used between appointments (especially Ca(OH)$_2$) and after a further 7 days (second microbiological sampling) for verification of the residual effect.

The instrumentation of groups 6 and 7 (irrigants) was initially to a size 30 K-file, and in the intracanal medicament (groups 1–5) and control (group 8) groups, it was to a size 50 K-file. This difference in the initial instrumentation was necessary because in the intracanal medicament groups (groups 1–5), the objective was to evaluate only the effectiveness of the medications and not the cumulative effect of instrumentation/irrigation and intracanal medicament. In the groups of irrigants (groups 6 and 7), the roots were initially instrumented up to a size 30 K-file and, after the contamination period, the instrumentation from a size 30 K-file up to a size 50 K-file was performed to verify the antimicrobial action of irrigants (NaOCl and CHX) during biomechanical preparation.

$Ca(OH)_2$ was evaluated because it is widely used in endodontics, although some controversies exist about its effectiveness against *E. faecalis* (Stevens & Grossman 1983, Ørstavik & Haapasalo 1990, Siqueira & Uzeda 1996, Valera *et al.* 2001, Ferguson *et al.* 2002). These researchers observed that CPMC was more effective than $Ca(OH)_2$ as an intracanal medication.

In this study, the most effective intracanal medication was $Ca(OH)_2$ + CPMC, as verified in previous studies (Leonardo *et al.* 1993, Siqueira & Uzeda 1996, Sukawat & Srisuwan 2002). The present study also confirmed that *E. faecalis* can be resistant to $Ca(OH)_2$ (Byström *et al.* 1985, Evans *et al.* 2002, Sukawat & Srisuwan 2002). Dentine has buffering potential, as in the proton donor that occurs in the hydroxyapatite hydrated layer (Nerwich *et al.* 1993), that can reduce the pH and $Ca(OH)_2$ effect inside dentinal tubules. Evans *et al.* (2002) verified that the proton pump of the *E. faecalis* cell, which carries protons to the interior of the cell acidifying the cytoplasm, is important for the survival of this microorganism in an alkaline environment. Presumably, when the alkalinity of the environment reaches pH 11.5 or higher, this 'lifeguard' mechanism is brought into action (Siqueira & Uzeda 1996). This mechanism, associated to the dentine buffer properties, can explain the resistance of *E. faecalis* to $Ca(OH)_2$ alone.

CPMC is a phenolic compound that has bactericidal activity. It disrupts bacterial cytoplasmic membranes, denatures proteins and inactivates enzymes. Besides, it liberates chlorine, a strong oxidizing agent that inactivates enzymes with sulphydryl groups (Siqueira *et al.* 1996). In contrast to previous studies, CPMC applied alone in this study had lower antibacterial activity in relation to $Ca(OH)_2$ (Stevens & Grossman 1983, Ørstavik & Haapasalo 1990, Siqueira & Uzeda 1996, Valera *et al.* 2001). These results can be because of differences among the applied methodologies. The methods that promote the direct contact of CPMC with the microorganisms seem to have more favourable results, such as those observed for agar diffusion tests (Stevens & Grossman 1983, Siqueira & Uzeda 1996). In this study, CPMC and FPMC were applied with paper points soaked in the medicament, and the microorganisms located inside the dentine tubules might not have direct contact with them. This could possibly explain the reduced antimicrobial effect observed in groups 2 and 5 (CPMC and FPMC, respectively). This method was selected in the present study because the *in vitro* conditions are more similar to the *in vivo* situation than those of direct-contact methodologies.

Even though tricresol formalin is not currently used as a long-term intracanal medication, it was tested because it is considered to be an effective bactericide. This medicament was not effective against *C. albicans*, corroborating the results obtained by Valera *et al.* (2001).

The effectiveness of NaOCl as an irrigant solution has been confirmed by several studies (Byström & Sundqvist 1983, 1985, Siqueira *et al.* 1997, Dametto *et al.* 2002). The present results showed that 2.5% NaOCl was effective in the first microbiological sampling, performed just after instrumentation, but it allowed microbial growth after 7 days of incubation. These results demonstrated that *C. albicans* and *E. faecalis* were able to recolonize the root canal after instrumentation and irrigation with 2.5% NaOCl. The 2.5% NaOCl concentration was selected because this is a commonly used concentration for endodontic treatment (Ørstavik & Haapasalo 1990, Siqueira *et al.* 1997, Leonardo & Leal, 1998).

On the other hand, 2.0% CHX had a strong antimicrobial action against *E. faecalis* resulting in negative microbial sampling after instrumentation (first microbial sampling) and after seven days (second microbial sampling), demonstrating that this substance has a residual effect (Jeansonne & White 1994, Leonardo & Leal 1999, Dametto *et al.* 2002), because it did not allow the recolonization of root canals after 7 days.

This study verified the effectiveness of irrigant solutions and intracanal medicaments on *C. albicans* and *E. faecalis* within root canals. When used alone, none of the agents was able to eliminate both the microorganisms. This study reinforces the importance of root-canal treatment in two sessions with the use of irrigant solutions and intracanal medications with antimicrobial properties.

Conclusions

In conclusion: (i) 2.0% CHX solution was a more effective irrigant solution than 2.5% NaOCl against *E. faecalis* and (ii) $Ca(OH)_2$ paste mixed with CPMC (Calen) was a more effective intracanal medicament than $Ca(OH)_2$ alone against *E. faecalis* and *C. albicans* inoculated in root canals.

Acknowledgements

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