Efficacy of chemo-mechanical preparation with different substances and the use of a root canal medication in dog's teeth with induced periapical lesion

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ABSTRACT

Objectives: to evaluate the effect of instrumentation, irrigation with different substances and the use of calcium hydroxide on bacterial load and microbiota profile in dog's teeth with pulp necrosis and periapical lesion. **Methods:** Fifty five root canals were divided into groups: I) Saline (SSL) (n=11); II) natrosol gel (n=11); III) 2.5% NaOCl (n=11); IV) 2% CHX-gel (n=11); V) 2% CHX-solution (n=11). Endodontic samples were cultured, microorganisms counted and the microbiota analyzed at different sampling times — s1, s2 and s3. **Results:** At s1, the mean CFU counts ranged from 5.5 x10^5 to 1.5 x 10^6. These values dropped significantly at s2 ($p<0.05$). No statistical significant difference was found between s2 and s3. Changes in root canal microbiota were found at s2 and s3. **Conclusion:** Regardless the use of calcium hydroxide as a root canal medication, 2.5% NaOCl and 2% CHX-gel demonstrated a potent antimicrobial activity against endododontic pathogens *in vivo*.

Keywords: Sodium hypochlorite. Chlorhexidine. Calcium hydroxide. Endodontic infection. Root canal medication.

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Introduction

Apical periodontitis is an infectious disease caused by microorganisms colonizing the root canal system.[1] One of the main goal in endodontic treatment is to eliminate or at least reduce the bacterial population within root canal to levels that are compatible with the healing process of periapical tissues.[2]

The antimicrobial efficacy of endodontic procedures has been evaluated over a known numbers of bacteria in root canals by culture[3,4,5,6,7] and molecular techniques.[8,9]

To an optimally disinfection of root canal system, endodontic treatment comprises both mechanical and chemical phases. The first involves the action of the instruments in dentin walls combined to the flow and backflow of the irrigant solution. It acts primarily on the main canal which harbors the largest number of bacterial cells, assuming a prior role in the root canal disinfection.[3]

However, due to the anatomical complexities in root canal system[3,4,10] and the restricted action of the instruments in the main root canal, the mechanical phase does not eradicate bacteria from the entire root canal system[3,11,12] requiring a chemical phase, which involves the use of potent antimicrobial agents to act deeply in dentin tubules.[3,4]

Several auxiliary chemical substances have been proposed over the years to be used during chemo-mechanical preparation, but sodium hypochlorite (NaOCl) remains the most widely used one. Recently, chlorhexidine has been tested as a potential substance.[5,9,13,14] Most antimicrobial comparisons between the two auxiliary chemical substances are demonstrated by in vitro studies[13,14,16-19] over a selected microorganism. Indeed, not only controversy exists among these studies but also limitation in the reproducing models of the infection (mono-infection) must be considered.

As a matter of fact, in vivo studies have also been inconsistent in their findings when comparing NaOCl and CHX; with NaOCl being more effective[9,20] or with no significant difference existing among them.[14,15]

The use of an inter-appointment root canal medication — calcium hydroxide [$Ca(OH)_2$] — has been recommended to help eliminate remaining bacteria strategically located in the root canal system after chemo-mechanical procedures.[4,10,21,22,23]

While some studies[4,11,12,24] had reported a further bacterial load reduction after the placement of $Ca(OH)_2$, others[10,21,22] demonstrated an increase in the proportion of positive cultures and bacterial counts. Indeed, its effectiveness in significantly increasing bacterial load reduction and the number of negative culture after chemo-mechanical procedures in clinical practice has been doubtful.[11,21,23,25]

Moreover, most in vivo studies[7,9,13,23] investigating the antibacterial effects of root canal procedures had provided only quantitative data, not determining its effect on the microbiota involved, which assumes special relevance to the establishment of therapeutic strategies.

This clinical study was conducted to evaluate the effect of instrumentation, irrigation with different substances and the use of calcium hydroxide on bacterial load and microbiota profile in dog's teeth with pulp necrosis and periapical lesion.

Materials and Methods

Root canal selection

Fifty five root canals (5 single root-canal premolars and 25 multiple root-canal premolars) from adult mongrel dogs were selected. Tooth shorter than 12 mm length and/or incompletely formed apices was excluded. The animals were first anesthetized with intravenous injection of 5% sodium thionembutal (10 mg/Kg body weight). An access opening was made with a high-speed diamond bur under irrigation, the pulp tissues were removed, and apical foramen was standardizing in 0.20 K-file diameter. Afterwards, the root canals remained open and exposed to the oral environment for 6 months to allow microbial contamination. An approval for the study protocol was obtained from the Ethics Committee of the Dental School of Piracicaba.

Microbial sampling

After isolating the tooth with a rubber dam, the crown and the surrounding structures were disinfected with 30% H_2O_2 (v/v) for 30 s, followed by 2.5% NaOCl for an additional 30 s. The disinfectant solutions were inactivated with 5% sodium thiosulphate in order to avoid interference with bacteriologic sampling.[9] Then the sterility control samples were taken from the toot surface with sterile paper points. An access cavity was prepared with sterile high-speed diamond burs under irrigation with sterile saline. Before entering the pulp chamber, the access cavity was disinfected by the same protocol as above and new sterility control

samples were taken of the cavity surface and streaking it on blood agar plates. For the inclusion of the tooth in the study, these control samples had to be negative. All subsequent procedures were performed aseptically. The pulp chamber were accessed with burs and rinsed with sterile saline, which was aspirated with suction tips. The first root canal sample (s1) was taken as follows: five sterile paper points were placed for 1 minute period into each canal to the total length calculated from the pre-operative radiograph and then pooled in a sterile tube containing 1 ml Viability Medium Göteborg Agar (VMGA III). Afterwards, the baseline samples (s1) were transported to the laboratory within 15 minutes for microbiological procedures.

Clinical procedures

After accessing the pulp chamber and subsequent microbial sampling (s1), teeth were randomly divided into groups according to the substances applied, as follows: I) saline solution (SSL) (n=11); II) natrosol gel (n=11); III) 2.5% NaOCl (n=11); IV) 2% CHX-gel (Endogel, Itapetininga, SP, Brazil) (n=11) and V) 2% CHX-solution (n=11). The pulp chamber was thoroughly cleaned with substances from each group. A K-file size 10 or 15 (Dentsply Maillefer, Ballaigues, Switzerland) was placed to the full length of the root canal calculated from the pre-operative radiographs. The coronal two-thirds of each canal was initially prepared using rotary files (GT® rotary files size 20, 0.06 taper - Dentsply Maillefer, Ballaigues, Switzerland) at 350 rpm, 4 mm shorter than the estimated length. Gates-Glidden burs sizes 5, 4, 3 and 2 (DYNA-FFDM, Bourges, France) were used in a crown-down technique reaching 6 mm shorter than the working length (1 mm from the radiographic apex). Afterwards, the working length was checked with a radiograph after inserting a file in the canal to the estimated working length, confirmed by the apex locator (Novapex, Forum Technologies, Rishon le-Zion, Israel). The apical preparation was performed using K-files ranging from size 35-45, followed by a step back instrumentation, which ended after the use of three files larger than the last filed used for the apical preparation. The working time of the chemo-mechanical procedure was established at 20 minutes for all cases.

In the CHX and natrosol gel groups, root canals were irrigated with a syringe (27-gauge needle) containing 1 ml of each substance before the use of each instrument, being immediately rinsed afterwards with 4 ml of saline solution. Particulary, in NaOCl-group, the use of each instrument was followed by an irrigation of the canal with 5 ml of 2.5% NaOCl solution. CHX activity was inactivated with 5 ml solution containing 5% Tween 80% and 0.07% (w/v) lecithin over a 1 min period. NaOCl was inactivated with 5 ml of sterile 5% sodium thiosulphate over a 1 min period. A second bacteriological sample was taken (s2), as previously described.

After drying the canal with sterile paper points, all teeth were dressed with a thick mix of a paste of calcium hydroxide (Merck, Darmstad, Germany) with sterile saline. The calcium hydroxide slurry was plugged in the canals with a lentulo spiral. Radiographs were taken to ensure proper placement of the calcium hydroxide in the canal. The access cavity was restored with 2 mm of Cavit™ (3M Dental Products, St Paul, MN, USA) and Filtek™ Z250 (3M Dental Products), in order to prevent coronal microleakage. After 14 days, teeth were aseptically accessed under rubber dam isolation and the calcium hydroxide was removed by the use of the master apical file and with sterile saline and careful filling the canal with the master apical file. A third bacteriological sample (s3) was taken, as previously described.

Culture technique

The transport medium containing the root canal samplings was shaken thoroughly in a mixer inside an anaerobic chamber for 60 s (Vortex, Marconi, São Paulo, SP, Brazil). The transport medium contained glass beads of 3 mm in diameter in order to facilitate mixing and homogenization of the sample prior to cultivation. Serial 10-fold dilutions were made up to 1:104 in tubes containing Fastidious Anaerobe Broth (FAB, Lab M, Bury, UK). Fifty µL of the serial dilutions $1:10^2$ and $10:10^4$ were plated, using sterile plastic spreaders, into 5% defibrinated sheep blood Fastidious Anaerobe Agar (FAA, Lab M), in which 1ml/l of hemin and 1ml/l of vitamin K1 were added, so as to culture non-selectively obligate anaerobes. Plates were incubated anaerobically (80% N_2, 10% H_2, 10% CO_2) at 37° C for 7 days (Peters LB 2002). Subsequently, 50 µL of each dilution were inoculated on BHI agar plates (Brain Heart Infusion agar, Oxoid, Basingstoke, UK), supplemented with 5% sheep blood, and incubated aerobically (37° C, air) for 24 and 48 h. After incubation, the total CFU value was counted using a stereomicroscope at 16 x magnifications (Zeiss, Oberkoren, Germany).

Microbial characterization

Preliminary characterization of microbial species were based on colony features (i.e. size, color, shape, height, lip, surface, texture, consistency, brightness and hemolysis) visualized under a stereoscopic lens (Lambda Let 2, Atto instruments Co., Hong Kong). Isolates were purified by subculture. Gram-stained and gaseous requirements were established by incubation for 2 days under aerobic and anaerobic environments.

Based on microbial colony features, Gram-stain and gaseous requirements, it was possible to determine the microbiota profile from root canals at different samplings moments (s1, s2, s3).

Statistics

Statistical comparisons were made between all groups (I-V) at the same samplings moments (s1, s2 or s3) and between s1, s2 and s3 in each group using the Kruskall-wallis test for non-parametric data (CFU counts, percentages of gram-positive rods and cocci, percentages of facultative and strict anaerobes species). When significant differences were found in the Kruskall-wallis test, Mann-Whitney test was performed to demonstrate where the differences were located. P-values <0.05 were considered statistically significant.

Results

Sterility check samples taken from the rubber dam, the crown and its surrounding structures tested before and after entry into the pulp chamber showed no microbial growth. The mean of the total colony forming unit (CFU) counts in the baseline samples (s1) ranged from 5.5 x10^5 to 1.5 x 10^6 (Table 1). At s1, no statistically significant difference was found between any of the mean CFU values found in all groups: GI) 9.3 x 10^5, GII) 5.5 x 10^5, GIII) 6.7 x 10^5, GIV) 6.4 x 10^5 and GV) 1.5 x 10^6 (all P>0.05) (Table 1). These values dropped significantly as a result of root canal instrumentation (s2): GI) 1.6 x 10^4, GII) 1.4 x 10^4, GIII) 7.6 x 10^2, GIV) 3.2 x 10^2 and GV) 2.6 x 10^3 (Table 1). At s2, statistically significant differences were found between all the mean CFU values (p<0.05), except when comparing GIII (NaOCl-group) with GIV (CHX-gel-group) (p>0.05) (Table 1), as both substances reduced almost 100% of the bacterial load (Fig 1).

After application of $Ca(OH)_2$ for 2 weeks (s3) bacterial mean CFU values dropped even lower than those at the end of the first visit (s2): GI) 6.7 x 10^3, GII) 5.3 x 10^3, GIII) 1.4 x 10^2, GIV) 1.8 x 10^2 and GV) 1.2 x 10^3 (Table 1). Higher and significant percentage levels of bacterial load reduction were found between s2 and s3 in group I (SSL), II (Natrosol-gel) and V (CHX-solution) (p<0.05) (Fig 1).

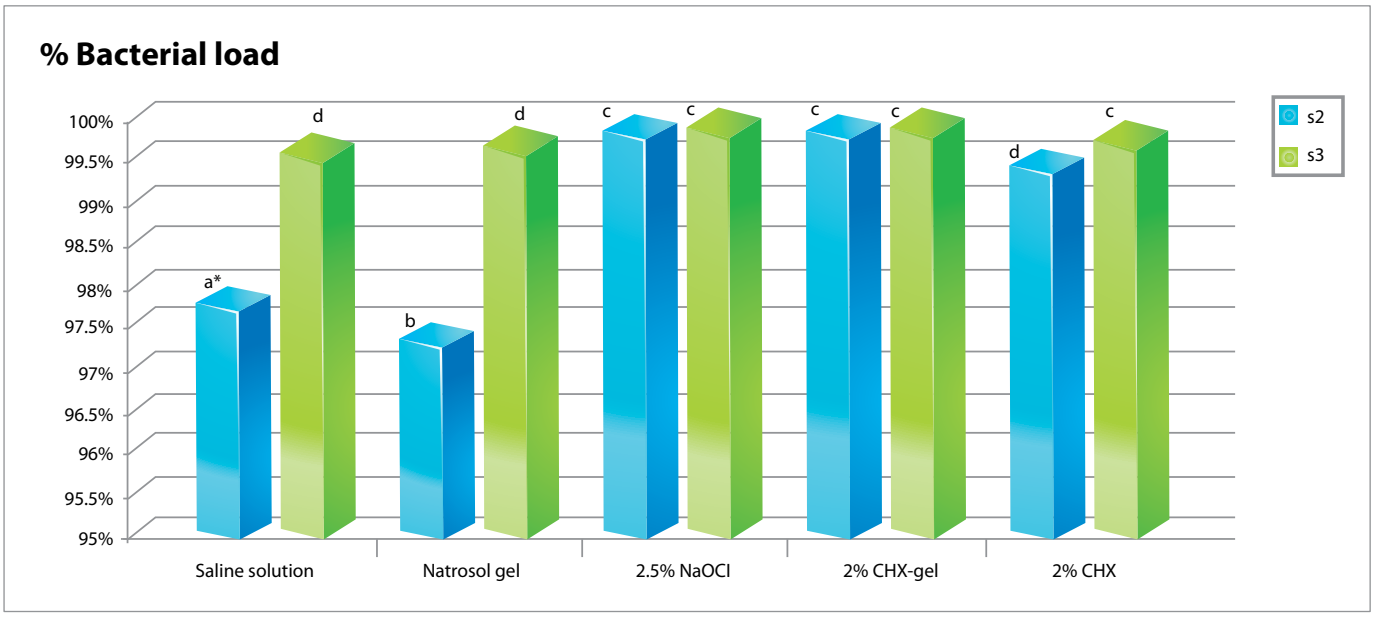

Figure 1. Mean percentage values of reduction in bacterial counts (CFU) from root canal samples obtained after root canal instrumentation (s2) and root canal medication (s3). *Same letters indicate no statistical difference among the groups (P > 0.05).

Nevertheless, no statistically significant difference in percentage levels of bacterial load reduction was found in groups III (NaOCl) and IV (CHX-gel) (Fig. 1) comparing s2 and s3.

In contrast to s2, at s3 no statistically significant difference was found in bacterial load between CHX-solution (GV) and NaOCl (GIII) or CHX-gel (GIV) ($p>0.05$) (Table 1). Distribution in mean percentage values of bacterial load reduction after root canal instrumentation (s2) and after root canal medication (s3) are shown in Figure 1.

A mixed microbiota, comprised predominantly by

Table 1. Quantity bacterial of UFC in 55 root canals with necrotic pulp and periapical lesions induced in the initial samples (S1) after root canal instrumentation (s2) and after intracanal medication (S3).

	Saline solution (GI)			Natrosol gel (GII)			2.5% NaOCl (GIII)			2% CHX-gel (GIV)			2% CHX-solution (GV)		
Samples	s1	s2	s3	s1	s2	s3	s1	s2	s3	s1	s2	s3	s1	s2	s3
H1	2.2^{D}	2.46^{C}	8.0^{A}	3.0^{D}	1.26^{C}	6.0^{A}	6.8^{D}	4.0^{A}	2.0^{A}	4.2^{D}	4.0^{A}	2.0^{A}	3.6^{D}	5.8^{B}	8.0^{A}
H2	8.6^{D}	1.96^{C}	3.94^{C}	2.6^{D}	1.88^{C}	8.0^{A}	3.4^{D}	1.0^{B}	2.0^{A}	5.4^{D}	4.0^{A}	2.0^{A}	2.4^{D}	3.6^{B}	1.2^{B}
H3	4.2^{D}	9.0^{B}	1.66^{C}	3.2^{D}	2.88^{C}	1.0^{B}	3.8^{D}	2.0^{A}	2.0^{A}	5.4^{D}	2.0^{A}	2.0^{A}	6.0^{D}	2.4^{C}	3.92^{B}
H4	4.0^{D}	2.9^{C}	1.0^{B}	3.6^{D}	1.08^{C}	4.0^{A}	6.8^{D}	8.0^{A}	2.0^{A}	3.0^{D}	2.0^{A}	2.0^{A}	5.8^{D}	3.6^{B}	2.14^{B}
H5	5.2^{D}	2.08^{C}	1.0^{B}	3.8^{D}	8.6^{B}	1.0^{B}	4.2^{D}	6.0^{A}	0	9.4^{D}	2.0^{A}	2.0^{A}	6.2^{D}	2.6^{B}	6.0^{A}
H6	2.12^{E}	1.36^{C}	2.0^{B}	5.6^{D}	1.44^{C}	3.08^{C}	1.22^{E}	4.0^{A}	2.0^{A}	6.4^{E}	2.0^{A}	0	4.4^{E}	2.2^{B}	1.0^{B}
H7	1.78^{E}	1.7^{C}	1.6^{B}	2.8^{D}	1.16^{C}	1.9^{C}	8.4^{D}	2.0^{B}	2.0^{A}	3.8^{D}	4.0^{B}	2.0^{A}	6.2^{D}	2.4B	1.0^{B}
H8	3.0^{D}	1.84^{C}	2.4^{B}	1.52^{E}	2.04^{C}	2.0^{B}	4.8^{E}	1.2^{B}	2.0^{A}	4.6^{C}	2.0^{B}	2.0^{A}	5.0^{D}	1.8B	1.0^{B}
H9	5.6^{D}	1.3^{C}	1.8^{B}	1.08^{E}	1.64^{C}	1.6^{B}	6.2^{E}	1.0^{B}	0	1.66^{E}	2.0^{B}	0	6.8^{E}	1.4B	4.0^{A}
H10	1.64^{E}	9.0^{B}	5.6^{B}	4.2^{D}	4.6^{B}	1.8^{B}	1.22^{E}	4.0^{A}	2.0^{A}	1.28^{E}	8.0^{A}	6.0^{A}	6.2^{E}	2.4B	8.0^{A}
H11	1.48^{E}	1.12^{C}	2.4^{B}	6.4^{D}	7.4^{B}	4.0^{A}	9.0^{D}	4.0^{A}	2.0^{A}	3.0^{D}	4.0^{A}	0	3.6^{D}	1.8B	4.0^{B}
Mean	9.3^{D}**a***	1.6^{C}**b**	6.7^{B}**e**	5.5^{D}**a**	1.4^{C}**c**	5.3^{B}**e**	6.7^{D}**a**	7.6^{A}**d**	1.4^{A}**d**	6.4^{D}**a**	3.2^{A}**d**	1.8^{A}**d**	1.5^{E}**a**	2.6^{B}**e**	1.2^{B}**d**

Different lowercase letters, in bold, represent differences in the statistical viewpoint ($p < 0.05$). A =10^2, B =10^3, C = 10^4, D = 10^5, E = 10^6.

Table 2. Frequency (on percentage mean values) of the profile of the microbiota of root canals with necrotic pulp and periapical lesion in the initial samples (S1) after root canal instrumentation (s2) and after root canal medication (s3) according to the tested groups (GI, GII, GIII, GIV, GV).

	s1						s2						s3					
	GI	GII	GIII	GIV	GV	Mean	GI	GII	GIII	GIV	GV	Mean	GI	GII	GIII	GIV	GV	Mean
Gram-positive cocci	100	81.8	90.9	90.9	100	92.7	72.7	81.8	81.8	81.8	81.8	79.98	79.98	72.7	72.7	45.5	100	76.4
Gram-negative cocci	27.3	72.7	63.6	36.4	72.7	54.54	54.5	18.2	27.3	0	45.5	29.1	29.1	27.3	0	0	0	7.28
Gram-positive rods	27.3	72.7	36.4	27.3	72.7	47.28	27.3	45.5	27.3	18.2	9.1	25.48	25.48	36.4	9.1	27.3	18.2	21.8
Gram-negative rods	36.4	18.2	9.1	45.5	81.8	38.2	100	90.9	54.5	45.5	100	78.18	78.18	27.3	0	18.2	0	21.8
Strict anaerobes	55.5	58.2	69.4	62.3	37.7	56.62	5.8	18.7	100	100	80	60.9	60.9	16.6	0	100	80	36.4
Facultative anaerobes	44.5	41.8	30.6	36.8	62.3	43.2	94.2	81.3	0	0	20	39.1	39.1	83.4	100	0	20	72.7

strict anaerobe bacteria, was found in the baseline samples (s1) (Table 2).

At s1, Gram-positive cocci bacteria predominated in all groups (GI, GII, GIII, GIV and GV). After chemo-mechanical preparation (s2), a high frequency of Gram-positive cocci and Gram-negative rods bacteria were found. At s3, regardless the auxiliary chemical substance applied during chemo-mechanical preparation, Gram-positive cocci bacteria predominated in all root canal samples (Table 2).

The microbiota profile at different sampling times (s1, s2 and s3), according to the groups tested (GI, GII, GIII, GVI and GV) are shown in Table 2.

Discussion

Culture procedure, used in this study, rather than contemporary techniques (molecular methods)[8,9] is a reliable method to evaluate the antimicrobial efficacy of root canal procedures, due to its capacity to detect viable bacteria afterwards. Additionally, correlation between non-cultivable bacteria and a favorable treatment outcome had been developed over the years.[22,25,26]

Most infecting bacteria (more than 97%) were removed only by the mechanical instrumentation and the flow/back-flow of the irrigant solution (saline solution). However, the addition of an auxiliary chemical substance exhibiting a potent antimicrobial activity is required in order to promote a deeper disinfection in dentin tubules.[3,4] Increased mean values in bacterial load reduction (almost achieving 100%) were found in teeth irrigated with 2.5% NaOCl or 2.0% CHX, demonstrating their potent antimicrobial activity against microorganisms involved in primary root canal infections.

Bacterial load in infected root canals was reduced from 10^5 to 10^2 UFC/ml after chemo-mechanical preparation with either 2.5% NaOCl or 2% CHX-gel. Typical results were shown by Vianna et al[9] detecting a reduction from 10^5 to10^1 UFC/ml in the 2.5% NaOCl-group and from 10^5 to 10^2 UFC/ml in the 2% CHX-gel-group. Alike, Siqueira et al[15] reported a reduction from 10^5 to 10^3 UFC/ml in the 2.5% NaOCl-group and from 10^5 to 10^2 UFC/ml in the 0.12% CHX-gel-group.

Regarding the antimicrobial activity, the present study, in agreement with previous in vivo[14,15] and in vitro studies,[13,16,19] showed no significant difference between the use of NaOCl and CHX-gel as an auxiliary chemical substance, even though a higher mean percent value of bacterial load reduction was found in teeth irrigated with 2.5% NaOCl. In contrast, Vianna et al[9] comparing in vivo the antibacterial efficacy of these two substances by molecular technique (RTQ-PCR) found 2.5% NaOCl to be more effective than 2% CHX-gel. However, the clinical significance in reducing bacterial DNA from infected root canals after chemo-mechanical procedures remains unclear, once dead cells may not implicate in the failure of the endodontic treatment.

Overall, it is reasonable to assume that 2.5% NaOCl and 2% CHX-gel have a potent antimicrobial activity in clinical practice and the choice between them should rely upon their particular and individual properties. CHX-gel seem to posses a residual antimicrobial activity that helps to prevent root canal reinfection.[27,28] In addition, its biocompatibility turns it the choice for teeth with open apices[13] and for patients who are allergic to bleaching solutions as NaOCl.[27] However, its inability to dissolve pulp tissues (an important advantage of NaOCl)[29] is its downside.

The antimicrobial activity of $Ca(OH)_2$ medication applied for 14 days was notable in teeth irrigated with an inert substance (SSL-group and natrosol gel-group). A significant increased reduction in the mean bacterial load was found in comparison with the values after instrumentation — from 1.6 x 10^4 to 6.7 x 10^3 CFU/ml in the SSL-group and 1.4 x 10^4 to 5.3 x 10^3 CFU/ml in the natrosol-group. Nevertheless, its efficacy in reducing bacteria load after chemo-mechanical procedures was consistent but not significant in teeth irrigated with a potent auxiliary chemical substance — from 7.6 x 10^2 to 1.4 x 10^2 UFC/ml in 2.5% NaOCl-group and from 3.2 x 10^2 to 1.8 x 10^2 UFC/ml in 2% CHX-gel.

Even different periods of application of $Ca(OH)_2$ have been found in the literature[4,6,23,25] most findings in the mean bacterial load reduction from "positive-culture" canals (often ≅10^2 UFC/ml) are consistent with our data after its use for 14 days, particularly in teeth irrigated with 2.5% NaOCl and 2% CHX-gel. Thus, the range in percent values of bacterial load reduction found after the placement of $Ca(OH)_2$ medication (97.42% to 99.90%) is also in agreement

with the ones previously reported by different authors (91.0-99.9%).[3,11,12]

After the placement of $Ca(OH)_2$ medication for 14 days, the number of root canals yielding negative culture increased, whereas[4] 'positive' samples showed an increase in the number of CFUs values when compared to s2. As a matter of fact, several studies[21,22,23,30] had demonstrated increasing values in bacterial counts after the use of $Ca(OH)_2$ medication. This fact may be explained by the presence of remained bacteria in the dentinal tubules that may escape from the direct action of $Ca(OH)_2$[10] and (re) infect the canal space; and the reduced action of the $Ca(OH)_2$ medication provided by the buffering effect of the dentine.

It is reasonable to assume from the present study that $Ca(OH)_2$ medication has a low ability in vivo to promote a significant bacterial load reduction, particularly in teeth irrigated with 2.5% NaOCl or 2% CHX-gel; and in helping eliminate bacteria in the majority of the infected root canals. Therefore, its is application in clinical practice should not only be to its antimicrobial activity but also to its other properties such as the ability to change the pH of dentin and cementum, the ability to depolymerize bacterial LPS of gram-negative bacteria and its hygroscopic action that eliminates exudates.

Overall, it is important to mention that the efficacy root canal procedures are not due only to the antimicrobial properties of the substances, but also to the susceptibility of root canal flora involved. Therefore, the knowledge of endodontic microbiota and its susceptibility to endodontic therapy is important to help achieving an optimal disinfection of the root canal system.

Regardless the auxiliary substance applied (inert or not) during instrumentation, a predominance of Gram-positive cocci and Gram-negative rods bacteria were found in the root canals, suggesting a non-selective pressure performed by any of the chemical substance tested (NaOCl or CHX). In contrast, after the use of $Ca(OH)_2$ medication, a predominance of Gram-positive cocci species was observed in all "positive" root canal samples. Such a critical finding must be considered in clinical practice, since Gram-positive cocci, particularly E. faecalis, is often implicated in persistent root canal infections, due to its high level of resistance to calcium hydroxide.

Conclusion

In conclusion, regardless the use of calcium hydroxide as a root canal medication, 2.5% NaOCl and 2% CHX-gel demonstrated a potent antimicrobial activity against endododontic pathogens in vivo.

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