Influence of the sodium hypochlorite on the healing process of the dog's teeth treated in single-visit

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ABSTRACT

Introduction: Elimination of bacteria from root canals is essential in the endodontic treatment of necrotic pulp teeth once bacteria not only cause, but also maintain, periapical lesions. **Objective:** The aim of this study was to analyze, *in vivo*, the influence of the irrigating solutions (1.0% NaOCl, 2.5% NaOCl, 0.9% sterile saline) in single-visit treatment of dogs' teeth with chronic periapical lesion. **Methods:** Forty root canals from three Beagle dogs were left exposed to the oral cavity to allow contamination and formation of the chronic periapical lesion. After that, the root canals were biomechanically prepared. During the instrumentation, three irrigating solutions were used: G1- 2.5% NaOCl; G2- 1.0% NaOCl; G3- 0.9% sterile saline. Control Group (G4) had no treatment and no coronal sealing. The root canals were filled with gutta-percha points and Sealapex. The crown openings were sealed with IRM® and amalgam. After six months, the animals were sacrificed and blocks of tissue histologically processed to be stained with hematoxylin and eosin, or Brown and Brenn. **Results:** There was no histological difference between the utilization of 1.0% or 2.5% sodium hypochlorite ($p>0.05$), but between them and sterile saline ($p<0.05$). **Conclusion:** It was concluded that the use of irrigating solutions with antibacterial potential (1% or 2.5% sodium hypochlorite) provided more favorable conditions for the healing process.

Keywords: Root canal treatment. Irrigating solution. Sodium hypochlorite. Biocompatibility. Healing process.

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Introduction

The essential role of microorganisms to develop and maintain the pulp and periapical diseases have been demonstrated in animal and human studies.[1,2,3]

Dental pulp and periapical tissues are commonly affected by a variety of microorganisms and their products. Even after microbial death, cellular components, such as lipopolysaccharide (LPS), persist for prolonged periods and can cause reactions resulting in chronic inflammation.[4]

Elimination of bacteria from root canals is an ideal in the endodontic treatment of teeth with a non-vital pulp and a chronic periapical lesion once bacteria not only cause, but also maintain periapical lesions.[5,6] Endodontic therapy requires the use of irrigating solutions to reduce microorganisms, remove debris and neutralize organic compounds, but due to the risk of leakage through the apical foramen, irrigants must be biocompatible and non-irritant to the periapical tissues.[7,8]

Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution because of its antibacterial action, dentinal bleaching and organic dissolution ability.[9,10,11] On the other hand, the high superficial tension of this chemical irrigant avoids its penetration into the irregularities of the canal system. Besides, its use increases the dentin hydraulic conductivity.[7,11-14] In high concentrations, it has a potent antimicrobial action due to the release of a large number of secondary chlorates, leading to tissue dissolution.[15,16,17] On the other hand, no difference was showed in the antibacterial activity of 1%, 2.5%, and 5% NaOCl in an *in vitro* study.[18]

Thus, the purpose of the present study was to evaluate *in vivo* the influence of the irrigating solutions (1.0% NaOCl, 2.5% NaOCl and 0.9% sterile saline) in single-visit treatment of dogs' teeth with chronic periapical lesion.

Material and methods

Forty root canals from 3 male Beagle dogs, aged one year were used in this study. Procedures were conducted according to the guidelines approved by the Research Committee of São Paulo State University, Brazil.

The animals were intramuscularly pre-anaesthetized with 2 ml of a mixture of xylazine (Rompum; Bayer do Brasil S/A, São Paulo, SP, Brazil) and ketamine hydrochloride (Ketalar; Park Davis-Aché Laboratórios Farmacêuticos S/A, São Paulo, SP, Brazil), in a 1:1 ratio, and anesthetized with sodium Nembutal (30 mg/kg body weight, Thionembutal, Abbott Ltda., Rio de Janeiro, RJ, Brazil).

Previously to the interventions, radiographs were taken to observe the normality of the structures. Coronal access were prepared with a #1090 cylindrical diamond bur (KG Sorensen, Barueri, SP, Brazil) and pulp extirpation with a size #15 K-file (Maillefer Instruments, Ballaigues, Switzerland), both performed under rubber dam isolation and antisepsis with 3% iodated alcohol solution (Asteriodine-Aster, Sorocaba, SP, Brazil). Pulp extirpation was performed with K files at the apical barrier. The root canals were left exposed to the oral cavity for 6 months to allow the formation of the chronic periapical lesion, which was radiographically confirmed.

After that, the root canals were explored with a #15 K-file. The root canals were biomechanical prepared up to a #40 K-file at the apical cementary barrier. During the biomechanical preparation, after each instrument change, one of three irrigating solutions was used: 2.5% NaOCl, 1.0% NaOCl or 0.9% sterile saline. The experimental groups were divided in four groups: G1- 2.5% NaOCl; G2- 1.0% NaOCl; G3- 0.9% sterile saline and G4- Control. The roots of the control group had the pulps removed and the root canal remained exposed to the oral cavity until the sacrifice of the animals.

After biomechanical preparation, a #30 K-file was once again used to remove dentin chips left in the apical foramen during instrumentation. After preparation, the root canals were irrigated, aspirated and dried, and 17% EDTA was placed and agitated for 3 min with a lentulo spiral. Irrigating solution was finally used for irrigation and the root canals were dried with sterile absorbent paper points (Tanari Industrial Ltda., Manaus, AM, Brazil).

The root canals of the Groups 1, 2 and 3 were filled with a gutta-percha points and Sealapex (Sybron Kerr, Romulus, Michigan, USA) using active lateral condensation technique, followed by radiographic confirmation. The crown openings were sealed with IRM® (Dentsply Ltda.) and amalgam (SS White Ltda.). The control group were not filled and remained exposed to the oral cavity until the sacrifice of the animals.

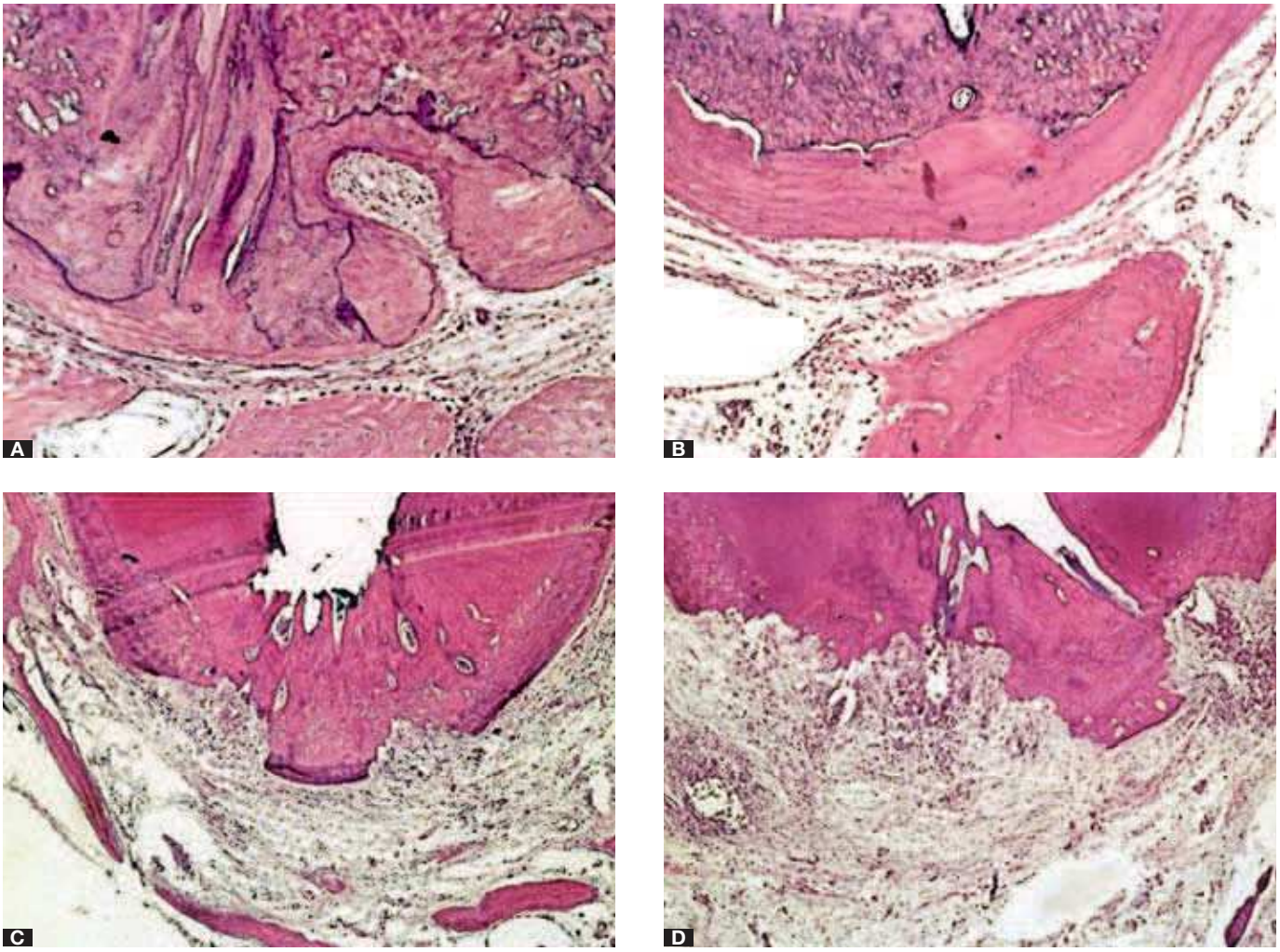

Figure 1. A) 2.5% sodium hypochlorite: Closure of apical ramifications with new cementum and periodontal ligament with few chronic inflammatory cells (H.E., 100x). **B**) 1.0% sodium hypochlorite: New cementum recovering the apex and the sealing of the apical ramifications, but the periodontal ligament shows few chronic inflammatory cells (H.E., 100x). **C**) Saline: Absence of new cementum, presence of resorption areas and severe inflammatory infiltrate (H.E., 40x). **D**) Control: Presence of chronic periapical lesion and resorption areas.

The animals were sacrificed with anesthetic overdose after 6 months and the maxilla and mandible were removed, fixed in 10% buffered formalin solution for 48 hours and demineralized in formic acid and sodium citrate solution. Blocks including the teeth and surrounding tissues were produced, and the specimens were embedded in paraffin wax, serially sectioned at 6 µm intervals, stained with hematoxylin and eosin (H.E.) and Brown and Brenn techniques. They were examined under light microscopy by a skilled examiner blinded to the groups.

The histomorphological parameters were examined and scored 1 to 4, 1 being the best result and 4 the worst, according to previous established criteria.[19] Data were submitted to statistical analysis by Kruskal Wallis tests at 5% significance level.

Results

Group 1 - 2.5% NaOCl solution

Connective tissue with cementum recovering the dentinal walls was observed in five cases. The connective tissue showed the presence of acute inflammatory infiltrated in 2 cases. In 2 specimens there was absence of acute inflammatory infiltrated while in the remaining it was observed a chronic inflammatory infiltrated of variable intensity. Complete biological sealing (complete sealing of the apical foramens with new cementum) was noted in only 2 cases and was completely absent in 2 specimens. The newly formed cementum covered all of the resorption areas except in 1 specimen. The periodontal ligament was completely organized in only 2 specimens. Brown and Brenn staining detected micro-organisms in 4 specimens.

Group 2 – 1.0% NaOCl solution

Connective tissue with cementum recovering the dentinal walls was observed in five cases. The connective tissue showed the presence of chronic inflammatory infiltrated of variable intensity in 6 cases. The new cementum was formed with a mean thickness of 84 µm which covered all of the resorption areas except in 1 specimen. The periodontal ligament presented completely organized in 4 specimens. Brown and Brenn staining detected microorganisms in 3 specimens.

Group 3 – 0.9% sterile saline

It was observed in 4 specimens that almost half of the delta canals presented invagination in the connective tissue infiltrated by inflammatory cells. In the remaining specimens, a connective tissue without inflammatory infiltrated and the presence of deposition of new cementum was observed. In 2 cases in which new cementum was not observed an active bone resorption was noted. The periodontium was completely organized in 2 specimens. Brown and Brenn staining detected microorganisms in 8 specimens.

Group 4 – Control group

The control group was characterized by the presence of chronic periapical lesion which involved the whole area between the apex of the tooth and the surrounding bony tissue with the presence of active resorption areas in the apical cement. Brown and Brenn staining detected microorganisms in 8 specimens.

Comparison among the groups

Statistical analysis showed that the best histological results were observed in Groups 1 and 2, which received 2.5% NaOCl and 1.0% NaOCl as irrigating solution respectively with no significant difference ($p>0.05$). However, between them and Group 3, which received saline as irrigating solution, and Group 4 (Control) there was a significant difference ($p<0.05$).

Table 1. Distribution of specimens in the groups according to the scores of the histomorphological parameters.

Histomorphological parameters	Scores	Groups			
		G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)
Thickness of newly formed cementum	1 - more than 60 µm	6	9	7	0
	2 - from 20 to 59 µm	4	1	1	0
	3 - from 1 to 19 µm	0	0	0	0
	4 - absence	0	0	2	10
Extension of newly formed cementum	1 - repair of all of the resorption areas or recovering of the pre-existent cementum	9	9	8	0
	2 - 1/2 to 2/3 repair of the resorption areas	1	0	0	0
	3 - 1/3 or less repair of the resorption areas	0	1	0	0
	4 - absence of cementum repairing resorption areas	0	0	2	10
Closure of the apical delta by cementum	1 - complete	2	5	2	0
	2 - complete in most of the ramifications	4	4	2	0
	3 - complete in few ramifications	2	0	4	0
	4 - absence	2	1	2	10
Cementum resorption	1 - absence or resorption areas completely repaired	9	9	8	0
	2 - resorption areas partially repaired	1	0	0	0
	3 - non-repaired resorption areas	0	0	0	0
	4 - active resorption areas	0	1	2	10
Bone resorption	1 - absence	10	10	6	0
	2 - inactive areas	0	0	3	0
	3 - few active areas	0	0	2	0
	4 - many active areas	0	0	0	10

Acute inflammatory infiltrate (intensity)	1 - absent or few cells	8	8	5	0
	2 - small: less than 50 inflammatory cells	0	2	4	0
	3 - moderate: between 50 and 250 inflammatory cells	1	0	1	10
	4 - severe: more than 250 inflammatory cells	1	0	0	0
Acute inflammatory infiltrate (extension)	1 - absent or few cells	8	8	5	-
	2 - small: less than 50 inflammatory cells	2	2	4	-
	3 - moderate: between 50 and 250 inflammatory cells	0	0	1	10
	4 - severe: more than 250 inflammatory cells	0	0	0	0
Chronic inflammatory infiltrate (intensity)	1 - absent or few cells	2	4	2	0
	2 - small: less than 50 inflammatory cells	3	3	1	0
	3 - moderate: between 50 and 250 inflammatory cells	1	1	5	0
	4 - severe: more than 250 inflammatory cells	4	2	2	10
Chronic inflammatory infiltrate (extension)	1 - absent or few cells	2	4	2	0
	2 - small: less than 50 inflammatory cells	6	3	2	0
	3 - moderate: between 50 and 250 inflammatory cells	1	3	4	0
	4 - severe: more than 250 inflammatory cells	1	0	2	10
Apical periodontal ligament space	1 - up to 200 µm	3	3	1	0
	2 - from 201 to 300 µm	3	5	3	0
	3 - from 301 to 400 µm	4	1	1	0
	4 - above 401 µm	0	1	5	10
Organization of periodontal ligament	1 - inserted from the cementum to the bone in the entire apical portion	2	4	2	0
	2 - inserted partially from the cementum to the bone of the apical portion	6	5	5	0
	3 - parallel to the surface of the tooth	0	0	1	0
	4 - without organization	2	1	2	10
Limit of filling	1 - 2 mm before the apical opening	0	1	0	-
	2 - at the level of the apical opening	8	7	8	-
	3 - beyond the apical opening	1	1	1	-
	4 - overfilling	1	1	1	-
Presence of debris	1 - absence	8	8	6	-
	2 - discreet presence	1	2	4	-
	3 - moderate presence	1	0	0	-
	4 - intense presence	0	0	0	-
Presence of giant cells	1 - absence	9	9	7	10
	2 - discreet - 1 to 3 cells	0	1	2	0
	3 - moderate - 4 to 6 cells	0	0	1	0
	4 - severe - 7 or more cells	1	0	0	0
Presence of microorganisms	1 - absent	6	7	2	0
	4 - present	4	3	8	10
Group x Group*		a	a	b	c

* Different letters indicate statistically significance at 5% (Kruskal-Wallis test).

G1: 2.5% NaOCl; G2: 1.0% NaOCl; G3: Saline; G4: Control.

Discussion

Sodium hypochlorite, in high concentrations, has a potent antimicrobial action due to the release of a large number of secondary chlorates, leading to greater tissue dissolution, thus being recommended for treatment of teeth with periapical lesions.[17] According to the present histopathological study, similar results were obtained with both concentration of NaOCl solutions (2.5% and 1%), which may be due to the fact that NaOCl solutions have more direct performance just on the bacteria contained in the main canal, but without action in the root canal

system as a whole, specially in the ramifications of the apical delta and inside the dentinal tubes. Some studies have demonstrated the reinfection of the main canal some days after obtaining high index of negative bacteriological tests with the instrumentation.[20,21]

On the other hand, although NaOCl has limited action in the root canal system as a whole, its performance promoted better results when compared with the 0.9% sterile saline solution. This result can be justified by the absence of microorganisms in most of the specimens where NaOCl solutions were used.

Several *in vitro* studies have been performed to check the antibacterial activity of NaOCl and showed that 4% NaOCl is effective against Enterococcus faecalis;[22] 4% NaOCl and 2.5% NaOCl were significantly greater than other tested agents;[23] there was no difference in the antibacterial activity of 1%, 2.5%, and 5% NaOCl.[18] These results corroborate with the present study that show no significant difference among the 1% and 2.5% NaOCl.

Although the present study has demonstrated the importance of the use of irrigants with antibacterial activity in the treatment of teeth with the presence of bacteria in the root canal, an ideal solution should act not only directly on the bacteria but also on the bacterial endotoxin. It has been demonstrated that endotoxin is present in higher concentration in infected teeth, mainly when there is chronic periapical reaction.[24] Although the NaOCl solutions can inhibit the action of some endotoxin, it is admitted that the NaOCl is not effective against all of them.[25] Therefore, the initial disinfection should be maintained or enlarged with the root canal dressing and the filling material.

The presence of the smear layer formed after the biomechanical preparation can also explain the similarity of results among those studies that evaluated different concentrations of NaOCl, wich is ineffective to remove it.[26-29]

NaOCl is also able to bleach the dentin and to dissolve organic material. These properties can increase the permeability[30,31] and decrease the superficial tension facilitating the diffusion of the ions of the filling material in the apical ramifications of the root canal,[32] which could lead to the best results in the groups where it was used.

Besides the irrigating solutions, the final result of the present study was conditioned to the use of filling material. Sealapex was used due to its biological properties and ability to stimulate deposition of mineralized tissue in the apical foramen.[33] Apical and periapical repair of dogs' teeth with chronic periapical lesion has been shown to occur with the use of Sealapex.[34,35]

In the present study, when a single-visit treatment of teeth with chronic periapical lesion was performed, it was demonstrated that the use of irrigating solutions with antibacterial potential provided more favorable conditions for the development of the healing process.

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposure of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965;18:340-8.
2. Moller AJ, Fabricius L, Dahlén G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res. 1981;89(6):475-84.
3. Sundqvist G. Ecology of the root canal flora. J Endod. 1992;18:427-30.
4. Silva L, Nelson-Filho P, Leonardo MR, Rossi MA, Pansani RA. Effect of calcium hydroxide on bacterial endotoxin in vivo. J Endod. 2002;28(2):94-8.
5. Leonardo MR, Almeida WA, Silva LAB, Utrilla LS. Histopathological observations of periapical repair in teeth with radiolucent areas submitted to two different methods of root canal treatment. J Endod 1995;21(3):137-41.
6. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J. 1997;30:297-306.
7. Ferraz CC, Gomes NV, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. Apical extrusion of debris and irrigants using two hand and three engine-driven instrumentation techniques. Int Endod J 2001;34(5):354-8.
8. Lambrianidis T, Tosounidou E, Tzoanopoulou M. The effect of maintaining apical patency on periapical extrusion. J Endod. 2001;27:696-8.
9. Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endod. 1988;14(3):125-7.
10. Andersen M, Lund A, Andreasen JO, Andreasen FM. In vitro solubility of human pulp tissue in calcium hydroxide and sodium hypochlorite. Dent Traumatol. 1992;8(3):104-8.
11. Estrela C, Estrela CRA, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113-7.
12. Barbosa SV, Safavi KE, Spangberg SW. Influence of sodium hypochlorite on the permeability and structure of cervical human dentine. Int Endod J. 1994;27:309-12.
13. Jiménez-Rubio A, Segura JJ, Llmas R, Jiménez-Planas A, Guerrero JM, Calvo JR. In vitro study of the effect of sodium hypochlorite and glutaraldehyde on substrate adherence capacity of macrophages. J Endod. 1997;23(9):562-4.
14. Chang YC, Huang FM, Tai KW, Chou MY. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92:446-50.
15. Georgopoulou M, Kontakiotis E, Nakou M. Evaluation of the antimicrobial effectiveness of citric acid and sodium hypochlorite on the anaerobic flora of the infected root canal. Int Endod J. 1994;27(3):139-43.
16. Ohara P, Torabinejad M, Kettering JD. Antibacterial effects of various endodontic irrigants on selected anaerobic bacteria. Endod Dent Traumatol 1993;9(3):95-100.
17. Ayhan H, Sultan N, Cirak M, Ruhi MZ, Bodur H. Antimicrobial effects of various endodontic irrigants on selected microorganisms. Int Endod J. 1999 32(2):99-102.
18. Siqueira JF Jr, Rôças IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. J Endod. 2000;26(6):331-4.
19. Bernabe PF, Holland R, Morandi R, Souza V, Nery MJ, Otoboni Filho JA, Dezan E Junior, Gomes-Filho JE. Comparative study of MTA and other materials in retrofilling of pulpless dogs' teeth. Braz Dent J. 2005;16(2):149-55.
20. Shih M, Marshall JF, Rosen S. The bactericidal efficiency of sodium hypochlorite as an endodontic irrigant. Oral Surg Oral Med Oral Pathol. 1970;29(4):613-9.
21. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J. 1985;18(1):35-40.
22. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. Int Endod J. 1997;30(4):279-82.
23. Siqueira JF Jr, Batista MM, Fraga RC, Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. J Endod 1998;24(6):414-6.
24. Pitts DL, Williams BL, Morton TH Júnior. Investigation of the role of endotoxin in periapical inflammation. J Endod. 1982;8(1):10-8.
25. Buttler TK, Crawford JJ. The detoxifying effect of varying concentrations of sodium hypochlorite on endotoxins. J Endod. 1982;8(2):59-66.
26. Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endod. 1987;13(4):147-57.
27. Cengiz T, Aktener BO, Piskin B. The effect of dentinal tubule orientation on the removal of smear layer by root canal irrigants. A scanning electron microscopic study. Int Endod J. 1990;23(3):163-71.
28. Goldman LB, Goldman M, Kronman J, Lin P. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol. 1981;52(5):197-204.
29. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod. 1975;1(7):238-42.
30. Andersen M, Lund A, Andreasen JO, Andreasen FM. In vitro solubility of human pulp tissue in calcium hydroxide and sodium hypochlorite. Endod Dent Traumatol 1992;83:104-8.
31. Estrela C, Estrela CRA, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113-7.
32. Taşman F, Cehreli ZC, Oğan C, Etikan I. Surface tension of root canal irrigants. J Endod. 2000;26(10):586-7.
33. Holland R, Souza V. Ability of a new calcium hydroxide root canal filling material to induce hard tissue formation. J Endod. 1985;11:535-43.
34. Tanomaru Filho M, Leonardo MR, Silva LAB, Utrilla LS. Effect of different root canal sealers on periapical repair of teeth with chronic periradicular periodontitis. Int Endod J 1998;31(2):85-9.
35. Leonardo MR, Almeida WA, Silva LAB, Utrilla LS. Histopathological observations of periapical repair in teeth with radiolucent areas submitted to two different methods of root canal treatment. J Endod. 1995;21(3):137-41.