Reduction in Bacterial Loading Using 2% Chlorhexidine Gluconate as an Irrigant in Pulpectomized Primary Teeth: A Preliminary Report

Ruiz-Esparza CL* / Garrocho-Rangel A ** / Gonzalez-Amaro AM *** / Flores-Reyes H **** / Pozos-Guillen AJ *****

Objective: *The aim of this study was to evaluate the reduction in bacterial loading using 2% chlorhexidine gluconate as an irrigating solution in pulpectomized primary teeth.* ***Study design:*** *A randomized, controlled clinical trial was performed that included primary teeth with pulp necrosis. Forty necrotic teeth were included, 20 irrigated with 2% chlorhexidine gluconate (experimental group) and 20 with sterile saline solution (control group); in all cases, 2 microbiological samples from within the canals were taken with sterile paper points, the first after the canal opening and before the first irrigation, and the second after instrumentation and final irrigation, before filling. All samples were evaluated by McFarland's scale.* ***Results:*** *The results were statistically analyzed by the Mann-Whitney U test. After analyzing samples before and after irrigation in the control group (saline), we found a significant decrease of bacterial load ($P < 0.0002$). The same occurred in the chlorhexidine group samples ($P < 0.0001$). When both groups were compared post-irrigation, a statistically significant difference was observed in favor of 2% chlorhexidine gluconate.* ***Conclusion:*** *Two percent chlorhexidine gluconate showed a greater reduction of intracanal bacterial loading compared with that observed with sterile saline solution. This irrigating solution is suggested as an alternative for pulpectomy of necrotic primary teeth.*

Keywords: *Pulpectomy, chlorhexidine, irrigating solution, primary teeth, deciduous.*
J Clin Pediatr Dent 35(3): 265–270, 2011

INTRODUCTION

One of the fundamental steps in a pulpectomy treatment in primary teeth is the bio-disinfection of the pulp root canal system by means of mechanical instrumentation and profuse irrigation. The main objective is the reduction of the pathogenic bacterial load to the minimum level within the root canals.[1-4]

In general, the rationale to irrigate is: (1) removal of the remains of inflamed or infected pulp tissue, shavings or dentin debris (smear layer), blood, exudates, food, and medications; (2) instituting detergent, antiseptic, and bleaching action; and (3) hydration and lubrication of the canal walls during instrumentation. Various techniques, irrigating solutions, and root canal filling materials have been used for treatment of chronically pulp-infected deciduous teeth. A number of solutions have also been used for irrigating the primary root canals during and after root canal preparation. The most widely used are physiological saline solution[5-7] and sodium hypochlorite (NaOCl).[8,9] Also, 10% urea peroxide with 15% EDTA,[10] Endo PTC combined with Dakin's solution,[11] and a mixture of NaOCl and hydrogen peroxide (H_2O_2)[12] have been used. Although NaOCl is both an antiseptic and a solvent,[13] its use has been controversial in primary teeth because of its citotoxicity and potential for injury to permanent tooth germs when it reaches the periapical tissues, due to the apical width of the of these teeth; besides, its smell and taste are unpleasant, exhibits a corrosive effect, and some allergic reactions have been reported.[14,15]

On the other hand, chlorhexidine is a potent antiseptic irrigant possessing low surface tension. It is commonly used

* Claudia L Ruiz-Esparza, DDS Resident, Pediatric Dentistry Posgraduate Program, Facultad de Estomatologí a, Universidad Autó noma de San Luis Potosí , Mé xico.
** Arturo Garrocho-Rangel, DDS, MS Associate Professor, Pediatric Dentistry Postgraduate Program, Facultad de Estomatologí a, Universidad Autó noma de San Luis Potosí , Mé xico.
*** Ana Ma Gonzalez-Amaro, MS Associate Professor, Endodontics Postgraduate Program, Facultad de Estomatologí a, Universidad Autó noma de San Luis Potosí , Mé xico.
**** Hector Flores-Reyes, DDS, PhD Associate Professor, Endodontics Postgraduate Program, Facultad de Estomatologí a, Universidad Autó noma de San Luis Potosí , Mé xico.
***** Amaury J Pozos-Guillen, DDS, PhD Associate Professor, Pediatric Dentistry Postgraduate Program and Endodontics Postgraduate Program, Facultad de Estomatologí a, Universidad Autó noma de San Luis Potosí , Mé xico.

Send all correspondence to: Amaury de Jesús Pozos Guillé n, Facultad de Estomatologí a, Universidad Autó noma de San Luis Potosí , Av. Dr. Manuel Nava #2, Zona Universitaria, C.P.78290; San Luis Potosí , SLP. Mexico.

Tel: 52 (444) 8262357 X 114.
Fax: 52 (444) 8139743.

in root canal therapy of permanent teeth because of its superior ability to penetrate accessory canals and dentin tubules to a depth of 100 µm without exhibiting caustic effects.[16-19] Chlorhexidine has also shown to be bacteriostatic and bactericidal for 48–72 hours against a wide range of aerobic and anaerobic bacteria such as *E faecalis, S aureus, and P intermedia*,[20,21] as well as *C albican*s, spores, and viruses.[22-24] It is biocompatible, almost non toxic, and is active against lipopolysaccharides.[16,25] Because of these properties, chlorhexidine is considered a highly effective intracanal irrigant in the endodontic treatment of permanent teeth, although its potential for use in pulpectomized primary teeth has not been completely evaluated. Therefore, the aim of this study was to evaluate the reduction in bacterial loading using 2% chlorhexidine gluconate as an irrigating solution in pulpectomized primary teeth.

MATERIALS AND METHODS

Study design and patients

Patients were recruited from the Pediatric Dentistry Postgraduate clinic at the Facultad de Estomatologia, San Luis Potosi University, Mexico. The study was approved by the ethics committee of the university. The objective of the study was explained to either the parents or legal guardians, and written informed consent was obtained. This controlled, randomized clinical trial included 40 patients of both sexes between 3 and 9 years old.

Inclusion criteria were as follows:

- Patients in good general health
- Primary teeth (anterior or posterior) containing at least one necrotic pulp canal, abscess, or sinus tract
- Presence of radiolucent area(s) in furcation or periapical region
- At least two thirds of root remaining
- Carious lesion(s) without direct exposure to the oral environment
- Sufficient tooth structure to support a rubber dam
- Sufficient isolation and sterility control in operative field to demonstrate no bacterial growth

Patients who had received antibiotics up to 2 weeks prior to the sampling or having any systemic compromise, nonrestorable teeth, perforated pulpal floor, excessive mobility, or pathological root resorption were excluded. Forty canal treatments were performed in necrotic primary teeth: 20 belonged to the experimental group, which were irrigated with 2% chlorhexidine gluconate (Consepsis, Ultradent Products Inc, South Jordan, UT, USA), and 20 to the control group, which were irrigated with sterile saline solution (Solución CS, Laboratorios PISA, S.A. Guadalajara, Mexico). All treatments were performed in a single visit. Sample size was calculated on the basis of a pilot study, consisting of 10 microbiological samples taken from necrotic primary root canals (5 corresponding to each irrigating solution), which were not included in the statistical analysis. Likewise, consistency and reliability tests for the diagnostic and results evaluator were carried out in an independent manner by means of an unweighted Kappa test, which resulted in a score of 0.90.[26] The sampling of patients was realized non probabilistically (consecutive cases), and the irrigant selected for each case was made from a list of random numbers generated by a computer.

Preclinical laboratory procedures

Prereduced thioglycolate tubes, supplemented with hemin (5 mg L-1) and menadione (1 mg L-1) (Oxoid LTD, Basingstoke, Hampshire, UK), were used as transport and growth media owing to their capacity to maintain the vitality of sampled bacteria.[27]

Isolation and operative field disinfection

The study procedure was performed by a single pediatric dentist; periapical radiographs of the selected teeth were taken using a standard paralleling technique. After antisepsis of the oral cavity, local anesthesia was induced using an inferior alveolar nerve block for the primary mandibular teeth and infiltration (palatal and buccal) for the primary maxillary teeth. Each treated tooth was cleaned with pumice and isolated with a rubber dam. Provisit (Casa Idea, SA de CV, Mexico) was placed along the tooth–rubber dam interface to prevent leakage of saliva into the operative field. To disinfect the operative field, we followed the protocol previously described.[28,29] Briefly, the tooth crown, surrounding rubber dam, and clamp were swabbed with 30% H_2O_2 (Fermont, Productos Quimicos, Monterrey, Mexico), followed by 5.25% NaOCl for 1 minute each; both solutions were inactivated with 10% sodium thiosulfate. Disinfection control samples were taken with sterile cotton pellets from the coronal surface of the tooth, rubber dam, and clamp, and immediately inoculated on blood agar plates (BBL, Becton Dickinson, Cuautitlan, Izcalli Mexico). The samples were then transferred to an aerobic incubator at 37º C for 48 hours.

The gross carious tissue was removed with a sterile round carbide bur (No. 3) cooled with sterile saline solution. The cavity and field were again disinfected as above. Then the pulpal roof was removed using a new bur of the same size, a sterile cotton pellet was placed on the floor of the pulp chamber to prevent penetration of disinfectants into the canals, and the root canal was accessed.

Collection of microbiological samples

Once the canals were exposed and after the canal's length was estimated using the preoperative periapical radiograph, the first microbiological sample was obtained from inside the canal (pre-irrigation); then 3 sterile absorbent paper points of a size compatible with the root canal diameter were sequentially placed for 30 seconds. If the canal was dry, then a small amount of sterile saline was used to wet the canal before the points were inserted. The retrieved paper points were immediately placed into the tube with thioglycolate. After sample collection, all teeth were treated conventionally. The usual instrumentation was done with FlexoFiles

(Dentsply Maillefer, Balaigues, Switzerland), together with one irrigation of 0.5 ml of the selected solution between each file. At the end of the instrumentation and before filling, the canal was irrigated for the last time and dried. At that time, a second microbiological sample was taken from the same canal, as previously described, with another 3 paper points; in the case of chlorhexidine, this last step was carried out after its inactivation with 10 mL sterile saline solution. Finally, the canal was filled with an iodoform paste (Vitapex®), and a postoperative X-ray was taken.

Laboratory procedures

The pre- and post-irrigation samples obtained were incubated for 24 hours at 37°C in an anaerobic chamber (85% N_2, 10% H_2, 5% CO_2) (Coy Laboratory Products, Grass Lake, MI, USA). Later, the bacteria present in these samples were counted by the turbidimetry method, through McFarland's scale pattern. This method estimates the number of bacteria in suspension (as CFU/mL) according to the degree of turbidity or density displayed by the different values of the scale.[30,31]

In addition, samples of the operative field (tooth crown, rubber dam, and clamp) in blood agar were also assessed, using the same method described above, to verify complete disinfection of the field before elimination of the carious tissue.

Before the microbiological phase of this research, McFarland's measurement scale of 10 degrees was developed in the laboratory.

Statistical analysis

Intra-group and inter-group comparisons (pre-irrigation samples *versus* post-irrigation samples) were performed. A nonparametric statistical analysis (Mann-Whitney *U* test) was carried out for analyzing the differences between groups. Alpha level was set at 0.05. The JMP IN v. 4.0.1(SAS Institute Inc, Cary, NC, USA) statistical program was used to analyze the data.[32]

RESULTS

In all, 40 canal treatments were performed on the pediatric patients, whose average age was 6.4 years. Of these, 80 microbiological samples were obtained: 40 from the experimental group (20 pre- and 20 post-irrigation) and 40 from the control group (20 pre- and 20 post-irrigation).

Basal conditions

Basal conditions (pre-irrigation) were similar in both groups in relation to the amount of bacteria present in necrotic canals. The number of colony-forming units (CFU)/mL from the pre-irrigation samples were quantified and compared. Analysis exhibited a *P* value of 0.2992 (Mann-Whitney *U* test), indicating that there was no statistical difference between the groups at the beginning of the study (ie, they were homogeneous before debridement).

Experimental group: before versus after irrigation

In the pre-irrigation samples corresponding to the experimental group, a median of 1.35×10^9 CFU/mL (range: 9×10^8 to 2.7×10^9) was obtained, with a mean of $1.5 \times 10^9 \pm 5.2 \times 10^7$ CFU/mL. For post-irrigation samples in the same group, a median of 0 CFU/mL (range: 0 to 1.5×10^7) was obtained, with a mean of $1.5 \times 10^6 \pm 4.6 \times 10^6$ CFU/mL (Figure 1). The difference between bacterial counts, or CFU/mL, before and after irrigation was statistically significant ($P < .0001$, Mann-Whitney *U* test).

Control group: before versus after irrigation

In the pre-irrigation samples corresponding to the control group, a bacterial count median of 1.65×10^9 CFU/mL (range: 9×10^8 to 2.7×10^9) was obtained, with a mean of $1.68 \times 10^9 \pm 4.7 \times 10^7$ CFU/mL. Post-irrigation, a median of 9×10^8 CFU/mL (range: 3×10^8 to 2.1×10^9) was obtained, with a mean of $1.06 \times 10^9 \pm 4.6 \times 10^7$ CFU/mL (Figure 2). The difference between bacterial counts, or CFU, before and after irrigation was considered statistically significant ($P < .0002$, Mann-Whitney *U* test).

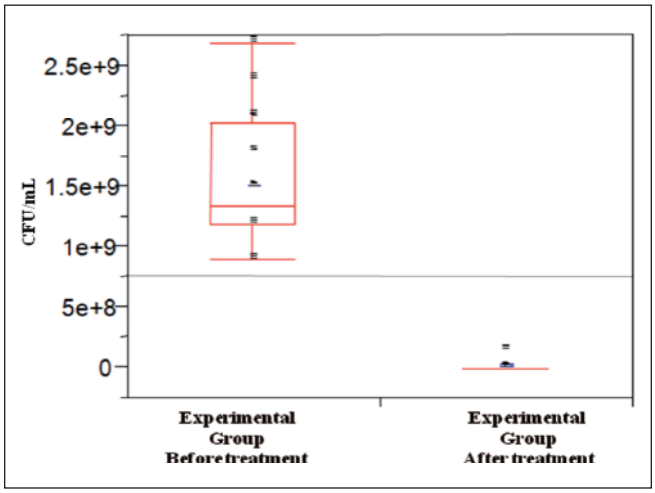

Figure 1. Box plots comparing bacterial quantifications before and after irrigation in the experimental group (2% chlorhexidine gluconate).

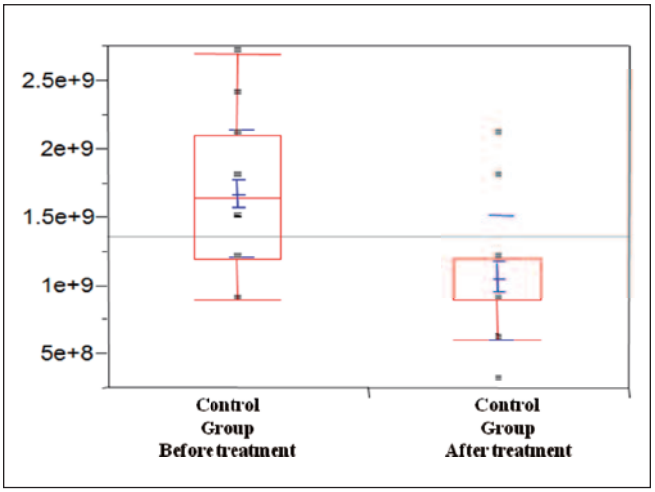

Figure 2. Box plots comparing bacterial quantifications before and after irrigation in the control group (sterile saline solution).

After irrigation: experimental versus control

Finally, the antimicrobial ability of both irrigating solutions employed in the study was compared through bacterial quantification, or CFU/mL, after irrigation. Analysis showed a significant difference in favor of the experimental group ($P < .0001$, Mann-Whitney U test).

DISCUSSION

One of the crucial requirements in pulp therapy is to disinfect the root canal before filling. Although mechanical instrumentation and the antiseptic properties of filling materials significantly reduce bacterial levels of necrotic canals, it is necessary use irrigating solutions to aid in the removal of the microbiota in areas inaccessible to instrumentation. Therefore, the clinician must pay particular attention to the biomechanical preparation of the complex pulp canal system characteristic of primary teeth (with multiple accessory canals and ramifications) to reduce the bacteria and their byproducts to a minimum, thus increasing the chances of a successful pulpectomy.[15] Anaerobic bacteria, mainly gram negative, have been identified in necrotic primary teeth; facultative mircroorganisms such as *E faecalis, S aureus*, and *C albicans* are considered highly resistant species and therefore possible causes of failed root canal treatments.[33]

Several studies have compared the properties of currently used endodontic irrigants. Most of these studies have been carried out on permanent teeth. Few have reported on the antimicrobial effect of irrigating solutions employed on primary root canals. The only study that reported the effect of chlorhexidine on primary pulp canals was done by Pascon *et al* who assessed the influence of 5 different irrigants (1% and 2% chlorhexidine, Dakin´ s solution, Dakin´ s solution + urea peroxide, and distilled water) on the permeability index (PI) of primary root dentin. They concluded that chlorhexidine, on its 2 concentrations, provided the highest permeability and penetration, both in root length and dentin depth. However, unlike our study, they did not quantify the bacterial residual load after irrigation with the tested solutions.[34]

Delany *et al* carried out a study similar to ours, but done *in vitro* and on recently extracted permanent teeth. The teeth were endodontically treated simulating clinical conditions, and when the effects of chlorhexidine and saline solution as control were compared, a significant difference was found ($P < 0.0049$) insofar as reduction of microorganisms when chlorhexidine was used as an irrigant after instrumentation. Their results were similar to ours, although in their case the dependent variable was measured in CFU/mL.[35]

Another study compared 2% chlorhexidine gluconate and NaOCl as intracanal irrigants in 62 extracted permanent teeth with pulp pathology; use of chlorhexidine resulted in fewer CFU than with NaOCl, although the difference was not statistically significant.[36] White *et al* compared 2 concentrations of chlorhexidine (2.0% and 0.12%) against *S. mutans* and demonstrated that the antimicrobial activity of chlorhexidine in both concentrations was maintained significantly for 72 hours. They, as in our study, irrigated after instrumentation and later inactivated the chlorhexidine with sterile water; they also obtained samples with paper points, although they collected more samples (6, 12, 24, 48, and 72 h) after treatment. Their results showed that 2% chlorhexidine maintains substantial antimicrobial activity when used as an endodontic irrigant.[37]

Leonardo *et al* measured the antimicrobial activity of 2% chlorhexidine in 22 root canals of 12 adult patients. They obtained samples with sterile paper points at the time of the canal opening. After instrumentation and irrigation, they sealed the root canal entrance for 48 hours, then withdrew the seal and took a second sample from the canal. When the samples were submitted for microbiological evaluation, they showed that the residual effect of chlorhexidine had lasted 48 hours.[16] On the other hand, Tanomaru Filho *et al* determined that the inflammation caused by chlorhexidine as an irrigating solution was not significant.[15]

Weber *et al* compared the residual antimicrobial activity in 94 root canals, using chlorhexidine, NaOCl, and buffered saline (as control); chlorhexidine was shown to be superior to the other 2 irrigants ($P < 0.001$), and its activity lasted up to 168 hours.[19] In another study,[38] using 24 infected and necrotic teeth with resorbing apical lesions (conventionally treated) and using pre-reduced thioglycolate as a storage medium. Chlorhexidine was found to be superior to the control irrigant ($P < 0.05$). However, they used a longer incubation time, which in our case was considered unnecessary, according to the results of the pilot study. Pre-reduced thioglycolate has been used to transfer microbiological samples from infected root canals to the laboratory, as suggested by Carlsson *et al* [39] This medium reduces oxygen, preventing the accumulation of superoxide radicals that would kill anaerobic bacteria; also, it contains small amounts of agar that prevent diffusion of oxygen into the medium.

Two other studies also showed similar antimicrobial activity of chlorhexidine compared with NaOCl. Their authors concluded that antimicrobial action is related to the type, concentration, and presentation form of the irrigants as well as the microbial susceptibility.[21,23] Likewise, Evanov *et al* noted that increasing the temperature of chlorhexidine from 37°C to 46°C causes significantly less growth of *E. faecalis* compared with use of saline solution, which was not associated with any increase in bactericidal effect even with the same temperature rise.[40]

An essential step in endodontic microbiology studies is the design and implementation of a preoperative disinfection protocol of the field, so that the taking of microbiological samples is carried out in the most aseptic possible conditions, avoiding the contamination that might confuse the results.[28] The disinfection protocol used in this study was a modification of the one described by Ng *et al.*[41]

Three endodontic studies shared the same operative field disinfection protocol that was used in our study. Wang *et al* concluded that 2% chlorhexidine in the form of a gel is an effective disinfectant of root canals, although as an intracanal drug it showed no significant difference in relation to

calcium hydroxide.[24] Vianna *et al* established *in vivo* that NaOCl exhibited a greater capacity to eliminate microorganisms and to remove cells from the root canal.[42] Siqueira *et al* employed 0.12% chlorhexidine solution alone as an irrigant, and together with calcium hydroxide as an intracanal drug for 7 days; their findings suggest that reduction of bacteria was significant using both preparations.[43]

The findings of this study demonstrate that 2% chlorhexidine reduced substantially the bacterial load in the root canals of deciduous teeth. Only a few *in vivo* studies have investigated the antimicrobial efficacy of chlorhexidine as an irrigant. In order to confirm these results, it will be necessary to carry out *in vitro* and *in vivo* studies. *In vitro* studies should will be consider the use of extracted primary teeth in order to evaluate the capacity of irrigant to remove smear layer and biofilm, including a greater variety of microorganisms, and its capacity to penetrate into dentinal tubules; however, it is worth emphasizing that is extremely difficult to obtain extracted primary teeth with complete roots. On the other hand, randomized controlled clinical trials are necessary for to evaluate: (1) the clinical and radiographic evolution of the necrotic primary teeth treated with pulpectomy and irrigated with 2% chlorhexidine; (2) the efficacy of this solution, and (3) the success of this pulp procedure in the primary dentition.

CONCLUSION

On the basis of the results obtained in this preliminary report, 2% chlorhexidine gluconate showed a greater reduction of intracanal bacterial loading compared with that observed with sterile saline solution. This irrigating solution is suggested as an alternative for pulpectomy treatment of necrotic primary teeth.

ACKNOWLEGEMENT

The authors thank Norman Wahl for his assistance in editing the manuscript. This work was partially supported by FAI-UASLP C10-FAI-05-31.58, PIFI 2009 and CONACYT 103311 grants.

REFERENCES

1. Onç ağ O, Hoşgö r M, Hilmioğlu S, Zekioğlu O, Eronat C, Burhanoğlu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J, 36: 423–32, 2003.
2. Cohen S, Burns RC. "Las ví as de la Pulpa." 8th ed. Madrid, Spain: Mosby, 254–59, 819–24, 2002.
3. Regan JD, Fleury AA. Irrigants in non-surgical endodontic treatment. J Ir Dent Assoc, 52: 84–92, 2006.
4. Zehnder M. Root canal irrigants. J Endod, 32: 389–98, 2006.
5. O'Riordan MW, Coll J. Pulpectomy procedure for deciduous teeth with severe pulpal necrosis. J Am Dent Assoc, 99: 480–82, 1979.
6. Thomas AM, Chandra S, Chandra S, Pandey RK. Elimination of infection in pulpectomized deciduous teeth: a short-term study using iodoform paste. J Endod, 20: 233–35, 1994.
7. Razi RS. Pulp therapy in the primary dentition. N Y State Dent J, 65: 18–22, 1999.
8. Goering AC, Camp JH. Root canal treatment in primary teeth: a review. Pediatr Dent, 5: 33–37, 1983.
9 Canoglu H, Tekcicek MU, Cehreli ZC. Comparison of conventional, rotary, and ultrasonic preparation, different final irrigation regimens, and 2 sealers in primary molar root canal therapy. Pediatr Dent, 28: 518–23, 2006.
10. Rifkin A. A simple, effective, safe technique for the root canal treatment of abscessed primary teeth. ASDC J Dent Child, 47:435–41, 1980.
11. Guedes-Pinto AC, De Paiva JG, Bozzola JR. Tratamento endodô ntico de dentes decí duos com polpa mortificada. Rev Assoc Paul Cir Dent, 35: 240–44, 1981.
12. Garcia-Godoy F. Evaluation of an iodoform paste in root canal therapy for infected primary teeth. ASDC J Dent Child, 54: 30–34, 1987.
13. Leonardo M, Bezerra L, Campos A. Tratamiento endodó ntico de dientes temporales con vitalidad pulpar (biopulpectomí a). In: Bezerra L, ed. Tratado de Odontopediatrí a. 1st ed. Caracas: Amolca, 632–37, 2008.
14. Mehdipour O, Kleier DJ, Averbach RE. Anatomy of sodium hypochlorite accidents. Compend Contin Educ Dent, 2007: 544–46, 2007.
15. Tanomaru Filho M, Leonardo MR, Silva LA, Aní bal FF, Faccioli LH. Inflammatory response to different endodontic irrigating solutions. Int Endod J, 35: 735–39, 2002.
16. Leonardo MR, Tanomaru Filho M, Silva LA, Nelson Filho P, Bonifá cio KC, Ito IY. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endod, 25: 167–71, 1999.
17. Ryan S. Chlorhexidine as a canal irrigant: a review. Compend Contin Educ Dent, 31: 338–42, 2010.
18. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J, 42: 288–302, 2009.
19. Weber CD, McClanahan SB, Miller GA, Diener-West M, Johnson JD. The effect of passive ultrasonic activation of 2% chlorhexidine or 5.25% sodium hypochlorite irrigant on residual antimicrobial activity in root canals. J Endod, 29: 562–64, 2003.
20. Buck RA, Cai J, Eleazer PD, Staat RH, Hurst HE. Detoxification of endotoxin by endodontic irrigants and calcium hydroxide. J Endod, 27: 325–27, 2001.
21. Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ. In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 97: 79–84, 2004.
22. Lin S, Zuckerman O, Weiss EI, Mazor Y, Fuss Z. Antibacterial efficacy of a new chlorhexidine slow release device to disinfect dentinal tubules. J Endod, 29: 416–18, 2003.
23. Ercan E, Ozekinci T, Atakul F, Gü l K. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: in vivo study. J Endod, 30: 84–87, 2004.
24. Wang CS, Arnold RR, Trope M, Teixeira FB. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. J Endod, 33: 1283–89, 2007.
25. Silva LA, Leonardo MR, Assed S, Tanomaru Filho M. Histological study of the effect of some irrigating solutions on bacterial endotoxin in dogs. Braz Dent, J 15: 109–14, 2004.
26. Landis JR, Koch G. The measurement of observer agreement for categorical data. Biometrics, 33: 159–74, 1977.
27. Carlsson J, Sundqvist G. Evaluation of methods of transport and cultivation of bacterial specimens from infected dental root canals. Oral Surg Oral Med Oral Pathol, 49: 451–54, 1980.
28. Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod, 33: 114–18, 2007.
29. Siqueira JF Jr, Rô ç as IN. *Catonella morbid* and *Granulicatella adiancens*: new species in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 102: 259–64, 2006.
30. McFarland J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc, 49: 1176–78, 1907.
31. Koneman E, Allen S, Dowel V, Sommers H. Pruebas de susceptibilidad a los antimicrobianos. In: Koneman E, ed. Diagnó stico microbioló gico. 6th ed. D.F Mexico: Panamericana, 380–93, 1992.
32. JMP-IN Version 4.0.1 (Academic) Copyright 1989–2000 SAS Institute Inc. Cary, NC.

33. Gomes BP, Lilley JD, Drucker DB. Associations of endodontic symptoms and signs with particular combinations of specific bacteria. Int Endod J, 29: 69–75, 1996.
34. Pascon FM, Puppin-Rontani RM. The influence of cleansers on the permeability index of primary tooth root dentin. J Clin Pediatr Dent, 3: 93–97, 2006.
35. Delany GM, Patterson SS, Miller CH, Newton CW. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg Oral Med Oral Pathol, 53: 518–23, 1982.
36. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod, 20: 276–78, 1994.
37. White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod, 23: 229–31, 1997.
38. Zamany A, Safavi K, Spå ngberg LS. The effect of chlohexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 96: 578–81, 2003.
39. Carlsson J, Sunquist G. Evaluation of methods of transport and cultivation of bacterial specimens from infected dental root canals. Oral Surg Oral Med Oral Pathol, 49: 451–54, 1980.
40. Evanov C, Liewehr F, Buxton TB, Joyce AP. Antibacterial efficacy of calcium hydroxide and chlorhexidine gluconate irrigants at 37 degrees C and 46 degrees C. J Endod, 30: 653–57, 2004.
41. Ng YL, Spratt D, Sriskantharajah S, Gulabivala K. Evaluation of protocols for field decontamination before bacterial sampling of root canals for contemporary microbiology techniques. J Endod, 29: 317–20, 2003.
42. Vianna ME, Horz HP, Gomes BP, Conrads G. In vivo evaluation of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue. Int Endod J, 39: 484–92, 2006.
43. Siqueira JF Jr, Paiva SS, Rô ç as IN. Reduction in the cultivable bacterial populations in infected root canals by a chlorhexidine-based antimicrobial protocol. J Endod, 33: 541–47, 2007.