Clinical Efficiency of 2% Chlorhexidine Gel in Reducing Intracanal Bacteria

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Abstract

This study evaluated the clinical efficacy of 2% chlorhexidine (CHX) gel on intracanal bacteria reduction during root canal instrumentation. The additional antibacterial effect of an intracanal dressing (Ca[OH]$_2$ mixed with 2% CHX gel) was also assessed. Forty-three patients with apical periodontitis were recruited. Four patients with irreversible pulpitis were included as negative controls. Teeth were instrumented using rotary instruments and 2% CHX gel as the disinfectant. Bacterial samples were taken upon access (S1), after instrumentation (S2), and after 2 weeks of intracanal dressing (S3). Anaerobic culture was performed. Four samples showed no bacteria growth at S1, which were excluded from further analysis. Of the samples cultured positively at S1, 10.3% (4/39) and 8.3% (4/36) sampled bacteria at S2 and S3, respectively. A significant difference in the percentage of positive culture between S1 and S2 ($p < 0.001$) but not between S2 and S3 ($p = 0.692$) was found. These results suggest that 2% CHX gel is an effective root canal disinfectant and additional intracanal dressing did not significantly improve the bacteria reduction on the sampled root canals. (J Endod 2007;33:1283–1289)

Key Words

Apical periodontitis, chlorhexidine, disinfection, intracanal medication, microbiology

Apical periodontitis is an inflammatory process on the periradicular tissues caused by the microbes present inside of the root canal system (1, 2). It has been shown that anaerobes are the primary etiological agent of apical periodontitis (2). One of the most important objectives in successful treatment of apical periodontitis is the elimination or reduction of intracanal bacteria. There is a strong relationship between the long-term treatment success and root canal filled after negative culture in teeth with apical periodontitis (3, 4). Sjögren et al (4) showed complete periapical healing in 94% of teeth with apical periodontitis that yielded negative culture after root canal instrumentation. In contrast, the samples with positive culture before root canal obturation had only 68% of complete periapical healing.

Root canal disinfection can be accomplished by mechanical and chemical means. The mechanical microbial control phase involves root canal preparation using hand and/or rotary instruments. The chemical microbial control phase involves the addition of treatment with antimicrobial agents during and after instrumentation. Mechanical preparation can reduce intracanal bacteria significantly but does not predictably eradicate them (5–8). On average, 40% to 60% of root canals have no cultivable bacteria after mechanical root canal instrumentation with sodium hypochlorite (NaOCl) solution (6–8). To obtain a more predictable microbial control, placement of intracanal medicaments such as calcium hydroxide is often recommended (5, 7–10).

Despite the fact that NaOCl effectively reduces recovery of bacteria from root canals, it is caustic if accidentally expressed into the periapical area or adjacent structures (11). In addition, it has a strong bleaching effect and foul odor. Chlorhexidine (CHX) gluconate has been suggested as an alternative irrigating solution that could replace NaOCl. CHX is a cationic biguanide that seems to act by adsorbing onto the cell wall of microorganism resulting in leakage of intracellular components. At low concentration, it has bacteriostatic effect. Although it is at a high concentration, CHX is bactericidal because of precipitation and/or coagulation of intracellular constituents (12). Its optimal antimicrobial activity is at pH 5.5 to 7.0 (13). CHX has a broad-spectrum antimicrobial activity, targeting both gram-positive and gram-negative microbes (14, 15). In general, in vitro studies suggested that CHX and NaOCl have comparable antibacterial effect when used in similar concentration (15, 16). In addition, CHX appeared to be a promising agent to be used as a final irrigant (17, 18). In a clinical study, Zamany et al (17) showed that a 2% CHX solution, used as a final irrigant, significantly decreased bacterial loads in root canals that had been irrigated with sodium hypochlorite during canal preparation. Additional advantages of CHX are its retentive character in root canal dentin (19) and its relatively low toxicity (20). Despite its advantages, CHX activity is pH dependent and is greatly reduced in the presence of organic matter (13). Unlike sodium hypochlorite, it lacks tissue dissolving properties (21).

Two percent CHX in a gel formulation (Endogel; Itapetininga, SP, Brazil) was proposed as an alternative to 5.25% NaOCl solution for root canal disinfection (22). This lubricant is composed of a gel base (1% Natrosol), which is a nonionic, highly efficient, inert, water-soluble agent that does not have antimicrobial activity (22) and 2% CHX gluconate at pH 7.0. Ferraz et al (22) showed that CHX gel produced a cleaner root canal surface and had an antimicrobial ability comparable with that obtained with the other solutions (5.25% NaOCl and 2% CHX liquid) tested. It appeared that CHX in a gel form required a much longer time to kill microorganisms than the corresponding concentration in a liquid form (16). In addition, 2% CHX gel has also proposed as intracanal medication (23) and a sodium perborate's vehicle for intracoronal bleaching (24).

Despite its promising results from in vitro studies, there are not enough clinical studies published so far on the effectiveness of 2% CHX gel. The objective of this study was to evaluate the effectiveness of 2% CHX gel to eradicate endodontic infection in patients with apical periodontitis. The additional antibacterial effect of $Ca(OH)_2$ mixed with 2% CHX gel as a paste for an intracanal dressing was also assessed.

Materials and Methods

In Vitro Evaluation of 2% CHX Gel Neutralizer

In vitro evaluation was performed based on the protocol described by Zamany and Spångberg (25) to assess the ability of 3% Tween 80 and 0.3% L-α-lecithin in activating 2% CHX gel.

Enterococcus faecalis was cultivated on blood agar medium for 24 hours. Colonies were harvested from the surface of the agar plate and suspended in a 0.43% solution of sodium chloride, centrifuged at about $400g$ for 10 minutes, washed twice, and resuspended. They were centrifuged and finally resuspended in 0.43% solution of sodium chloride. A stock cell suspension with a density of 2×10^4 viable cells per milliliter was prepared. The cell density was determined by measuring light absorbance with a spectrophotometer at a wavelength of 580 nm according to MacFarland's scale (Remel, Lenexa, Kan). Utility cell suspensions were prepared by diluting the stock cell suspension in 0.43% solution of sodium chloride. Each cell suspension was used within 30 minutes of preparation.

Three percent (3%) Tween 80 plus 0.3% L-α-phosphatidylcholine (L-α-lecithin) was prepared by dissolving 0.75 g of L-α-lecithin (Sigma Chemical) in 3 mL of Tween 80 and adjusting the final volume by adding 97 mL of sterile saline solution.

One milliliter of 2% CHX gel (Endogel) was tested against 9 mL of 3% Tween 80 plus 0.3% L-α-lecithin at room temperature (25°C) for 5 minutes in a sterile test tube containing glass beads (diameter, 3 mm); 0.1 mL of bacterial cell suspension containing 2×10^4 viable cells was added to this mixture. At 10 and 60 minutes, 3 glass beads and 0.1-mL aliquots were withdrawn and spread over the surface of two blood agar plates by means of the glass beads, which were incubated at 37°C for 72 hours. The numbers of colony-forming units (CFUs) on the blood agar plates were recorded. The experiment was repeated and the mean CFUs from two experiments were calculated and recorded. To test the effectiveness of 2% CHX gel, 0.1 mL of bacterial cell suspension was tested against the mixture of 9 mL of sterile saline and 1 mL of 2% CHX gel, which was equilibrated for 5 minutes. To confirm that 3% Tween 80 plus 0.3% L-α-lecithin did not have antimicrobial effect, 0.1 mL of bacterial cell suspension was tested against the mixture of 9 mL of sterile saline and 1 mL of the neutralizing agent, which was equilibrated for 5 minutes. For the positive control, 0.1 mL of bacterial cell suspension was added to the mixture of 10 mL of sterile saline. For the negative controls, the mixture of 9 mL of sterile saline and 1 mL of 2% CHX gel or mixture of 9 mL of sterile saline and 1 mL of the neutralizing agent was tested without adding bacteria suspension.

Subject Recruitment and Qualification

Patients presenting to the University of North Carolina School of Dentistry graduate Endodontic clinic for evaluation and treatment of infected pulps with apical periodontitis were considered for this study. Approval for the project was obtained from the University of North Carolina School of Dentistry Committee on Investigation Involving Human Subjects. The primary investigator conducted all clinical and sampling procedures. The nature of study, complications, and associated risks were fully explained to the patients or patients' guardians and consents were obtained before initiation of treatment.

The mesiobuccal roots of the mandibular first and second molars, the distal roots of maxillary first and second molars, the buccal roots of maxillary first premolars, and single-rooted teeth were sampled and included in the study. Tooth selection criteria included the following: a radiographic periapical radiolucent lesion, a negative response to thermal or electric pulp testing, enough crown structure for adequate isolation, and no history of previous endodontic treatment on the tooth of study. Exclusion criteria for the study included the following: teeth with unfavorable conditions for rubber-dam application, vital pulp tissue observed during the treatment, or immature teeth with open apices.

Treatment Group Assignment

Qualified subjects were accepted into the study in a nonrandom consecutive sample and treated with ProFile 0.04 taper nickel-titanium files (Dentsply/Tulsa Dental, Tulsa, OK) and 2% CHX gel (Endogel) as a disinfectant. On the completion of root canal instrumentation at the end of the first appointment, all teeth were dressed with $Ca(OH)_2$ mixed with 2% CHX gel for at least 2 weeks. In this study, 43 patients diagnosed with apical periodontitis were included as the test subjects and 4 patients with irreversible pulpitis as the negative control subjects.

Bacteria Sampling

Each tooth was isolated with rubber dam, which was disinfected with 30% hydrogen peroxide until no further bubbling of the peroxide occurred. If difficulty occurred in attaining a bubble-free status, Oraseal Putty (Ultradent Products Inc, South Jordan, UT) was placed around the neck of the tooth and the process repeated. All surfaces were then coated with tincture of iodine and allowed to dry. Gross caries removal and initial access form were accomplished with sterile high-speed and low-speed burs. The rubber dam and surrounding tooth structure were disinfected with iodine tincture before completing the access with another sterile bur. This protocol has been shown to be effective in surface disinfection (7, 8). After access was achieved, cases were selected for sterility testing of the operating field. One in every five teeth was tested throughout the experimental period to ensure our disinfection protocol worked properly. The tooth surface was swabbed with a 5% sodium thiosulfate solution to inactivate the iodine tincture so that residual iodine would not influence bacteriologic sampling. To evaluate the efficacy of the disinfection procedure, a sterile cotton pellet was moistened in 5% sodium thiosulfate solution and used to swab the access cavity. The swab was then transferred to a vial containing 1 mL of liquid dental transport medium (LDTM) (Anaerobe Systems, Morgan Hill, CA) for processing for bacterial culture. Sterile saline was used to flush debris within the chamber. A description of the 3 samples (S1, S2, and S3) taken in the study is found in Table 1.

Bacteria samples were collected from the mesiobuccal canals of mandibular molars, distobuccal canals of maxillary molars, buccal canals of upper premolars, and single-rooted teeth. In multirooted teeth, orifices of canals not used for bacteriologic sampling were sealed with Cavit (ESPE, Norristown, PA) until all samples collected. A sterile orifice opener (Dentsply/Tulsa Dental) was used to initiate the access into the canals of interest. Sterile saline was again used to flush any debris from the chamber. The chamber was dried with sterile cotton pellets and/or paper points before placement of LDTM into the canals of interest with a sterile tuberculin syringe. The canals were instrumented with a sterile stainless steel K-file (Kerr, Romulus, MI) size #15 (ISO) to within 1 mm

TABLE 1. Description of Microbiological Samples Taken for Each Tooth

S1:	Initial sample
S2:	Postinstrumentation sample
S3:	Postdressing sample

TABLE 2. Apical Preparation Size Corresponding to Canal and Tooth Type

Tooth Type	Canal	Apical Size	Taper
Incisors	Central	#60	.04
Single-rooted premolars	Central	#60	.04
Maxillary premolar	Buccal	#40	.04
Maxillary molar	Distobuccal	#40	.04
Mandibular molar	Mesiobuccal	#40	.04

of the estimated working length. The LDTM remaining in the canal was soaked up and transferred to the LDTM vial with sterile xx-fine paper points (Mynol; Block Drug Corp, Jersey City, NJ) placed as close to working length as possible. This constituted the initial sample (S1). All samples were submitted to the laboratory within 24 hours.

The working length was established to the root terminus by using an apex locator (Root ZX; J. Morita, Irvine, CA) and confirmed by radiograph (0.5–1.0 mm short of radiographic apex). The canals and pulp chamber were filled with 2% CHX gel using 30-G Maxi-Probe irrigation needles (Dentsply Internatioanl Inc, York, PA) before instrumentation. Sterile ProFile 0.04 taper nickel-titanium files (Dentsply/Tulsa Dental) were used to instrument the canals by a crown-down technique to standardized apical sizes. Rotary instrumentation was used with an Aseptico ITR Electric Torque Control Motor (Dentsply/Tulsa Dental) rotating at 300 rpm. After each file, the canals were irrigated with saline using 30-G Maxi-Probe irrigation needles followed by refilling the canals with 2% CHX gel. Root canal instrumentation was continued in the presence of 2% CHX gel. This procedure was repeated for each file until the final preparation. After the instrumentation, the canal spaces were filled with 2% CHX gel for 2 additional minutes followed by final rinse with 10 mL of physiologic saline. The apical preparation size based on the tooth type is shown in Table 2.

The neutralizing agent (3% Tween 80 and 0.3% L-α-lecithin) for 2% CHX gel (Endogel; Itapetininga, Sao Paulo, Brazil) was prepared and tested as shown by Zamany and Spangberg (25). The canals were slowly flushed with 2 mL of 3% Tween 80 plus 0.3% L-α-lecithin (Sigma Corp, St. Louis, MO) for 5 minutes to neutralize 2% CHX gel. The canals were then flushed with 2 mL of sterile saline and dried with sterile paper points. By using a new set of sterile instruments, the canals were filled with LDTM, and the final file sizes were placed to the working length. Files were pumped five times with minimal reaming motion. The entire canal content was absorbed with sterile paper points and transferred to the LDTM sample vial. This constituted the postinstrumentation sample (S2). All samples were processed for microbiology within 24 hours. Once bacterial samples were collected, Cavit (ESPE, Norristown, PA) was removed from the orifices of the uninstrumented canals. Those canals were further instrumented with rotary files and 2% CHX gel as described earlier.

The canals were again filled with 2% CHX gel for 2 minutes and then with saline and dried with paper points. A mixture of Ca(OH)$_2$ and 2% CHX gel was placed into all canals with a Lentulo spiral filler (Caulk, Milford, DE), and the access cavity was sealed with IRM (Dentsply

International Inc, York, PA). The intracanal medication of the Ca(OH)$_2$ mixed with 2% CHX gel was placed for a minimum of 2 weeks.

At the second appointment, under rubber dam isolation, the tooth was accessed with the strict aseptic protocol described above. Intracanal medicament was passively removed with a K-file and sterile saline irrigation. The canals of no interest were again sealed with Cavit until the sample was collected from the canal of interest. Neutralization of the Ca(OH)$_2$/2% CHX gel dressing was accomplished with 2 mL of 0.5% citric acid followed by 2 mL of 3% Tween 80/0.3% L-α-lecithin introduced into each canal with a sterile tuberculin syringe with a 30-G Maxi-Probe irrigation needle (Dentsply International Inc). The canals were irrigated again with sterile saline and dried. As described previously, LDTM was introduced and collected, constituting the postdressing sample (S3).

Four additional teeth diagnosed with irreversible pulpitis and no radiographic signs of an apical lesion were treated identically to the test group. These served as a negative control to detect sample contamination potential.

The laboratory procedures were performed at the University of North Carolina Dental Microbiology Laboratory. The vials with the paper point samples were agitated with a vortex gently before aliquot disbursement. Sample dilutions of 10, 100, and 1000-fold were prepared under anaerobic conditions using sterile glassware. Petri dishes with anaerobic Brucella sheep blood agar supplemented with heme and vitamin K were quantitatively inoculated by spiral plating (Spiral Systems; Interscience, Hanover, MA) of undiluted sample as well as each of the three dilutions. Plates were incubated at 37°C for 7 days in an anaerobic chamber (Coy Anaerobic Systems) containing 10% hydrogen, 85% nitrogen, and 5% CO$_2$. After incubation, the colony-forming units (CFUs) were obtained by using a Nikon 69229 stereoscope (Nikon, Melville, NY) at 10× magnification.

Statistical Analysis

A log$_{10}$ transformation of the CFU values was performed to normalize the data. Repeated-measures analysis of variance was used to detect significance in bacteria reduction among the S1, S2, and S3. The number of positive culture was also recorded at S1, S2, and S3. Fisher exact tests were used to detect the differences in the number of positive cultures between S1 and S2, S1 and S3, and S2 and S3. The level of significance was set at 0.05 for all analyses.

Results

Three percent Tween 80 plus 0.3% L-α-lecithin was found to be an effective inactivating agent, ensuring full recovery of *E. faecalis* in the presence of 2% CHX gel (Table 3). No test organisms were recovered when *E. faecalis* was tested against 2% CHX gel; 3% Tween 80/0.3% L-α-lecithin yielded a full recovery of *E. faecalis*.

Forty-three test subjects and four negative control subjects were included in the study. The log$_{10}$ mean values and standard deviations at S1 to S3 are presented in Table 4. Percentages of root canals with positive culture

TABLE 3. In Vitro Evaluation of 2% CHX Gel Neutralization

	E. faecalis	Neutralizer	2% CHX Gel	2% CHX Gel + *E. faecalis*	Neutralizer + *E. faecalis*	Neutralizer + 2% CHX Gel + *E. faecalis*
Bacterial growth	+++	−	−	−	+++	+++

− no growth; +, low growth; ++, medium growth; +++, high growth.

Neutralizer = 3% Tween 80 plus 0.3% L-α-lecithin.

TABLE 4. Log_{10} Mean Values (CFU/mL) and Standard Deviations (SD) at S1, S2, and S3

	S1		S2		S3	
	Mean	SD	Mean	SD	Mean	SD
Size #40	5.59	1.83	0.36	1.15	0.14	0.59
Size #60	5.64	1.69	0.32	0.94	0.41	1.27
Total	5.62	1.74	0.34	1.04	0.26	0.96

at S2 and S3 are summarized in Table 5 and Figure 1. All four negative control subjects showed no bacteria growth at S1, S2, and S3.

Bacteria were initially detected in 39 of the 43 test teeth. Four test teeth with no initial bacterial growth were excluded from further analysis. Of the teeth with positive culture at S1, 18 teeth were prepared to #60 and 21 teeth were prepared to #40. The mean log_{10} value was 5.62 ± 1.74 CFU/mL (S1).

Of the teeth that were culture positive at S1, 35 of 39 (89.7%) were negative for culture at S2. For the size #40 and #60 groups, 19 of 21 (90.5%) and 16 of 18 (88.9%) had a negative culture, respectively. There was no statistical difference in the numbers of negative culture between these two groups at S2 (p = 0.871). The mean log_{10} value was below the limits of detection (<1.32 CFU/mL) of the assay (S2). A significant decrease in bacterial numbers from S1 to S2 was observed (p < 0.0001).

The average number of days of calcium hydroxide therapy was 19 days, with a range of 14 to 29 days. Three samples in S3 were lost during culturing. Of the remaining samples, 33 of 36 (91.7%) were free of bacteria at S3. For the size #40 and #60 groups, 18 of 19 (94.7%) and 15 of 17 (88.2%) had a negative culture, respectively. There was no statistical difference in the numbers of negative culture between these two groups at S3 (p = 0.481). All four samples that were culture positive at S2 were free of bacteria at S3, but three samples that were culture negative at S2 had recoverable CFUs at S3. The mean log_{10} value again was below the limits of detection of the assay (S3). A significant decrease in bacterial numbers from S1 to S3 (p<0.0001) was observed, but there was no significant difference from S2 to S3 (p = 0.349).

The Fisher exact test showed a significant difference in the numbers of positive culture between S1 and S2 (p < 0.001) and between S1 and S3 (p < 0.001). There was no statistical difference in the numbers of negative culture between S2 and S3 (p = 0.692).

Discussion

The antimicrobial effectiveness of 2% CHX (solution or gel form) was well investigated in vitro. It appeared that CHX and NaOCl have similar antimicrobial activity against the common organisms isolated from the root canal system (16). There are several advantages for the clinical use of CHX as root canal disinfectant over NaOCl, including low toxicity (20), substantivity (19), more tolerable odor and taste, and nonbleaching. Despite its promising results from in vitro studies, published clinical studies have been insufficient. In this study, we evaluated the bacteria reduction ability of 2% CHX gel as a root canal disinfectant in patients with chronic apical periodontitis.

TABLE 5. Percentage of Samples with Negative Culture at S2 and S3

Groups	Initial Sample Size*	S2 (%)	S3 (%)†
Size #40	21	19/21 (90.5)	18/19 (94.7)
Size #60	18	16/18 (88.9)	15/17 (88.2)
Total	39	35/39 (89.7)	33/36 (91.7)

*Four samples did not show initial bacteria growth, and they were excluded from analysis.
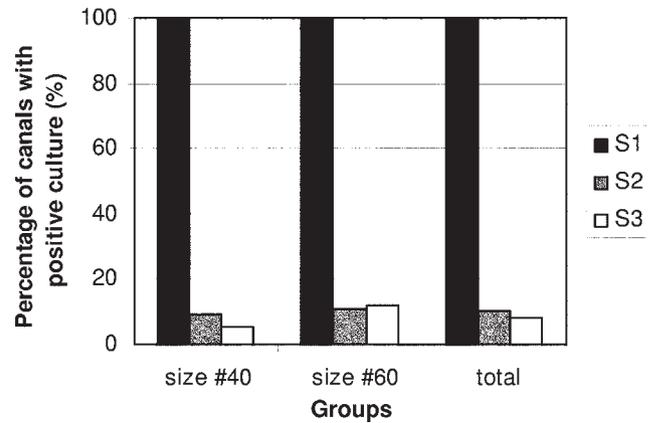†Three samples were lost during culturing (S3).

Figure 1. Percentage of samples with positive culture for each group at the different culturing points (S1 = initial sample, S2 = postinstrumentation sample, and S3 = postdressing sample).

An effective method of inactivating 2% CHX solution was shown previously by Zamany and Spangberg (25). The authors used L-α-lecithin, Tween 80, and sodium thiosulfate in different proportions to prepare six potential inactivating solutions. Inactivating agents and 2% CHX solution were tested against *Enterococcus faecalis* on blood agar plates. They showed that the combination of 3% Tween 80 and 0.3% L-α-lecithin was the most effective inactivating agent. However, whether 3% Tween 80 plus 0.3% L-α-lecithin can effectively neutralizes 2% CHX gel (Endogel; Itapetininga, Sao Paulo, Brazil) has not been evaluated yet. To address this, an in vitro experiment modified from the protocol described by Zamany and Spangberg (25) was performed. Our data confirmed that 3% Tween 80 plus 0.3% L-α-lecithin was a superior neutralizing agent for 2% CHX gel and inactivated the gel within 5 minutes. The presence of 1% Natrosol (Itapetininga, SP, Brazil) did not interfere with the neutralization process. It appeared that 3% Tween 80 plus 0.3% L-α-lecithin requires the same amount of time to inactivate either the gel or solution form of CHX.

There are several advantages of using the CHX gel over the solution; the 2% CHX gel used in this study contains 1% natrosol, 17% EDTA, and 2% CHX, which is the same formulation that was used by Ferraz et al (22). Based on the scanning electron microscopy study, Ferraz et al (22) showed that, when the root canal walls were treated with 2% CHX gel, almost all dentinal tubules were opened. Those authors believed that 2% CHX gel was able to clean the root canal walls and their anatomic complexities effectively because of the viscosity of the gel, which promoted a better mechanical cleansing of the root canal and better removal of dentin debris and the remaining tissue. In addition to the mechanical properties, 2% CHX gel also has good antimicrobial activity and a lubricant action during instrumentation (22). Hence, compared with the solution form, the gel form can potentially disinfect the dentinal tubules and anatomic complexities during instrumentation. Another advantage comes from its lubrication action, which leads to less stress on rotary files during instrumentation. In addition, 2% CHX gel eliminates the need for the use of root canal lubricant because it functions more like lubricant than irrigation solution.

Although numerous in vitro studies evaluated the antimicrobial properties of 2% CHX gel, there is only one clinical study available in the literature. Vianna et al (26) evaluated the microbial reduction after chemomechanical preparation of human root canals containing necrotic pulp tissue. Thirty-two single-rooted teeth with necrotic pulp and apical periodontitis (from 32 patients) were selected for this study. One group (n = 16) was irrigated with 2.5% NaOCl during the root canal instrumentation, whereas the other group (n = 16) was irrigated with 2% CHX gel. Bacterial load was assessed by the use of real-time quan-

titative-polymerase chain reaction and traditional culturing techniques. Real-time quantitative-polymerase chain reaction showed that the bacterial reduction was greater in the NaOCl group than in the CHX group. According to culture technique, 75% of cases were free of bacteria after chemomechanical preparation in the NaOCl group, whereas 50% of cases were bacteria free in the CHX group. The authors concluded that 2.5% NaOCl was a more effective root canal irrigant than 2% CHX gel. In contrast, our results showed that 2% CHX gel was an effective root canal disinfectant, and close to 90% of cases were void of bacteria after the chemomechanical preparation. This disparity is probably caused by the differences in the methodology between two studies. We speculated that both apical enlargement and irrigation technique attributed to higher bacterial reduction in this study. All single-rooted teeth were prepared to the standardized size of #60/.04 using rotary NiTi instruments, whereas Vianna et al (26) prepared them to the apical size of #35–45 using K-files followed by step-back instrumentation. Previous studies have shown that uniform reduction of bacteria occurred with progressive filing (6, 7). Hence, a higher load of bacterial reduction is expected for a larger apical preparation. In addition, the irrigation methods used were different between two studies. Vianna et al (26) stated that the use of each instrument was followed by irrigation with a syringe containing 1 mL of 2% CHX gel and immediately after with 4 mL of saline. In contrast, we filled the root canal with 2% CHX gel and instrumented with a rotary NiTi file followed by rinse with physiologic saline. This procedure was repeated for each file until the final preparation. In addition, the root canals were treated with 2% CHX gel for 2 minutes after the instrumentation followed by copious rinse with physiologic saline. The advantage of our approach is that it allows 2% CHX gel to have sufficient contact time and physical contact with the root canal walls. Unlike 2% CHX solution, the gel form requires a longer contact time for effective disinfection. In vitro studies showed that 2% CHX solution and 5.25% NaOCl had similar antimicrobial activities and required about the same amount of time to eliminate microorganisms (16). However, 2% CHX gel requires much longer time to eliminate microorganisms compared with either 2% CHX solution or 5.25% NaOCl (16). Using the method from this study, the canals were soaked with 2% CHX gel for approximately 45 minutes during the entire treatment, which allowed sufficient time for proper root canal disinfection.

In addition to the contact time, adequate delivery of 2% CHX gel to the root canal system is an important factor, particularly in curved roots. Unlike the solution, the gel form does not have a good flowing property. It requires additional attention to deliver the gel to the root canal system. This was accomplished in our study by several ways. First, root canal preparations were performed with rotary NiTi instruments in the canals filled with 2% CHX gel. In this way the gel was physically delivered throughout the root canal system as the rotary files were making contact with the walls. Second, larger apical preparation would allow adequate lubrication of the apical third (6, 7, 18). Third, we used a 30-G Maxi-Probe irrigation needle to deliver 2% CHX gel. Because of its small diameter, the needle was able to be placed close to the working length and allowed the proximity to the anatomic foramen (18).

On average, 40% to 60% of root canals have no cultivable bacteria after chemomechanical root canal preparation with NaOCl solution (6-8). Shuping et al (7) evaluated the extent of bacterial reduction with NiTi rotary instrumentation and 1.5% NaOCl irrigation in 42 patients with apical periodontitis. Bacterial samples were taken before and after the instrumentation, and they were cultured anaerobically for 7 days. The authors found that 62% of canals were rendered bacteria free after instrumentation with 1.5% NaOCl. By using a similar clinical protocol, McGurkin-Smith et al (8) obtained 40% of canals sampled bacteria free after instrumentation and a strict irrigation protocol using 5.25% NaOCl and EDTA. It seems that increasing the NaOCl concentration from 1.5%

to 5.25% did not increase the percentage of canals sampled bacteria free. On the other hand, our results showed that 89.7% of canals prepared with 2% CHX gel cultured bacteria free. It appears that 2% CHX gel is a more effective disinfectant than 1.5% to 5.25% NaOCl when it is used as in this study (Fig. 2). Nonetheless, direct comparison among these studies is not possible even though they were performed at the same institution following similar clinical procedures and treatment philosophy. Currently, efforts have been made to investigate comparatively the efficacy of 2% CHX gel versus 5.25% NaOCl as well.

To obtain a more predictable microbial control, the placement of intracanal medicament of $Ca(OH)_2$ is often recommended (5, 7–10). Studies consistently showed that $Ca(OH)_2$ can help to further eliminate surviving bacteria in the root canals (5, 7–10). However, a minimum of two-visit root canal treatment is required when using $Ca(OH)_2$ intracanal dressing because it is ineffective when used as short-term medicament (10). Although $Ca(OH)_2$ is a good antimicrobial agent, it is ineffective against some species of microorganisms such as *E. faecalis* (10, 27), which is found in the case of persistent root canal infection.

To improve the antimicrobial efficacy of $Ca(OH)_2$ against *E. faecalis*, the combination of $Ca(OH)_2$ and 2% CHX gel was used in this study. Gomes et al (28) showed that $Ca(OH)_2$ mixed with 2% CHX gel had better antimicrobial activity than $Ca(OH)_2$ manipulated with sterile water in an in vitro study. In addition, Sirén et al (29) showed that $Ca(OH)_2$ was unable to kill *E. faecalis* in the dentine, but $Ca(OH)_2$ combined with CHX effectively disinfected the dentine. Other studies also support that the mixture of $Ca(OH)_2$ and CHX solution is more effective in eliminating *E. faecalis* than $Ca(OH)_2$ alone (30). In a clinical study, Siqueira et al (31) showed that the application of a 7-day intracanal dressing with $Ca(OH)_2$/CHX gel can further increase the number of root canals yielding negative cultures. Another randomized clinical trial showed that the antibacterial efficacy of $Ca(OH)_2$, 2% CHX gel, and $Ca(OH)_2$/2% CHX gel was comparable when used as intracanal dressing (23). Zerella et al (32) evaluated the effectiveness of $Ca(OH)_2$/CHX intracanal dressing in retreatment cases. At the beginning of the third appointment, 40% of teeth cultured positively culture after treated with $Ca(OH)_2$ alone, whereas only 20% of teeth cultured positively after being treated with $Ca(OH)_2$/CHX. They did not find statistical difference between those two groups, probably because of the small sample size. Interestingly, all cases that initially harbored *Enterococcus*
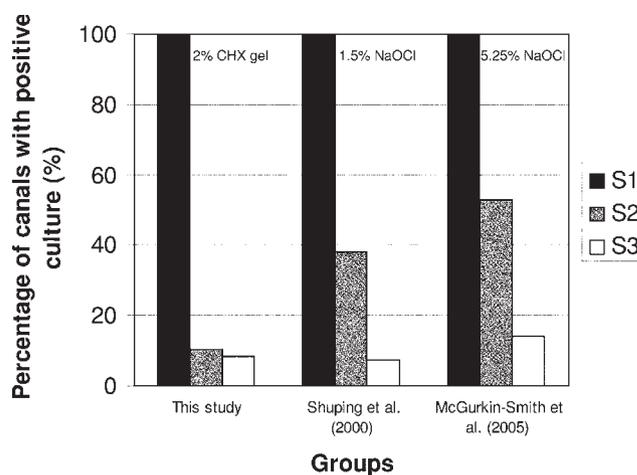

Figure 2. Percentage of samples with positive culture at the different culturing points from the selected studies (S1 = initial sample, S2 = post-instrumentation sample, and S3 = postdressing sample).

Clinical Research

species were successfully disinfected with the $Ca(OH)_2$ and CHX combination (32). Some authors argue that the presence of $Ca(OH)_2$ can reduce the antimicrobial efficiency of CHX (32). However, the mixture of $Ca(OH)_2$/CHX might still be more beneficial than $Ca(OH)_2$ alone.

To neutralize $Ca(OH)_2$/2% CHX gel intracanal dressing, we rinsed the canals with saline followed by the addition of 0.5% citric acid to inactivate $Ca(OH)_2$ component and Tween 80/L-α-lecithin to inactivate 2% CHX gel component. Previous studies have shown 0.5% citric acid was effective in neutralizing calcium hydroxide from the root canal system (7, 8). Tween 80/L-α-lecithin was also shown to inactivate the CHX component of the dressing (23, 31).

In our study, placement of $Ca(OH)_2$/2% CHX gel intracanal dressing for at least 2 weeks rendered 91.7% of canals bacteria free in teeth with apical periodontitis. This value is consistent with the previous reports (Fig. 2). Shuping et al (7) had 92.5% of canals bacteria free when placing for $Ca(OH)_2$ alone for at least 1 week. McGurkin-Smith et al (8) had 86% of canals bacteria free after 2 weeks of $Ca(OH)_2$ alone. Interestingly, unlike those studies, we did not find significant improvement of the root canal disinfection with additional intracanal dressing (Fig. 2). This does not imply that $Ca(OH)_2$/2% CHX gel intracanal dressing is ineffective. Rather, it showed that 2% CHX gel is an effective root canal disinfectant.

The purpose of two-visit root canal treatment using intracanal dressing (such as $Ca[OH]_2$) is to predictably control the root canal infection (33). This is important because the negative culture before the root canal filling is related to long-term root canal treatment success (3, 4). However, some studies questioned the effectiveness of calcium hydroxide to disinfect the canals and reported a residual flora in the canals after more than 1 week of the intracanal dressing (34, 35). In some cases, residual bacteria in the canal grew in number even in the presence of calcium hydroxide (34, 35). We had a similar finding in our study. Three samples showed no bacteria growth after root canal preparation with 2% CHX gel, but the bacteria were detected after 2 weeks of $Ca(OH)_2$/2% CHX gel intracanal dressing. A possible explanation is that a low number of bacteria (under the detection limit by culturing) might reside in anastomosing systems such as isthmus between mesiobuccal and mesiolingual canals of mandibular molars. These bacteria grew in number to a detection level over a 2-week period of calcium hydroxide dressing. Alternatively, there could be contamination in bacteriologic sampling or culturing step.

Ideally, one-visit root canal treatment is desirable if predictable root canal disinfection can be achieved. Our results showed that 2% CHX gel is an effective root canal disinfectant, and additional intracanal dressing did not significantly improve the disinfection. Hence, we can speculate that one-visit root canal treatment with 2% CHX gel can be performed without compromising its long-term success. Nonetheless, further investigations and comparative studies are required to support this hypothesis. In summary, our results indicate that 2% CHX gel can be an effective root canal disinfectant, and it is worthy of further investigation.

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ERRATUM

In the article titled "Redefining the Persistent Infection in Root Canals: Possible Role of Biofilm Communities," which published in J Endod 2007;33 (6): 652–662, the author, Luis Chávez de Paz, DDS, MS, PhD, is listed in PubMed and in Elsevier's Article Locator as "L. de Paz." The correct identification is "Chavez de Paz LE." The Journal of Endodontics regrets this error.