

Efficacy of Instrumentation Techniques and Irrigation Regimens in Reducing the Bacterial Population within Root Canals

José F. Siqueira, Jr., DDS, MSc, PhD, Isabela N. Rôças, DDS, Sandra R. L. D. Santos, DDS, Kenio C. Lima, DDS, MSc, Fernando A. C. Magalhães, and Milton de Uzeda, DDS, MSc, PhD

The purpose of this study was to compare the in vitro intracanal bacterial reduction produced by using two instrumentation techniques and different irrigation methods. Root canals inoculated with *Enterococcus faecalis* were prepared by using the following techniques and irrigants: alternated rotary motions (ARM) technique, hand nickel-titanium files and 2.5% sodium hypochlorite (NaOCl) as irrigant; ARM technique and combined irrigation with 2.5% NaOCl and citric acid; ARM technique and combined irrigation with 2.5% NaOCl and 2% chlorhexidine gluconate; and Greater Taper rotary files, using 2.5% NaOCl as irrigant. Controls were instrumented by using the ARM technique and irrigated with sterile saline. Canals were sampled before and after preparation. After serial dilution, samples were plated onto *Mitis-Salivarius* agar, and the colony forming units that were grown were counted. All test techniques and solutions significantly reduced the number of bacterial cells within the root canal ($p < 0.05$). There was no significant difference between the experimental groups ($p > 0.05$). Nonetheless, all of them were significantly more effective than the control group ($p < 0.05$). These findings support the importance of using antimicrobial irrigants during the chemomechanical preparation, regardless of the solutions or instrumentation techniques used.

The main goal of biological research applied to a clinical discipline is to provide a solid scientific basis for the diagnosis and treatment of a determined disorder, helping to solve clinical problems and enhancing the efficacy of the therapy. Because microorganisms are essential for the development of periradicular diseases and are the major causative factors associated with endodontic treatment failures, endodontic research assumes special importance in finding

methods and materials to predictably eradicate the root canal infection.

Among the procedures involved in the control of endodontic infection, irrigation can play an important role in the elimination of microorganisms from the root canal. Irrigants are used during the endodontic treatment to flush out loose debris, to lubricate the dentinal walls, to dissolve organic matter in the canal, and to be antimicrobial (1). It has been demonstrated that when no irrigant is used during instrumentation, approximately 70% more debris seemed to remain in the root canals when compared with irrigated canals (2). Cleaning and disinfecting intracanal procedures are highly dependent on the mechanical and chemical effects of the irrigants. Mechanical effects during irrigation are generated by the flow and backflow of irrigant solution in the root canal (3). Despite the type of irrigant used, the bacterial population inside the root canal is significantly reduced by the mechanical effects of irrigation (3). Nevertheless, studies have revealed that chemical compounds that possess antimicrobial effects can significantly help the mechanical effects to eliminate microorganisms (4–6).

During World War I, Dakin (7) introduced the widespread use of a 0.5% to 0.6% sodium hypochlorite (NaOCl) solution for antiseptic of open and infected wounds. Based on this report, NaOCl was recommended as an irrigant by Coolidge (8) in 1919. In 1936, Walker (9) introduced the use of double-strength chlorinated soda (5% NaOCl) solution as root canal irrigant. To date, NaOCl has been used worldwide as an irrigant in endodontic practice, and no study has hitherto definitively shown that another substance is more effective. Studies have generally shown that NaOCl has a broad-spectrum antimicrobial activity, because it can rapidly kill vegetative bacteria, spore-forming bacteria, fungi, protozoa, viruses, and bacterial spores (10, 11).

Regardless of the NaOCl concentration used as irrigant, studies have demonstrated that microorganisms may survive the effects of chemomechanical preparation (4–6, 12). Remaining microorganisms can jeopardize the outcome of the endodontic therapy. If they gain access to the periradicular tissues, treatment can inevitably result in failure. Other irrigants and different strategies have been recommended to enhance both the cleaning and antimicrobial capabilities of irrigation (6, 13–16). For instance, the combination of NaOCl with other substances, such as hydrogen peroxide, citric acid, or EDTA has been a commonly recommended strategy. Few

studies have directly compared the antimicrobial effectiveness of some combinations of NaOCl with other solutions (4, 6, 16).

Newer instruments and techniques have also been proposed for root canal preparation. Rotary nickel-titanium instruments with increased tapers and different designs have been recently developed. Although it has been demonstrated that these newer instruments and techniques improve the shaping of the root canal, few studies have evaluated their capability in eliminating the root canal infection. Such studies have reported that rotary and hand instrumentation techniques were equally effective for reducing intracanal bacteria (3, 17). Greater Taper (GT) rotary files (Dentsply, Ballaigues, Switzerland) have been recently introduced in the market. The four GT rotary files have the same size 20 noncutting tip and four predefined tapers (0.06, 0.08, 0.10, and 0.12). Three GT accessory files have also become available and have the same taper (0.12) and different tip diameters (35, 50, and 70). To our knowledge, the instrumentation technique using GT rotary files has not been evaluated for its efficacy in eliminating intracanal bacteria.

The purpose of this study was to compare the in vitro intracanal bacterial reduction produced by two instrumentation techniques and different irrigation regimens.

MATERIAL AND METHODS

Fifty extracted human lower bicuspid with a single root canal, checked by radiographs, were selected for this study. Conventional access preparations were made and the root canals were instrumented 1 mm beyond the apical foramen with K-type files up to size 20, under irrigation with tap water. Working length was established at the apical foramen. Following root canal preparation, the enlarged apical foramen was sealed by means of epoxy resin to prevent bacterial leakage. To make both handling and identification easier, the teeth were then mounted vertically in plaster blocks and sterilized overnight by ethylene oxide gas. Specimen preparation was as reported previously (3, 5).

Sterilized plaster blocks containing the teeth were opened in a laminar air flow cabinet. A suspension was prepared by adding 1 ml of a pure culture of *Enterococcus faecalis* (ATCC 29212), which was grown in brain-heart infusion broth (BHI) (Difco, Detroit, MI) for 24 h, to fresh BHI. Each root canal was completely filled with the *E. faecalis* suspension by using sterile, 1-ml tuberculin syringes. Sterile #15 K-type files were used to carry the bacterial suspension to the entire root canal length. Blocks were then placed inside sterile plastic bags and incubated at 37°C for 24 h.

The root canals were divided into four experimental groups, accordingly, to the technique and irrigation method used. In group 1, 10 root canals were irrigated with 2.5% NaOCl during instrumentation. In group 2, 10 canals were alternately irrigated with 2.5% NaOCl and 10% citric acid. In group 3, 10 root canals were alternately irrigated with 2.5% NaOCl and 2% chlorhexidine gluconate. The control group consisted of 10 root canals that were irrigated with 0.85% sterile saline solution. Root canals from experimental groups 1 to 3 and the control group were prepared by using Nitiflex files (Maillefer, Ballaigues, Switzerland) in the alternated rotary motions (ARM) technique, as described by Siqueira (1). Briefly, a #25 Nitiflex file was inserted into the root canal to a point where it bound slightly and then turned clockwise with no more than one-quarter rotation. It was then turned counterclockwise with light apical pressure. Counterclockwise rotation

was also no more than one-quarter turn. These motions were repeated continuously until the file reached the working length. ARM was maintained in this position for a few seconds. The file was withdrawn 1 to 2 mm, still oscillating, then replaced to the working length. This continuous oscillation associated with the up and down motion was repeated until the file was able to slide easily to the working length. Each sequentially larger file was worked in a similar fashion. Apical preparation was completed by enlargement through a #40 Nitiflex file. In experimental group 4, 10 root canals were prepared by instrumentation with GT rotary files in a crown-down manner. Instruments were used in a profile electric handpiece, adjusted to 185 rpm. Canals were enlarged to their full lengths by using GT rotary files with 0.10 and 0.12 tapers. Apical preparation was performed by using a GT rotary file 0.12 taper. A 2.5% NaOCl solution was used as irrigant. Irrigant was delivered into the canals by using a 3-ml plastic syringe with a 23-gauge needle. Each set of instruments was used to prepare no more than three root canals.

The root canals were sampled before and after instrumentation. Canals were filled with sterile 0.85% saline solution and each sample was taken by using three paper points. After initial sampling, the root canals were irrigated with 1 ml of the tested solutions. Canals were irrigated with 1.5 ml of the tested solutions after each file size. In the groups where combined solutions were used, each substance was used after each size file and the last irrigation was always performed with NaOCl. Although standardization was difficult in group 4, frequent irrigation was always performed. Regardless of the test group, a total volume of 7 ml of irrigant was always used for each tooth. Residual NaOCl was inactivated by rinsing the canals with 2 ml of a 5% sodium thiosulphate solution. A final irrigation was performed by using 1 ml of 0.85% sterile saline solution. Canals were then sampled by using three paper points.

After each sampling, paper points were transferred to tubes containing 1 ml of 0.85% saline solution and vortexed for 1 min. After 10-fold serial dilutions in sterile 0.85% saline solution, aliquots of 0.1 ml were plated onto *Mitis-Salivarius* agar plates and incubated at 37°C for 48 h. The colony forming units that were grown were counted and then transformed to log numbers to confer homoscedasticity to the populations.

Data, obtained from samples that were taken before and after instrumentation, were analyzed statistically for differences inside groups by using the paired *t* test and between groups by ANOVA and the Tukey's test. The significance level was established at 5% ($p < 0.05$).

RESULTS

Data, obtained from the comparison between the samples that were taken before and after root canal preparation, showed that all test techniques and irrigation regimens significantly reduced the number of bacterial cells in the root canal ($p < 0.05$).

The mean log number of bacterial cells in the initial samples from group 1 (root canals irrigated with 2.5% NaOCl and instrumented by the ARM technique) was 6.97 ± 0.64 . After preparation, the mean values of the number of bacterial cells decreased to 2.76 ± 0.59 . The mean reduction of bacteria in log numbers was 60.3% (range, 48% to 70.7%).

Instrumentation and alternate irrigation with NaOCl and citric acid provided a decrease of 78.4% (range, 54.2% to 100%) in the number of viable bacteria in the root canal. For this group, the

means of the bacterial cell numbers at the initial and final samples were 6.1 ± 0.79 and 1.3 ± 1.12 , respectively. Thorough elimination of bacterial cells occurred in four specimens after chemomechanical preparation.

Initial samples from the root canals irrigated with NaOCl/chlorhexidine gluconate contained a mean log number of bacterial cells of 6.68 ± 0.31 , whereas the mean number of bacteria in the final samples was 2.46 ± 0.84 . The mean reduction for this group was 62.8% (range, 52.7% to 100%). One specimen showed thorough elimination of bacteria.

Instrumentation with GT rotary files and irrigation with 2.5% NaOCl provided a decrease of 66.5% (range, 55.7% to 100%) in the number of viable bacteria in the root canal. For this group, the means of the bacterial cell numbers at the initial and the final samples were 6.74 ± 0.73 and 2.27 ± 0.93 , respectively. One specimen was rendered free of living bacteria after chemomechanical preparation.

Instrumentation and irrigation, using saline (control group), showed a mean bacterial reduction of 38.3% in log numbers. The mean number of bacterial cells in the canals before and after preparations were 5.86 ± 0.74 and 3.6 ± 0.67 , respectively.

Comparisons between groups failed to find a significant difference between the four experimental groups ($p > 0.05$). However, all groups showed a statistically significant increase in bacteria elimination when compared with the control group (saline solution) ($p < 0.05$).

DISCUSSION

Enterococci have been demonstrated to possess the ability to multiply after standard-chemomechanical procedures (18). In addition, they may survive even under unusual environmental stresses, such as low nutrient availability and may be extremely resistant to medications used during the root canal therapy (1). This probably explains why *E. faecalis* has been commonly isolated from teeth with failed endodontic treatment (19). The use of *E. faecalis* in this study is justified because of its reported resistance to chemomechanical procedures, its supposed involvement in endodontic failures, and because it is relatively easy to culture and manipulate.

Clinical and laboratory studies could not demonstrate any significant difference in antibacterial effect between NaOCl concentration ranging from 0.5% to 5% (5, 6, 12). Apparently, the frequency and the volume of irrigation with NaOCl can compensate for the effects of concentration. Although NaOCl is considered a strong and rapid disinfectant, studies have shown that bacteria can survive its antibacterial effects during irrigation in most canals, independently of the concentration used.

To maximize the effects of NaOCl, association with other solutions has been recommended and tested. Baumgartner and Mader (15) observed that irrigation with a combination of NaOCl and EDTA effectively cleaned the root canal walls, removing pulpal remnants, smear layer (from instrumented root canal walls), and predepositin (from uninstrumented walls). Byström and Sundqvist (6) have found that the combined use of EDTA and 5% NaOCl was more efficient than the use of NaOCl alone. However, living bacteria were still present in approximately half of the cases at the second appointment and in few cases at the third appointment. Svec and Harrison (13) have demonstrated that a combination of NaOCl and hydrogen peroxide was significantly more effective in cleansing the root canal than saline solution. Studies (4, 20) have

shown that the use of alternate irrigation with NaOCl and hydrogen peroxide was no more effective than other methods with regard to chemical (antibacterial) and mechanical effects. Kuruvilla and Kamath (16) reported that the use of NaOCl and chlorhexidine gluconate combined within the root canal resulted in a significant reduction of postirrigant positive cultures when compared with each substance alone. Baumgartner et al. (14) found that irrigation regimen, which used a combination of NaOCl and citric acid, was effective in removing the smeared layer from the surface of the prepared root canal walls.

NaOCl exerts its antimicrobial activity by being a highly active oxidizing agent that destroy the activity of proteins, in particular, those containing amino acids cysteine and methionine, which possess sulfhydryl groups (1). NaOCl can also have deleterious effects on bacterial DNA that involve the formation of chlorinated derivatives of nucleotide bases. In addition, NaOCl has been reported to induce bacterial membrane disruption (11). It is well known that the agent responsible for the antimicrobial effects of NaOCl is undissociated hypochlorous acid (HOCl), not chlorine. The dissociation of HOCl to the less effective hypochlorite ion (OCl^-) is higher as the pH increases. Between pH 4 and 7, chlorine exists predominantly as HOCl, the active moiety, whereas above pH 9, OCl^- predominates. Disinfection by NaOCl is optimal at around pH 6, because the concentration of HOCl is optimal and its dissociation is minimal (11). However, NaOCl solutions are usually prepared in high pH to be more stable. Because antimicrobial effects are pronounced in low pH conditions, due to the higher concentration of undissociated HOCl, it would seem that the acidification of the NaOCl solution by citric acid during a combined use could theoretically improve the disinfection capability of NaOCl. However, our findings do not support this theory, because there was no significant difference in antibacterial efficacy when comparing NaOCl alone or combined with citric acid. This probably occurred because the alternate use of NaOCl and citric acid was insufficient to significantly reduce the pH of NaOCl, because the irrigation with each solution may have removed the excess of the previously used solution, and neutralized or diluted the residual amount of the previous one. In other words, each solution probably exerted its own effects without directly being affected by the previous one. The same could have occurred for the association of NaOCl with chlorhexidine.

The results of this study demonstrated that all irrigation regimens were significantly effective in eliminating bacteria from the root canals. However, neither citric acid nor chlorhexidine used in combination with NaOCl offered better results when compared with NaOCl alone. Therefore, from the antimicrobial point of view, there is no apparent benefit to associate other irrigants with NaOCl.

We observed strong dentin pigmentation when associating NaOCl with chlorhexidine. Pigments were not eliminated even after the last irrigation with NaOCl. The clinical significance of such pigmentation has not been reported.

Some factors should be taken into account to maximize the effects of irrigation. The needle delivering the solution must come in close proximity to the material to be removed. Even narrow canals, prepared to a minimum diameter, can be effectively flushed of debris when the coronal two-thirds are tapered to allow for placement of the irrigation needle close to the apical third. The use of smaller needles is more effective than larger ones (20).

Because chemomechanical preparation is a relatively brief procedure, it seems that the antibacterial effectiveness is limited to main root canal, regardless of the type of irrigant, concentration,

combinations, and volume used. This can be particularly critical for recently developed rotary techniques, which have been reported to reduce the time of the chemomechanical procedure. Theoretically, the lesser the time that the irrigant remains in the canal, the lesser is its antibacterial effectiveness. However, our data do not support such a statement, because there was no significant difference when comparing the experimental groups.

Recent technological advances have provided rotary instruments and instrumentation systems that can significantly improve the shaping of the root canal, particularly curved canals. Nevertheless, evidence suggests that rotary instrumentation does not provide better results in either cleaning or disinfection capabilities when compared with hand techniques (3, 17). More importantly, nickel-titanium instruments can predictably enlarge curved root canals, while maintaining the original path, to sizes not routinely attainable with stainless steel files. Large preparations can incorporate more anatomical irregularities and allow the removal of a substantial number of microbial cells from the root canal. In addition, because effective irrigation might not occur consistently unless the canals are sufficiently enlarged (3, 20), larger preparations may enhance the effects of irrigation in the apical third.

The results of this and other studies (3–6) support the suggestion that larger preparations (as large as the root anatomy permits) and frequent and abundant irrigation with antimicrobial substances (such as NaOCl) play an important role in maximizing the effectiveness of chemomechanical preparation. However, despite the irrigation regimen or instrumentation technique, most specimens still contained living bacteria. This confirms that instruments and irrigants failed to penetrate into confined areas of the root canal system. It has been demonstrated that even when the highest standards are followed, endodontic failure can still occur, inasmuch as there are regions in the root canal system that cannot properly be disinfected and obturated with existing instruments, techniques, and materials. Because bacteria surviving instrumentation and irrigation can put at risk the outcome of endodontic treatment, the use of an effective, antibacterial, intracanal medication should be still recommended, despite recent technological advances.

Drs. Siqueira and Uzeda and Mr. Magalhães are members of the Oral Microbiology Laboratory, Institute of Microbiology, Federal University of Rio de Janeiro, RJ, Brazil. Dr. Siqueira is chairman and professor of Endodontics, and Dr. Rôças is professor of Endodontics, Department of Dentistry, Estácio de Sá University, RJ, Brazil. Dr. Santos is practicing in Niterói, RJ, Brazil. Dr. Lima is professor of Preventive Dentistry, Federal University of Rio Grande do Norte, Natal, RN, Brazil. Address requests for reprints to Dr. José F. Siqueira, Jr., R. Herotides de Oliveira 61/601, Icarai, Niterói, RJ, Brazil 24230-230.

References

1. Siqueira JF Jr. Tratamento das infecções endodônticas. Rio de Janeiro: MEDSI, 1997.
2. Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigating solutions. *J Endodon* 1975;1:127–35.
3. Siqueira JF Jr, Lima KC, Magalhães FAC, Lopes HP, Uzeda M. Mechanical reduction of the bacterial cell number inside the root canal by three instrumentation techniques. *J Endodon* 1999;25:332–5.
4. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal. *Int Endod J* 1997;30:279–82.
5. 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 Endodon* 2000; 26:331–4.
6. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985;18:35–40.
7. Dakin HD. On the use of certain antiseptics substances in treatment of infected wounds. *Br Med J* 1915;28:318–20.
8. Coolidge ED. The diagnosis and treatment of conditions resulting from diseased dental pulps. *J Nat Dent Assoc* 1919;6:337–49.
9. Walker A. Definite and dependable therapy for pulpless teeth. *J Am Dent Assoc* 1936;23:1418–24.
10. Siqueira JF Jr, Batista MMD, Fraga RC, Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented Gram-negative anaerobes and facultative bacteria. *J Endodon* 1998;24:414–6.
11. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 1999;12:147–79.
12. Cvek M, Nord CE, Hollender L. Antimicrobial effect of root canal debridement in teeth with immature root: a clinical and microbiologic study. *Odontol Revy* 1976;27:1–10.
13. Svec TA, Harrison JW. Chemomechanical removal of pulpal and dental debris with sodium hypochlorite and hydrogen peroxide vs. normal saline solution. *J Endodon* 1977;3:49–53.
14. Baumgartner JC, Brown CM, Mader CL, Peters DD, Shulman JD. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. *J Endodon* 1984;10:525–31.
15. Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. *J Endodon* 1987;13:147–57.
16. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. *J Endodon* 1998;24:472–6.
17. Dalton C, Orstavik D, Phillips C, Pettiet M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. *J Endodon* 1998;24:763–7.
18. Gomes BPFA, Lilely JD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. *Int Endod J* 1996;29:235–41.
19. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:86–93.
20. Abou-Rass M, Piccinino MV. The effectiveness of four clinical irrigation methods on the removal of root canal debris. *Oral Surg Oral Med Oral Pathol* 1982;54:323–8.