Comparative Evaluation of the Antimicrobial Efficacy of a 5% Sodium Hypochlorite Subsonic-activated Solution

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Abstract

Introduction: The study evaluated the efficacy of subsonic agitation of sodium hypochlorite (NaOCl) in reducing bacterial load in the root canal. **Methods:** Root canals of 112 extracted human single-root teeth were preflared using K-Flexofiles (Dentsply Maillefer, Ballaigues, Switzerland) up to #20 and then shaped using ProTaper S1-S2-F1-F2-F3 (Dentsply Maillefer) at the working length. Irrigation was performed with 33 mL of 5% NaOCl, alternating with 10 mL of 10% EDTA. After ethylene oxide sterilization, the root canals were infected with 30 μL of *Enterococcus faecalis* culture and randomly assigned to four groups (n = 25) of different irrigation regimens plus positive and negative controls. Irrigation was performed with 2 mL of 5% NaOCl. In the NaOCl 15 group, the irrigant was left in place for 15 seconds, and in the NaOCl 30 group it was left in place for 30 seconds. In the EndoActivator (EA; Dentsply Tulsa Dental Specialties, Tulsa, OK) 15 and EA 30 groups, NaOCl was subsonically agitated with EA for 15 and 30 seconds, respectively. The residual bacterial count was then evaluated. Differences among groups were analyzed with one-way analysis of variance and the post hoc Bonferroni test ($p < 0.05$). **Results:** A statistically significant difference was evidenced among groups ($F_3 = 9.01$, $p < 0.001$). The standard irrigation groups (NaOCl 15 and 30) showed higher microbial counts than the EA 30 group ($p < 0.05$). **Conclusion:** Thirty seconds of NaOCl subsonic agitation with EndoActivator appears to be slightly more effective in reducing bacterial load in the root canal compared with NaOCl irrigation alone. *(J Endod 2010;36:1358–1360)*

Key Words

Disinfection, EndoActivator, endodontic irrigants, sodium hypochlorite, subsonic

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Bacteria and their byproducts play a relevant role in the onset and perpetuation of pulpal and periradicular disease (1). Root canal treatment aims to eliminate remnants of pulp tissue, bacteria, and microbial toxins from the infected canal system and to prevent reinfection in order to achieve long-term success (2–4). Clinical studies have shown a more favorable long-term prognosis of specimens that were culture negative before obturation versus culture-positive specimens (94% vs 68%) (5), whereas other studies have failed to show any significant difference concerning healing (6). However, there is general consensus that successful elimination of the causative agents from the root canal system is the key to health (7).

Chemical-mechanical treatment of the root canal system has shown its efficacy in reducing bacterial load (8), even though bacteria may persist despite these efforts (9) because of the complexity of the root canal system (10–12). Sodium hypochlorite (NaOCl) has been widely used as an irrigant since its introduction in endodontics by Walker in 1936, and it is still considered an effective disinfectant agent (13). NaOCl used at concentrations ranging from 0.5% to 6% is a potent antimicrobial agent and effectively dissolves organic debris. Numerous irrigation regimens have been proposed to enhance the effectiveness of NaOCl in disinfecting the root canal system, including in combination with sonic and ultrasonic instrumentation (14). Both cavitation and acoustic streaming may help to enhance debridement and disinfection (15) of complex root canal systems (16). However, ultrasonic instrumentation with metal active tips may lead to canal transportation, ledges, zipping, and stripping (17), especially in very curved canals (18).

Recently, a device known as the EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK) (19) has been introduced; it is designed to enhance hydrodynamic phenomena by means of the subsonic activation of a passive smooth polymer tip, which is inserted into the root canal full of irrigating solution. The objective of this study was to evaluate the efficacy of subsonic activation of NaOCl in reducing bacterial load in the root canal.

Materials and Methods

One hundred twelve extracted human single-root teeth with a fully formed apex (upper central incisors and canines with substantially equal canal curvature and morphology) that had not undergone prior endodontic treatment were used. After debriding the root surface, specimens were immersed in a 5% solution of NaOCl (Niclor 5; OGNA, Muggiò, Italy) for 1 hour and then stored in saline solution until preparation. Each specimen was sectioned to obtain a residual root length of 15 mm. Each root canal was preflared using K-Flexofiles (Dentsply Maillefer, Ballaigues, Switzerland) up to #20 and then shaped using ProTaper S1-S2-F1-F2-F3 (Dentsply Maillefer) at the working length. The working length was established under microscopic vision (OPMI Pro Ergo; Carl Zeiss, Oberkochen, Germany) at 10× magnification when the tip of the instrument was visible at the apical foramen. Irrigation was performed with a 30-gauge needle syringe using 33 mL of 5% sodium hypochlorite at 50°C (Niclor 5; OGNA, Muggiò, Italy) and alternating with 10 mL of 10% EDTA (Tubuliclean, OGNA); the total irrigation time was 10 minutes per specimen. After drying with paper points, the roots were inspected under the microscope at 10× magnification to verify the absence of cracks and canal cleanliness. Root surfaces were sealed with varnish and sticky wax; each specimen was fixed with cyanoacrylic cement onto an Eppendorf tube, which was placed in a plastic support box. Specimens were placed in envelopes and sterilized with ethylene oxide. This is a volatile gas that does not alter the structure of materials with which it

comes into contact and does not produce a temperature increase. It leaves no residue at the end of the sterilization cycle, even inside the dentin tubules, not influencing the growth or vitality of bacteria inoculated subsequently (20–23). The procedure was as follows: 6 hours at 40°C, 3 hours at 70% to 75% humidity, a 6-hour application of 10% ethylene oxide, and total removal of the gas from the envelope by repeated replacement of the air content.

The sterilized roots were placed under a laminar flow biohazard cabinet (CLANLAF VFR 1206; Capriolo, Brescia, Italy). The root canals were infected with a standard volume of 30 μL of a pure culture of *Enterococcus faecalis* ATCC 29212, which was previously grown in brain-heart infusion (Oxoid, Milan, Italy) medium broth for 24 hours and adjusted spectrophotometrically to an optical density of 0.15 at 620 nm (Genesys 20 Spectrophotometer; Thermo Electron Corporation, Madison, WI) to match the turbidity of 3×10^7 CFU as confirmed by colony counts in triplicate. Specimens were further incubated aerobically at 37°C for 2 hours to allow penetration of *E. faecalis* into the root canal dentine. Two additional specimens were used as negative controls and 10 as positive controls. The remaining 100 samples were randomly subdivided into four groups (n = 25) using a random numbers table.

Irrigation Protocols and Microbe Count

Specimens in the NaOCl 15 group (n = 25) were irrigated for 40 seconds with 2 mL of a 5% NaOCl solution at room temperature with a 30-gauge needle syringe 2 mm short of the apex. NaOCl was left in the root canal for 15 seconds before removal with 5 mL of saline solution. Specimens in group NaOCl 30 (n = 25) followed the same procedure, but the NaOCl was left in the root canal for 30 seconds before removal.

Specimens in the EndoActivator (EA; Dentsply Tulsa Dental Specialties, Tulsa, OK) 15 group (n = 25) were irrigated for 40 seconds with 2 mL of a 5% NaOCl solution at room temperature with a 30-gauge needle syringe 2 mm short of the apex. NaOCl was left in the root canal and immediately activated subsonically for 15 seconds, inserting the EA 15/.02 polymer tip into the root canal 2 mm short of the apex; the irrigant was then removed with 5 mL of saline solution. The EA driver was set at 10.000 cpm. Specimens in the EA 30 group (n = 25) followed the same procedure, except that the NaOCl was activated with EA for 30 seconds. Positive controls (n = 10) were irrigated for 40 seconds with 2 mL of sterile water.

Subsequent to each irrigation treatment, the root canals were dried at working length and sampled with sterile paper points. The paper points were transferred to tubes containing 1 mL of 0.85% saline solution and vortexed for 1 minute. After 10-fold serial dilutions, aliquots of 0.1 mL were plated onto brain-heart infusion medium agar and incubated at 37°C under aerobic conditions for 24 hours. The colony-forming units (CFUs) grown were counted and then transformed into actual counts based on the known dilution factors.

Statistical Methods

The Kolmogorov-Smirnov test for normality revealed a normal data distribution. Statistical analysis was conducted with a model of one-way analysis of variance test and a post hoc Bonferroni test for multiple comparisons. Differences were considered statistically significant when $p < 0.05$. All statistical analyses were performed using the SPSS for Windows 12.0 software package (SPSS Inc, Chicago, IL).

Results

Descriptive statistics of the postirrigation microbe count and the percentage of bacterial load reduction are summarized in Table 1. The inferential analysis revealed a statistically significant difference among groups ($F_3 = 9.01$, $p < 0.001$). The multiple comparisons post hoc analysis evidenced a statistically significant difference between standard NaOCl irrigation groups 15 and 30 and group EA 30 in which the NaOCl was activated with the subsonic device for 30 seconds ($p < 0.05$). The bacterial load reduction compared with positive controls (mean $1.26 \pm 1.05 \times 10^7$ CFU, 61.5% reduction) ranges from 98.6% (NaOCl 15) to 99.6% (EA 30) with slight differences among groups.

Discussion

The need to improve root canal disinfection is increasingly attracting interest because even modern nickel-titanium rotary instrumentation only act on the central portion of the root canal system, leaving potential niches untreated (21–24). Thus, in recent decades, endeavors have been made to enhance the efficacy of irrigant solutions through innovative irrigant delivery devices and agitation techniques, both manual and machine assisted (19).

Sonic activation has been shown to be an effective method to remove the oral biofilm and enhance root canal disinfection (25). However, the performance of subsonic agitation appears to be less effective compared with ultrasonic activation of irrigant solutions (26). This may be attributed to the different acoustic streaming velocity and frequency, which positively influence debris removal from the qualitative standpoint. However, other studies found no difference between the 2 systems (27) and reported similar penetration of the solution into extracted teeth accessory canals (28), whereas the EA promoted less extrusion of the irrigant over the apex (29).

The advantages of sonic agitation of the irrigant solution have been analyzed, reporting significantly better debridement of the root canal walls compared with manual agitation with endodontic files (27). The EA system has been reported to effectively clean debris from lateral canals, remove the smear layer, and dislodge clumps of simulated biofilm within the curved canals of molar teeth (30). Another recent study (31) compared the effects of different ultrasonic tips and the EA system on necrotic pulp dissolution and transportation of the main canal using epoxy resin–modified models with simulated accessory canals and 2.5% NaOCl irrigant. The results showed that ultrasonic activation dissolved more tissue than did sonic activation or passive irrigation; the EA sonic system with passive polymer tip and ultrasonically activated nickel-titanium tips caused no detectable canal transportation. However, these studies did not consider the influence of the type of irrigation on root canal disinfection.

TABLE 1. Descriptive Statistics of the Postirrigation Microbe Count (105CFUs) and Bacterial Load Reduction (%)

							95% CI		
Group	N	Mean	STD	Median	Min	Max	Lower	Upper	Bacterial load reduction (%)
NaOCl-15″	25	3.75	3.00	2.2	1.4	8.5	2.47	5.04	98.6
NaOCl-30″	25	3.47	2.17	3.4	0.53	6.2	2.46	4.47	98.7
EA-15″	25	2.34	1.20	2.25	1.12	5.08	1.83	2.85	99.1
EA-30″	25	1.01	0.84	0.67	0.43	3.17	0.67	1.35	99.6

CFU, colony-forming units; CI, confidence interval; EA, Endoactivator.

The test hypothesis of this study was that sonic activation of NaOCl associated with a standard irrigation regimen enhances disinfection. The potential of the system, used in combination with a 5% sodium hypochlorite, to reduce bacterial load in the root canal was investigated. It was tested on clean root canal systems. This was achieved by root canal chemomechanical instrumentation and debridement, exploiting the well-known efficacy of standard irrigation protocols alternating NaOCl and EDTA (20) in removing smear layer and organic debris from the root canal system. It was hoped to suggest a possible improvement of disinfection because otherwise untreated niches may be open to the hydrodynamic action of the activated solution.

The bacterial model used *E. faecalis* to test the efficacy of the irrigation protocols under comparison. *E. faecalis* is not particularly demanding from the nutritional standpoint, is resistant to extreme challenges, and has frequently been isolated in cases of endodontic failure (32, 33) because it can penetrate the dentine tubules and escape chemomechanical treatment of the root canal system (34).

None of the protocols tested in this study completely eradicated microorganisms. The results show a significant improvement of root canal disinfection in the EA 30 group in which 30 seconds of agitation was applied compared with irrigation alone. For the EA 15 group, in which activation was only for 15 seconds, there was no difference versus irrigation alone. The EndoActivator driver was always used at the maximum power setting of 10,000 cpm; thus, comparative data concerning the efficacy of the device at lower power settings are not available. This point remains to be investigated.

A recent study using an *E. faecalis* infection model (35) investigated the intracanal disinfection performance of three different irrigation techniques: conventional irrigation with NaviTip needles (Ultradent, South Jordan, UT), the EndoActivator system, and the EndoVac system (Discus Dental, Culver City, CA). The importance of chemomechanical preparation in reducing bacterial load was confirmed, but no significant differences were found in the three techniques, which performed similarly.

In conclusion, within the limits of this study, sonic activation for 30 seconds of a 5% NaOCl solution appears to be slightly more efficacious in disinfecting the root canal compared with a standard irrigation regimen with needles and also compared with sonic activation for only 15 seconds. However, in the study conditions, the difference in bacterial load reduction among groups did not appear to be impressive enough to allow clinical extrapolation of the results. In our opinion, the interesting potential of sonic activation systems should be further investigated through clinical studies aimed to establish a correct irrigation protocol.

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