Comparative evaluation of the antimicrobial efficacy of a 5% sodium hypochlorite subsonic-activated solution.

Comparative evaluation of the antimicrobial efficacy of a 5% NaOCl subsonic-activated solution.

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Abstract

Introduction: The study evaluated the efficacy of subsonic agitation of NaOCl in reducing bacterial load in the root canal. **Methods:** Root canals of 106 extracted human single-root teeth were preflared using K-Flexofiles up to #20 then shaped using ProTaper S1-S2-F1-F2-F3 at the working length. Irrigation was with 33 mL of 5% sodium hypochlorite, alternating with 10 mL of 10% EDTA. After ethylene oxide sterilization, the root canals were infected with 30µl of *Enterococcus faecalis* culture, and randomly assigned to four groups (n=25) of different irrigation regimens plus positive and negative controls. Irrigation was with 2 mL of 5% NaOCl. In group A the final NaOCl flush was left in place for 15 seconds; in group B for 30 seconds; in groups C and D the NaOCl was subsonically-agitated with EndoActivator™ respectively for 15 and 30 seconds. The residual bacterial count was then evaluated. Differences among groups were analyzed with one-way ANOVA and post-hoc Bonferroni's test ($p<0.05$). **Results:** A statistically significant difference was evidenced among groups ($F=9.01$; $df=3$; $p<0.001$). The standard irrigation groups (1 and 2) showed higher microbial counts than group 4 ($p<0.05$). **Conclusion:** 30 seconds of NaOCl subsonic agitation with EndoActivator™ appears to be more effective in reducing bacterial load in the root canal compared to NaOCl irrigation alone.

Key Words

sodium hypochlorite, subsonic, disinfection, endodontic irrigants.

Bacteria and their by-products play a relevant role in the onset and perpetuation of pulpal and periradicular disease (1-ref. JOE 2006). 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 (2-3-4:1-3 JOE review2009). Clinical studies have demonstrated a more favorable long-term prognosis of specimens that were culture-negative before obturation, versus culture-positive specimens (94% vs 68%) (5-REF JOE 5y follow up), while other studies have failed to show any significant difference concerning healing (6- 10 ET). However, there is general consensus that successful elimination of the causative agents from the root canal system is the key to health (7- 12 ET).

Chemical-mechanical treatment of the root canal system has demonstrated its efficacy in reducing bacterial load (8-ref JOE 2006), even though bacteria may persist despite these efforts (9-ref JOE 2006) due to the complexity of the root canal system (10-11-12: 17-19 JOE review2009). 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- ref Bergenholtz). NaOCl used at concentrations ranging from 0.5% to 5% 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- Martin 143 ETp89). Both cavitation and acoustic streaming may help to enhance debridement and disinfection (15- 147 ETp89) of complex root canal systems (16- 158 Archer ETp90). However, ultrasonic instrumentation with metal active tips has been shown to suffer from a risk of canal transportation, ledges, zipping and stripping (17- 164 ET), especially in very curved canals (18- 165 ET).

Recently a device known as the EndoActivator^{TM}(19- 95JOE) has been introduced; it is designed to enhance hydrodynamic phenomena by means of the subsonic activation of a passive smooth polymer tip, that 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 and six extracted human single-root teeth with fully-formed apex (upper central incisors and canines with substantially equal canal curvature and morphology) that had not undergone prior endodontic treatment were utilized. After debriding the root surface, specimens were immersed in a 5% solution of NaOCl (Niclor 5, OGNA, Muggiò, Italy) for 1 h and then stored in saline solution until preparation. Each specimen was sectioned to obtain a residual root of length 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, Ballaigues, Switzerland) at the working length. The working length was established under microscopic vision (OPMI Pro Ergo, Carl Zeiss, Oberkochen, Germany) at 10X magnification, when the tip of the instrument was visible at the apical foramen. Irrigation was performed with a 22 gauge needle syringe: 33 mL of 5% sodium hypochlorite at 50°C (Niclor 5, OGNA, Muggiò, Italy), alternating with 10 mL of 10% EDTA (Tubuliclean, OGNA, Muggiò, Italy), total irrigation time 10 min per specimen. After drying with paper points, the roots were inspected under the microscope at 10X magnification to verify 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 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- 23JBA). The procedure was as follows: 6 h at 40° C, 3 h at 70% - 75% humidity, 6 h 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, USA). The root canals were infected with a standard volume of 30μL of an overnight culture of *Enterococcus faecalis* ATCC 29212, at a turbidity of 50-55 OD absorbance [3-5 x 10^7 colony forming units/mL (CFU/mL)] in Brain Heart Infusion (BHI; Oxoid, Milan, Italy) medium broth and further incubated aerobically at 37°C for 2 h to allow penetration of *E. faecalis* into the root canal dentine. Two additional specimens were used as negative controls and four 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 group A (n=25) were irrigated with 2 mL of a 5% NaOCl solution at room temperature with a 30 gauge needle syringe, 2 mm short of the apex. A final flush of NaOCl was left in the root canal for 15 seconds before removal with 5 mL of saline solution. Specimens in group B (n=25) followed the same procedure but the NaOCl was left in the root canal for 30 seconds before removal.

Specimens in group C (n=25) were irrigated similarly, and the final flush of NaOCl was activated subsonically for 15 seconds, inserting the EndoActivator™ (Dentsply Tulsa Dental Specialties, Tulsa, OK) 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 EndoActivator™ driver was set at 10.000 cpm. For specimens in group D (n=25) the same procedure was followed, except that the NaOCl was activated for 30 seconds.

The root canals were then dried with paper points. To evaluate the bacterial load remaining in the root canal, each specimen was subjected to an appropriate number of 1:10 serial dilutions in sterile distilled water and plated on BHI medium agar. Plates were incubated at 37°C for 24h under aerobic conditions. The microbe counts are reported as CFU/mL.

Statistical methods

The Kolmogorov-Smirnov test for normality revealed a normal data distribution. Statistical analysis was conducted with a model of One-way ANOVA test and post-hoc Bonferroni's 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 post-irrigation microbe count are summarized in Table 1. The inferential analysis revealed a statistically significant difference among groups ($F=9.01$; $df=3$; $p<0.001$). The multiple comparisons post-hoc analysis evidenced a statistically significant difference between standard irrigation groups 1 and 2, and group 4 in which the NaOCl was activated with the subsonic device for 30 seconds ($p<0.05$).

Discussion

The need to improve root canal disinfection is increasing attracting interest, since even modern NiTi rotary instrumentation only act on the central portion of the root canal system, leaving potential niches untreated (21-24: 20-22 JOE review). 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 (25- review JOE).

Sonic activation has been shown to be an effective method to remove the oral biofilm and enhance root canal disinfection (26- 87revJOE). However, the performance of subsonic agitation appears to be less effective compared to ultrasonic activation of irrigant solutions (27- Sabins JOE 2003). 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 two systems (28- Jensen 22 JOE Anas09) and reported similar penetration of

the solution into extracted teeth accessory canals (29- 20 JOE Anas09), whereas the EndoActivator promoted less extrusion of the irrigant over the apex (30- 21 JOEAnas09).

The advantages of sonic agitation of the irrigant solution have been analyzed, reporting significantly better debridement of the root canal walls compared to manual agitation with endodontic files (28). The EndoActivator 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 (31- 76). Another recent study (32- Anas JOE ott 2009) compared the effects of different ultrasonic tips and the EndoActivator System on necrotic pulp dissolution and trasportation 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 EndoActivator sonic system with passive polymer tip and ultrasonically activated NiTi tips caused no detectable canal transportation. However, these studies did not consider the influence of the type of irrigation on root canal disinfection.

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 final flush of 5% sodium hypochlorite, to reduce bacterial load in the root canal was investigated. It was tested on clean root canal systems, achieved by root canal chemo-mechanical instrumentation and debridement, exploiting the well-known efficacy of standard irrigation protocols alternating NaOCl and EDTA (20- Berutti JOE96), in removing smear layer and organic debris from the root canal system. It was hoped to suggest a possible improvement of disinfection, since 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 (33,34- 35,36 JBA Pasqui) since it can penetrate the dentine tubules and escape chemo-mechanical treatment of the root canal system (35- 37idem).

None of the protocols tested in this study completely eradicated microrganisms. However, the results demonstrate a statistically-significant improvement of root canal disinfection in group 4, in which 30 seconds agitation was applied, compared to irrigation alone. For group 3, in which activation was only for 15 seconds, there was no difference versus irrigation alone. The EndoActivator^{TM} 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 (36- BRITO JOE ott2009), investigated the intracanal disinfection performance of three different irrigation techniques: conventional irrigation with NaviTip needles, the EndoActivator system and the EndoVac system. The important role of chemo-mechanical 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 more efficacious in disinfecting the root canal compared to a standard irrigation regimen with needles, and also compared to sonic activation for only 15 seconds. However, no extrapolation of the results may be indicated, and further clinical studies will be required to establish a correct irrigation protocol enhanced by the use of sonic activation systems.

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Group	N	Mean	STD	Median	Min	Max	95% C.I.		Bacterial load reduction (%)
							Lower	Upper	
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

TABLE 1. Descriptive statistics of the post-irrigation microbe count (10^5CFU) and bacterial load reduction (%); EA = Endoactivator