

The effect of different temperature and concentration of sodium hypochlorite on the elimination of *E. Faecalis* using rotary instrumentation and intermittent passive ultrasonic irrigation

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الغرض من هذه الدراسة تقييم تأثير المتغيرات المختلفة لهيبوكلوريت الصوديوم في المختبر درجة حرارة السائل، تركيز (السائل) في قدرته على إزالة البكتيريا الإشريكية البرازية، باستخدام أدوات تحضير الأقماع الدوّارة والإرواء فوق الصوتي السلبي المتقطع في أقنية متفاوتة الإنخراط. طريقة البحث: تم تحضير ٧٢ سن مقلوع وحيد الجذر باستخدام المبرد K-file إلى مقاس ٢٠ ثم زرعت بالبكتيريا الإشريكية البرازية. قسمت الأقماع بالتساوي إلى خمسة مجموعات اختبارية ومجموعة واحدة شاهده. تم تحضير الأقماع في المجموعة الشاهدة باستخدام الأدوات الدوّارة (٤.٠ profile) وتم إروؤها بهيبوكلوريت الصوديوم بتركيز ٢.٥٪ و درجة حرارة الغرفة ٢٥° بتحرك سائل الإرواء بجهاز الاهتزاز فوق الصوتي بشكل متقطع. في المجموعة الأولى تم تشكيل الأقماع إلى إنخراط ٠.٠٦، في المجموعة الثانية و الثالثة كانت درجة حرارة هيبوكلوريت الصوديوم ٣٧° و ٤٥° و في المجموعتين الرابعة و الخامسة كان تركيز هيبوكلوريت الصوديوم ١٪ و ٥٪. تم زرع القنوات في درجة حرارة ٣٧° لمدة ٤٨ ساعة و أخذت العينات باستخدام الأقماع الورقية. تم أخذ عينات من الأقماع قبل وبعد التحضير وتم قياس منطقة النمو البكتيري. النتائج: ظهر تزايد واضح في تعداد البكتيريا في المجموعة الشاهدة بالمقارنة مع المجموعات ١، ٢، ٣، ٤، ٥. كذلك هناك تناقص واضح في النمو البكتيري في المجموعة ٥ مقارنة بالمجموعة ٤. الخلاصة: أظهرت النتائج أن تحفيز محلول هيبوكلوريت الصوديوم بزيادة التركيز و درجة الحرارة بالإضافة إلى الإرواء فوق الصوتي السلبي المتقطع مهم في تعزيز القدرة على إزالة البكتيريا الإشريكية البرازية من القناة الجذرية.

OBJECTIVE: The purpose of this study was to compare the intracanal bacterial reduction using rotary instrumentation and intermittent passive ultrasonic irrigation (IPUI) with different concentrations and temperatures of NaOCl in different canal tapers. **MATERIALS and METHODS:** The root canals of seventy-two extracted single-rooted teeth were instrumented up to size #20k file and inoculated with *E. faecalis*. The teeth were divided into 5 experimental groups and one control. The root canals in the control group were shaped to a 0.04 taper using ProFile rotary files, with 1.5 minute of IPUI by NaOCl at a concentration of 2.5% and room temperature of 25°C for 30 seconds at a time at three intervals. In Group I, the canals were shaped to a 0.06 taper, and in Groups II and III - the temperature of NaOCl used was 37°C and 45°C respectively, and in Groups IV and V - the concentrations of NaOCl were 1% and 5% respectively. The canals were incubated at 37°C for 48 hours and bacterial samples were obtained using paper points and plated on agar plates. The zones of bacterial growth were measured and statistical analysis was performed. **RESULTS:** There was significantly more bacterial growth in the control group than in Groups I, II, III and V. Furthermore, there was a significant reduction in bacterial growth in Group V compared to Group IV. **CONCLUSIONS:** The result of this study showed that significant bacterial reduction in contaminated root canals could be obtained using intermittent passive ultrasonic irrigation combined with 2.5% NaOCl at 37°C in canals prepared to a taper of 0.06. In addition, complete bacterial eradication could be obtained using IPUI with 2.5% NaOCl at 45°C or 5% NaOCl at room temperature (37°C).

INTRODUCTION

Bacteria persisting within the root canal system are the major cause of endodontic treatment failures.^{1,2} Irrigation is an important aspect of root canal preparation and is used to improve the efficiency of the preparation in removing debris and microorganisms from the root canal system.^{3,4} NaOCl has long been used as an effective irrigating solution and it

remains the irrigant of choice in modern endodontic therapy.⁵⁻⁷ With the reduced preparation time made by the advent of rotary instruments, the irrigant of choice should be one that exerts its microbial activity quickly against resistant microorganisms found in the root canal system. Alternatively, certain factors that effectively enhance the cleansing activity of a known irrigant can be utilized.

Passive ultrasonic irrigation (PUI) of the NaOCl has been shown to enhance its penetration and solvent action,^{8,9} allowing the irrigant to reach inaccessible areas of the root canal system that have potential

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to harbor microorganisms such as dentinal tubules, fins and ramifications.

Whereas a few studies have shown that PUI should be utilized for 3 minutes after root canal preparation,¹⁰ other studies advocated that 1 minute of PUI¹¹ was sufficient to cleanse the canal of microorganisms. Previous investigations by the author¹² have shown the efficacy of using PUI in an intermittent fashion (IPUI) for irrigation, and could possibly increase the potency of NaOCl through frequent maintenance of a chlorine reserve that is sufficient to eliminate bacteria as suggested by Cameron¹³ in 1987 and Siquera *et al.*¹⁴ in 2000.

In addition, canal diameter and taper have been shown to play a role in the efficiency of the irrigant in bacterial reduction.¹⁵⁻¹⁸ Likewise, the concentration of NaOCl has been studied and several concentrations have been advocated.^{19,20} In addition, the temperature of NaOCl has been shown to have an effect on the efficacy of the bactericidal potential of NaOCl.^{21,22}

Utilizing the aforementioned factors to increase the potency of NaOCl irrigation in combination with intermittent ultrasonic irrigation (IPUI) in rotary instrumented canals might reduce bacterial colonization in comparison with standard rotary instrumentation and PUI with a reduced time factor.

The purpose of this *in vitro* study was to compare the intracanal bacterial reduction using rotary instrumentation and IPUI with NaOCl in three situations:

1. In canals with a taper of 0.04 and 0.06
2. With three different concentrations of NaOCl (1%, 2.5% and 5%)
3. With three different temperatures of NaOCl (25°C, 37°C and 45°C)

MATERIALS and METHODS

Seventy-two extracted single rooted maxillary and mandibular premolar

human teeth free of caries, cracks and root canal treatment were selected for this study. Conventional access preparations were made using a high speed round bur under water coolant spray. The working length was established by subtracting 1 mm from the length of size 15 k file when it extruded just beyond the apical foramina. The coronal aspect of each canal was flared using Gates Glidden drills sizes 2 and 3 (Dentsply Maillefer, Switzerland). The root canals were instrumented up to size 20 k file under irrigation with tap water to unify canal diameter before beginning the experiment. After root canal preparation, the enlarged apical foramen was sealed with composite to prevent bacterial leakage. The teeth were randomly divided into 4 experimental groups and 1 control group with an equal average of canal lengths. To make both handling and identification easier, the teeth were mounted vertically in plastic blocks with 12 roots per block totaling 5 plaster blocks, representing groups. The plaster blocks were sterilized under ultraviolet (UV) light overnight. All procedures thereafter were performed under a laminar airflow hood (Baker Company, Maine, USA) using sterilized instruments. Random samples were taken from each group, plated on agar plates and incubated at 37°C for 48 hours to determine absence of bacterial growth and effectiveness of the sterilization by UV light. This served as a negative control group. A bacterial suspension was prepared by adding 1 ml of pure cultured of *E. faecalis* (ATCC-29212) grown in Brain Heart infusion broth (Oxoid, England) to fresh Brain Heart infusion broth. The cultures of *E. faecalis* were obtained from Microbiology Laboratory of King Abdulaziz University Hospital, Riyadh, Kingdom of Saudi Arabia. Each root canal was completely filled with *E. faecalis* suspension using 1 ml tuberculin syringes. The blocks were then placed inside sterile plastic bags and incubated at 37°C for 48 hours.

All the root canals were sampled before instrumentation to verify presence of bacteria. Under the laminar airflow hood using sterilized instruments, the control group was prepared with 0.04 taper profile rotary files (Dentsply, Tulsa, USA) in a crown down sequence starting with file #20 until a #40 file fit at working length. After each instrument, the canals were irrigated with 2 ml of 2.25% solution of NaOCl at room temperature (25°C), using a disposable syringe and a 21-gauge needle (Bu Kwang Medical Inc., Korea) that was inserted as far into the prepared root canal as possible without binding. Ultrasonic P5 (Piezon Master 400, EMS, Nyon, Switzerland) with a NiTi file #25 was inserted passively to 2/3 the working length in conjunction with copious amounts of irrigant and oscillated for 30 seconds once after size 30k file preparation, once after the end of preparation (at size #40), and once after NaOCl was left *in situ* to complete 15 minutes. With each root canal, a total volume of 10 ml of NaOCl was used with a total contact time of 15 minutes.

In Group I, the canals were prepared to a 0.06 taper with Profile 0.06 rotary instrument under the same conditions of the control group. In Group II, the irrigant (NaOCl) was heated to 37°C in a water bath prior to use, following the same instrumentation protocol as the control group. In Group III, the irrigant (NaOCl) was heated to 45°C in a water bath prior to use, following the same instrumentation protocol as the control group. In Group IV, the concentration of the NaOCl was 1% while the instrumentation protocol was the same as for the control group. In Group V, the concentration of the NaOCl was 5% while the instrumentation protocol was the same as the control group.

NaOCl was inactivated, the canals were filled with Brain Heart Infusion broth, and samples were taken by inserting sterile paper points to a full length of the canals

and rotating them. To prevent cross-contamination of the samples, sterile paper points were used, and the tweezers used to manipulate these paper points were sterilized by open flame before using. Each paper point was plated onto an agar plate and incubated at 37°C for 48 hours. The widest area of bacterial growth around the paper points was measured in mm (zones of bacterial growth) and recorded for each sample.

Measurements were analyzed statistically for differences between groups using ANOVA and Tukey's tests. The significance level was established at 5% ($P < 0.05$).

RESULTS

Negative control group of randomly plated samples showed no growth. Mean zones of bacterial growth of each group are shown in Table 1 for samples taken immediately after instrumentation of the root canals.

The Wilks' Lambda Multivariate test showed that there was a significant difference between groups effect { $F(3,44) = 4.506, P < .01$ }. Post hoc comparisons utilizing Tukey's HSD test showed that there was significantly more bacterial growth in the control group than in Groups 1, 2, 3 and 5. Furthermore, there was a significant reduction in bacterial growth in Group 5 compared to Group 4.

Table 1. Mean zone of bacterial growth (mm) in each group immediately after instrumentation.

Groups	Mean	Standard deviation	Number of specimens (N)
Control	20.35 mm	13.32	12
Group 1	0.25 mm	0.62	12
Group 2	8.14 mm	12.6	12
Group 3	0.00 mm	0.00	12
Group 4	10.37 mm	11.5	12
Group 5	0.00 mm	0.00	12

Table 2. Significant differences in zones of bacterial growth between groups immediately after instrumentation.

Multiple comparisons						
Tukey HSD						
(I) Group	(J) Group	Mean difference (I-J)	Standard Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
0.00	1.00	9.9667*	1.8518	0.000	4.5289	15.4045
	2.00	5.9250*	1.8934	0.030	0.3650	11.4850
	3.00	10.2167*	1.8518	0.000	4.7789	15.6545
	4.00	4.9042	1.8518	0.100	-0.5336	10.3420
	5.00	10.9250*	1.8518	0.000	5.4872	16.3628
1.00	0.00	-9.9667*	1.8518	0.000	-15.4045	-4.5289
	2.00	-4.0417	1.8934	0.283	-9.6017	1.5184
	3.00	0.2500	1.8518	1.000	-5.1878	5.6878
	4.00	-5.0635	1.8518	0.082	-10.5003	0.3753
	5.00	0.9583	1.8518	0.995	-4.4795	6.3961
2.00	0.00	-5.9250*	1.8934	0.030	-11.4850	-0.3650
	1.00	4.0417	1.8934	0.283	-1.5184	9.6017
	3.00	4.2917	1.8934	0.223	-1.2684	9.8517
	4.00	-1.0208	1.8934	0.994	-6.5809	4.5392
	5.00	5.0000	1.8934	0.102	-0.5600	10.5600
3.00	0.00	-10.2167*	1.8518	0.000	-15.6545	-4.7789
	1.00	-0.2500	1.8518	1.000	-5.6878	5.1878
	2.00	-4.2917	1.8934	0.223	-9.8517	1.2684
	4.00	-5.3125	1.8518	0.059	-10.7503	0.1253
	5.00	0.7083	1.8518	0.999	-4.7295	6.1461
4.00	0.00	-4.9042	1.8518	0.100	-10.3420	0.5336
	1.00	5.0625	1.8518	0.082	-0.3753	10.5003
	2.00	1.0208	1.8934	0.994	-4.5392	6.5809
	3.00	5.3125	1.8518	0.059	-0.1253	10.7503
	5.00	6.0208*	1.8518	0.022	0.5830	11.4586
5.00	0.00	-10.9259*	1.8518	0.000	-16.3628	-5.4872
	1.00	-0.9583	1.8518	0.995	-6.3961	4.4795
	2.00	-5.0000	1.8934	0.102	-10.5600	0.5600
	3.00	-0.7083	1.8518	0.999	-6.1461	4.7295
	4.00	-6.0208*	1.8518	0.022	-11.4586	-0.5830

Based on observed means

*The mean difference is significant at 0.5 level

DISCUSSION

E. faecalis was chosen for the study because of its high resistance to a wide range of microbial agents,²³ its presence in association with persistent apical periodontitis,²⁴ its difficult elimination from the root canal with use of chemomechanical procedures,²⁵ and for its ease in culturing and manipulation.²⁶

In this study, measurement of bacterial growth on blood agar was used to evaluate the amount of bacteria sampled from the canals. Although this was a subjective evaluation, it allowed a

comparative evaluation between different irrigation methods because the bacterial inoculation, sampling and measurement were standardized. In addition, this model enabled easy handling and testing with limited means. However, the antibacterial effectiveness of irrigants in root canal therapy may be quite different compared to mixed cultures present in a dynamic biological system, as usually occurs *in vivo*. Thus, direct extrapolations to clinical conditions must be exercised with caution.

The present study showed that increasing the taper of the root canal with IPUI resulted in significant reduction in bacterial growth within the canal compared to the control. This is in agreement with Lee *et al.*,¹⁷ Van der Sluis *et al.*¹⁸ and, Abou Rass and Piccinino.²⁷ Increasing the taper of the canal allows deeper penetration of the irrigating needle and the ultrasonic file that has a major effect on the efficacy of the irrigation. Therefore, it is suggested that keeping the diameter of the apical foramina to a desirable size while increasing the taper of the canal will enhance the efficacy of IPUI with NaOCl.

The desirable concentration of NaOCl should be one that has low toxicity and adequate antibacterial effects. In the present study, 5% NaOCl completely eliminated bacterial presence and NaOCl 2.25% significantly reduced bacterial growth, while NaOCl 1% had no effect with the use of IPUI of the same taper. This is in agreement with Berber *et al.*,¹⁹ Abou Rass and Oglesby,²⁸ and Gomes *et al.*²⁹ in non-ultrasonic irrigation studies. It is in disagreement with Siquera *et al.*¹⁴ who observed no significant difference between 1%, 2.5% and 5.25% concentrations of NaOCl in eliminating *E. faecalis*, and Huque *et al.*²⁰ who suggested that the most effective concentration of NaOCl was 12%. Siquera *et al.*¹⁴ mentioned that other factors such as frequent exchange of the irrigant might maintain the

antibacterial effectiveness of the NaOCl solution compensating for the effects of concentration. In addition, Cameron³⁰ reported that the most effective regime with ultrasonic energy was to activate each and every dose of irrigant placed in the canal. In this study, the irrigant was activated three times, this may have had some role in potentiating the effects of 5% NaOCl.

The results of the present study agree with those of Cunnigham and Joseph,²² Abou Rass and Oglesby,²⁸ and Cunnigham and Balekjian³¹ in that increasing the temperature of NaOCl increased the germicidal activity of the irrigant. Additionally in the present study, increasing the temperature to 45°C proved more effective with complete bacterial eradication. Ultrasonic activation of 2.5% sodium hypochlorite warms the solution, an action that is advantageous since it has been shown that warming this irrigant improved its tissue dissolving.²² In this study, raising the temperature of the solution to body temperature (37°C) reduced bacteria more significantly than the control, but did not completely eliminate bacteria. Little is known on the amount of temperature increase during PUI, although it is suggested not to exceed 5°C and therefore pre-warming the solution to an acceptable level can effectively enhance the bactericidal activity of NaOCl. Additional studies are needed to verify the effective temperature needed to eradicate bacteria without damaging the PDL, with consideration to the temperature increase that IPUI adds to the irrigant.

The result of this study showed that combining factors known to enhance NaOCl with ultrasonic irrigation improved its action. These synergistic effects enabled the irrigant solution to penetrate and cleanse the complex morphology of the root canal space, and potentiate the effects of the irrigant, as well as the ultrasonic energy produced by the ultrasonic device.

CONCLUSIONS

Use of passive ultrasonic irrigation of NaOCl for 30 second periods intermittently throughout canal preparation of infected root canals can be significantly enhanced by changing irrigant variables, as well as canal taper. Complete bacterial eradication can be achieved with IPUI and 5% NaOCl at room temperature or 2.5% NaOCl at 45°C. In addition, significant bacterial reduction in contaminated root canals can be attained using IPUI with 2.5% NaOCl at 37°C or when canal taper is increased to 0.06.

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