The effect 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 لهيبوكلورايت الصوديوم في المختبر (طريقة الإرواء ، تماس السطح مع سائل الإرواء، تكرار تغيير السائل . و الحجم الكلي للسائل) في قدرته على إزالة البكتيريا الإشريكيه البرازية ،باستخدام أدوات تحضير الأقنية الدّوارة والإرواء فوق الصوتي السلبي المتقطع. طريقة البحث: تم تحضير ٦٠ سن مقلوع وحيد الجذر باستخدام المبرد K_file إلى مقاس ٢٠ ثم زرعت بالبكتيريا الإشريكيه البرازيه. تم تحضير الأقنية باستخدام الأدوات الدّوارة (profile ٠.٠٤ إلى مقاس ٤٠ وتم إرواؤها ب ٢ مل من هيبوكلورايت الصوديوم بتركيز ٢.٢٥٪. ثم تم تحريك سائل الإرواء بجهاز الاهتزاز فوق الصوتي لمدة ٣٠ ثانية بعد المبرد مقاس ٣٠ و مقاس ٤٠. و تم البقاء على هيبوكلورايت الصوديوم داخل القناة في نهاية التحضير لمدة خمس دقائق لتكتمل مدة التحضير الكلي إلى ١٥ دقيقة. كانت كمية هيبوكلورايت الصوديوم المستخدمة ١٠ مل.قسمت الأقنية بالتساوي إلى أربع مجموعات اختباريه ومجموعة واحدة شاهده. استخدم المص في المجموعة الأولى لإيصال سائل الإرواء داخل القناة. أما في المجموعة الثانية كانت مدة تماس سائل الإرواء ٣٠ دقيقة. في المجموعة الثالثة تم استخدام سائل الإرواء كل دقيقتين وفي المجموعة الرابعة كان الحجم الكلي لسائل الإرواء ٢٠ مل وفي مجموعة الشاهدة استخدمت الطريقة الكيموميكانيكية لتحضير القناة بدون استخدام الجهاز فوق الصوتي السلبي المتقطع. تم اخذ عينات من الأقنية قبل وبعد التحضير وتم قياس منطقة النمو البكتيري. النتائج: ظهر تناقص واضح في تعداد البكتيريا في المجموعات ١،٢،٤ بالمقارنة مع المجموعتين٣ و٥. الخلاصة: أظهرت النتائج أن عملية الغسل وزيادة كمية سائل الإرواء. وزيادة مدة تماسه بالتضافر مع استخدام الأدوات الدّوارة لتحضير القناة والجهاز فوق الصوتي السلبي المتقطع قد عززت القدرة على إزالة الإشريكيه البرازية من القناة الجذرية.

OBJECTIVE: The purpose of this study was to evaluate *in vitro* the effect of different variables (delivery system, surface contact with the irrigant, the frequency of changing the irrigant and the total volume of the irrigant) of sodium hypochlorite on the elimination of *E. faecalis* using rotary instrumentation and intermittent passive ultrasonic irrigation (IPUI). ***MATERIALS and METHODS:*** Sixty extracted single-rooted teeth were instrumented up to size 20 K-file, then inoculated with *E. faecalis*. The root canals were prepared up to a # 40 using 0.04 profile rotary files and irrigated with 2 ml of 2.25% NaOCl. Ultrasonic P5 with NiTi file # 25 was oscillated for 30 seconds after # 30 file, at the end of the preparation, and after NaOCl was left in situ for 5 minutes to complete 15 minutes of preparation. A total volume of 10 ml of sodium hypochlorite was used. The root canals were divided equally into four experimental and one control groups. In Group I, pipette was used to deliver the irrigant; Group II, the total contact time of irrigant was 30 minutes; Group III, the canal was flushed every 2 minutes; Group IV, the total volume of the irrigant was 20 ml and in Group V (control), chemo-mechanical preparation was carried out without IPUI. The root canals were sampled before and after instrumentation and the zone of bacterial growth were measured in mm and recorded. ***RESULTS:*** A significant bacterial reduction was demonstrated in Groups I (P=0.020), II (P=0.001), and IV (P=0.000) as compared to Groups III and V. ***CONCLUSIONS:*** The results of this study showed that the flushing action, the larger volume of sodium hypochlorite irrigant and the increase in the contact time of the irrigant combined with rotary instrumentation and IPUI enhanced the elimination of *E. faecalis* from the root canal.

INTRODUCTION

Complete debridement and disinfection of the root canal system are considered to be essential for predictable long-term success in endodontic treatment. Residual pulpal tissue, bacteria and dentin debris may persist in the irregularities of the root canal system, even after meticulous mechanical preparation is carried out.[1] Several irrigant solutions have been recommended for use to aid in the mechanical preparation. Sodium hypochlorite (NaOCl) is the most commonly used irrigant. It has been proven to be an excellent irrigating solution, due to its tissue dissolving capability and anti-microbial activity.[2]

Most of dentin debris is an inorganic matter that cannot be dissolved by NaOCl. Therefore, removal of dentin debris relies mostly on the flushing action of irrigant. The enhancement of the flushing action of an irrigant solution by using ultrasonic is well documented.[3-7] Passive ultrasonic irrigation (PUI) was first described by

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Weller *et al.*,[8] in which ultrasonic file oscillate freely in the root canal without cutting action. PUI relies on the transmission of acoustic energy from an oscillating file to an irrigant in the root canal. The energy is transmitted by means of ultrasonic waves and can induce acoustic streaming and cavitation of the irrigant.[9-11] The cleaning efficacy of PUI implies the effective removal of dentin debris, microorganisms (planktonic or in biofilm) and organic tissue from the root canal. Due to the active streaming of the irrigant its potential to contact a greater surface area of the canal wall will be enhanced.[12] Two flushing methods can be used during PUI, namely a continuous flush of irrigant from ultrasonic handpiece or an intermittent flush method using syringe delivery. In the intermittent flush method, the irrigant is injected into the root canal by a syringe, activated by the ultrasonic file and replenished several times after each ultrasonic activation.

Previous studies showed that both mechanical and chemical action of the irrigant were dependent upon the efficiency of the delivery system,[13] the tissue surface area in contact with the irrigating solution,[14, 15] the frequency of changing the solution[15] and the total volume of the irrigating solution.[16, 17] Utilizing the aforementioned factors that increase the potency of NaOCl irrigation in combination with intermittent passive ultrasonic irrigation (IPUI) in rotary instrumented canals might reduce bacterial colonization in comparison with standard rotary instrumentation and irrigation.

The purpose of this study was to evaluate *in vitro*, the effect of different variables (delivery system, surface contact with the irrigant, the frequency of changing the irrigant and the total volume of the irrigant) of sodium hypochlorite on the reduction of *E. faecalis* using rotary instrumentation and intermittent passive ultrasonic irrigation.

MATERIALS AND METHODS

Sixty extracted single-rooted human teeth were selected for this study. Conventional access preparations were made and the working length was established by subtracting 1 mm from the length of size 15 K-file when extended just beyond the apical foramina. The coronal aspect of each canal was flared using Gates Glidden drills sizes 2 and 3 (Dentsply Maillefer, Ballaigues, Switzerland). The root canals were prepared up to size 20 K-file under irrigation with tap water. After root canal preparation, the enlarged apical foramen was sealed with composite to prevent bacterial leakage. To make both handling and identification easier, the teeth were mounted vertically in plaster blocks (each block contains 12 teeth) and sterilized under ultraviolet (UV) light overnight. All other 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 UV light sterilization. This served as a negative control group. A bacterial suspension was prepared by adding 1 ml of a pure culture of *E. faecalis* (ATCC-29212) in brain heart infusion broth (Oxoid, England) for 24 hours. The cultures of *E. faecalis* were obtained from Microbiology Laboratory, King Abdulaziz Hospital, Riyadh, Kingdom of Saudi Arabia. Each root canal was completely filled with *E. faecalis* suspension using sterile 1 ml tuberculin syringe. The blocks were then placed inside sterile plastic bags and incubated at 37°C for 48 hours.

The samples were equally divided into four experimental and one control groups (12 teeth each). In Group V (control), the root canals were prepared using 0.04 taper profile rotary files (Dentsply

Maillefer, Switzerland) in a crown-down sequence, starting with # 25 until # 40 file fit at working length. Ten ml of 2.25% solution of NaOCl (MA Abudawood & Partners for Industry Co., Jeddah, KSA) at room temperature of 25°C was used as an irrigant for a total period of 15 minutes. After each instrument, canal irrigation with 2 ml was carried out using a disposable syringe and a 21-gauge needle (Bu Kwang Medical Inc., Korea) that was inserted into the prepared root canal as far as possible without binding. The canal was then flooded with a final 2 ml of the irrigant and allowed to sit for 5 minutes (to complete 15 minutes). In Group I, pipette (Medical Disposable Industrial Complex, Riyadh, KSA) was used to deliver the irrigant, following the same instrumentation protocol as the control group. In addition, NiTi file # 25 attached to an ultrasonic P5 (Piezon Master 400, EMS, Nyon, Switzerland) was inserted passively to full working length in conjunction with copious amounts of irrigant and oscillated for 30 seconds after size 30 K-file and at the end of the preparation (by size #40K). In order to complete 15 minutes of contact time, the canal was then flooded with 2 ml of NaOCl and allowed to sit for 5 minutes. The ultrasonic frequency employed under these conditions was approximately 30 kHz and the displacement – amplitude varied between 20 and 30 µm according to the manufacturer's instructions. In Group II, the total contact time of the irrigant was 30 minutes following the same instrumentation protocol as the control group, while ultrasonic P5 was oscillated for 30 seconds after preparation by # 30 K-file, at the end of the preparation and then every 5 minutes for 30 minutes. In Group III, the root canal was flushed every 2 minutes, following the same instrumentation protocol as the control group and the same IPUI protocol as Group I. In Group IV, the total volume of the irrigant was 20 ml. After each instrument, canal irrigation with 4 ml was carried out following the same instrumentation protocol as the control group and the IPUI protocol as Group I.

All the root canals were sampled before instrumentation to verify presence of bacteria, if any, and then immediately after instrumentation. NaOCl was inactivated and then the canals were filled with Brain Heart Infusion broth and samples were taken by inserting sterile paper points to the full length of the canals and allowed to saturate. Each paper point was plated onto an agar plate and incubated at 37°C for 48 hours. To prevent cross-contamination of the samples, a sterile tweezer was used to manipulate the paper points. 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 using ANOVA and Tukey's tests. The significance level was established at 5% ($P<0.05$).

RESULTS

Negative control group of the random plated samples showed no bacterial growth after sterilization by UV light. Mean zones of bacterial growth of each group are shown in Table 1. One-way ANOVA showed that there was a significant difference in bacteria reduction ($P=0.000$) between the groups. Post Hoc Tukey Test showed a significant bacterial reduction in Group I ($P=0.020$), II ($P=0.001$), and IV ($P=0.000$) compared to Groups III and V (Table 2).

DISCUSSION

Enterococcus faecalis is found most prevalently in persistent infections and especially in failed root canal therapy.[18] This microorganism was chosen for the study because of its high resistance to

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

Groups	Mean	Standard Deviation	N
Group 1	9.3333	7.7499	12
Group 2	5.5833	4.1001	12
Group 3	13.3333	8.7004	12
Group 4	4.7500	5.6145	12
Control group	20.3500	13.3245	12
Total	10.6700	10.0338	60

Table 2. Post Hoc Turkey's Test showed a significant difference in zone of bacterial growth between the groups

Groups	Groups	Mean Difference	Standard Error	Sig.
Group 1	2.00	3.7500	3.4720	.816
	3.00	-4.0000	3.4720	.778
	4.00	4.5833	3.4720	.680
	5.00	-11.0167 (*)	3.4720	**.020**
Group 2	1.00	-3.7500	3.4720	.816
	3.00	-7.7500	3.4720	.183
	4.00	.8333	3.4720	.999
	5.00	-14.7667 (*)	3.4720	**.001**
Group 3	1.00	4.0000	3.4720	.778
	2.00	7.7500	3.4720	.183
	4.00	8.5833	3.4720	.112
	5.00	-7.0167	3.4720	.270
Group 4	1.00	-4.5833	3.4720	.680
	2.00	-.8333	3.4720	.999
	3.00	-8.5833	3.4720	.112
	5.00	-15.6000 (*)	3.4720	**.000**
Control	1.00	11.0167 (*)	3.4720	.020
	2.00	14.7667 (*)	3.4720	.001
	3.00	7.0167	3.4720	.270
	4.00	15.6000 (*)	3.4720	.000

* The mean difference is significant at the .05 level

a wide range of microbial agents,[19] its presence in association with persistent apical periodontitis,[20] difficulty of its elimination from the root canal with use of chemo-mechanical procedures,[21] and finally for ease of culturing and manipulation.[22]

The root canals in this study were enlarged to the diameter of a # 40 instrument to comply with the recommendation of Ram[23] who showed that the removal of debris seemed to be affected by the canal diameter rather than the type of solution used. This would logically apply to ultrasonic irrigation of the canals as suggested by Walmsley,[24] as was done in the present study. Narrow canals may compromise the efficacy of ultrasonic irrigation. Therefore, such canals may need to be enlarged to ensure free oscillation of the ultrasonic file.[6, 25]

Cameron[26] reported that the most effective regime with ultrasonic energy was to activate every dose of irrigant placed in the canal. In the present study, the irrigant was activated three times intermittently, as this seemed clinically practical.

In the current study, measures of bacterial growth on blood agar were 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 enables easy handling and testing with limited means. However, one must bear in mind that the anti-bacterial effectiveness of irrigants in root canal therapy may be quite different compared to mixed cultures present in a dynamic biological system, as usually seen *in vivo.* Thus, direct extrapolations to clinical conditions must be exercised with caution because of the obvious limitation of *in vitro* studies.

In the present study, the microbiological samples collected within the root canals with paper points were obtained just after biomechanical preparation in order to evaluate the chemico-mechanical action immediately after the instrumentation and IPUI.

The result of this study showed that the use of pipette to deliver the irrigant with the ultrasonic activation reduced bacterial colonies when compared with the control group. This could be due to the flushing

action of pipette that was potentiated by IPUI. Baker *et al.*[14] showed that the flushing action of the irrigant solution was more important during the cleaning process than its ability to dissolve tissue.

Several studies[27-29] showed a strong relationship between the contact time of the irrigant and the antimicrobial action of NaOCl. Similar result was observed in the present study, in which a considerable bacterial reduction was achieved by increasing the contact time of the irrigant to 30 minutes with 3 minutes of IPUI. In addition, other studies[30, 31] showed that NaOCl removed significantly more smear layer and dentin debris from the root canal during PUI for 3 minutes.

These results can be explained by the greater increase in temperature of sodium hypochlorite solution,[32, 33] which in turn increased its germicidal activity[13, 32, 34] and capacity to dissolve organic material.[15]

On the other hand, Sabins *et al.*[35] showed no significant difference between 30 and 60 seconds of PUI in dentin debris removal from the root canal. In their study, the NaOCl was injected in the root canal by a syringe and not refreshed during ultrasonic activation as in the present study.

The result of this study also showed that increasing the volume of the irrigant significantly reduced bacteria colonies in root canal. This agreed with the reports of other studies.[16, 17, 36] and this could be explained by the fact that chlorine is responsible for the dissolving and antimicrobial capacity of NaOCl. However, chlorine is consumed rapidly during the first phase of tissue dissolution, probably within 2 minutes.[15] Therefore a large volume of NaOCl for continuous replenishment is essential. The statistical results of the present study showed no significant difference between Groups II and IV with respect to bacterial reduction, which means that larger volumes of irrigant with 15 minutes contact time and 1.5 minutes IPUI compensated for the effects of increasing the contact time (30 minutes) and 3 minutes IPUI. This finding is inconsistent with other studies.[37, 38] Druttman and Stock[37] concluded that the irrigant replacement in the root canal system was more likely to be influenced by time than by the volume used. In addition, Passarinho-Neto *et al.*[38] showed that 5 minutes of PUI removed more dentin debris from the root canal than 3 minutes when the volume was the same for both groups. Such disagreement could be explained by the flushing methods of PUI. In the above studies they used a continuous flush of irrigant, where a frequent replenishment was not possible. In the present study the irrigant was injected in the root canal by a syringe and replenished several times after each ultrasonic activation.

Surprisingly, flushing the canal every 2 minutes in combination with IPUI did not have any impact on bacterial reduction. Such result could be due to technical errors during the sampling procedure after instrumentation. Otherwise, no scientific explanation could be given. Meanwhile, the control group showed that rotary instrumentation without intermittent passive ultrasonic irrigation was not able to eliminate a significant number of bacterial colonies when compared to Groups I, II and IV. This finding was consistent with Shuping *et al.*[39] who showed that after rotary instrumentation and NaOCl irrigation, 61.9% of the tested canals were rendered bacteria-free. Such result emphasized the importance of ultrasonic irrigation to reduce the number of bacteria inside the root canal.

The enhanced performance of NaOCl using IPUI could be explained by the fact that during IPUI, the ultrasonically oscillating file activated the irrigant and led to detachment of microorganisms, dentin debris and organic tissue from the root canal which got absorbed or dissolved in the irrigant,[8, 15] after which,

the root canal was flushed with 2 ml of fresh irrigant that removed the remnants from the root canal.

The method used in this study just permitted evaluation of the bacteriological conditions of the main root canal but did not deflect on absence of bacterial growth in the dentinal tubule. Further studies are recommended to evaluate the effect of IPUI on microbial reduction inside the dentinal tubule.

CONCLUSIONS

Within the limitation of this study, it was concluded that:

1. The flushing action, the larger volume of irrigant and increasing the contact time of the irrigant combined with IPUI and rotary instrumentation increased the elimination of *E. faecalis* from the root canals.
2. IPUI with larger volume of irrigant reduced bacterial colonization from the main root canal space in a short period of time.

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