

The effect of intermittent passive ultrasonic irrigation and rotary instruments on microbial colonies of infected root canals

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الغرض من هذه الدراسة تقييم مدى فعالية ازالة جراثيم الاشريكية البرازية المتواجدة في الأقفنية الجذرية عند استخدام الارواء فوق الصوتي بمحلول هيبوكلورايت الصوديوم بتركيز ٢,٢٥٪ لمدة دقيقة ونصف متقطعة، خلال التحضير اليدوي للأقفنية، و بشكل مستمر عند تحضير الأقفنية بأدوات الارذاذ الدوارة. طريقة البحث: تم ملء ثمانية و اربعون سن بجراثيم الاشريكية البرازية و تقسيمها الى اربع مجموعات اختيارية. تم تحضير هذه المجموعات اما يدويا بطريقة الدرجة الراجعة او تحضيرها يدويا بالاضافة الى استخدام الارواء فوق الصوتي بمحلول هيبوكلورايت الصوديوم بتركيز ٢,٢٥٪ لمدة دقيقة ونصف أو تحضيرها بالأدوات الدوارة فقط، أو تحضيرها بالأدوات الدوارة مع استخدام الارواء فوق الصوتي المتقطع بمحلول هيبوكلورايت الصوديوم بتركيز ٢,٢٥٪ لمدة دقيقة ونصف. النتائج: ظهر تزايد واضح في تعداد الجراثيم في المجموعة المحضرة يدويا فقط بالمقارنة مع المجموعة المحضرة يدويا و المروية بالفوق الصوتي السليبي و ظهر فرق بسيط بين المجموعة المحضرة يدويا و مروية مقابل المجموعة المحضرة بالادوات الدوارة. ولا فرق يذكر بين المجموعات المحضرة اليا بالادوات الدوارة. الخلاصة: اظهرت النتائج ان الارواء الفوق صوتي المتقطع للقنوات بهيبوكلورايت الصوديوم ٢,٢٥٪ لمدة دقيقة ونصف (نصف الوقت المقترح سابقا) خلال تحضير القنوات يدويا يقلل من التعداد الجرثومي للاشريكية البرازية بشكل كبير بينما لا يوجد فرق في تعداد الجراثيم عند تحضير الأقفنية اليا.

OBJECTIVE: To study the effectiveness of reduction of *E. faecalis* in root canals with passive ultrasonic irrigation (PUI) of 2.25% NaOCl for 1.5 min intermittently during hand instrumentation and continuously after rotary instrumentation. **MATERIALS and METHODS:** Forty-eight extracted single rooted teeth were filled with *E. faecalis* suspension and divided into 4 groups. They were either hand instrumented alone using the stepback technique, hand instrumented with PUI of the 2.25% NaOCl intermittently for a total of 1.5 min during the instrumentation, rotary instrumented with ProFile 0.04 alone, or rotary instrumented with PUI of the irrigant for 1.5 min. **RESULTS:** There was significantly more bacterial growth in the hand instrumented group than in the hand instrumented group with PUI, and marginal significant difference in the hand instrumented group with PUI compared to the rotary instrumented group. No differences were found between the rotary instrumented groups. **CONCLUSIONS:** It was concluded that intermittent use of PUI of 2.25% NaOCl for a total of 1.5 min (half of the current recommended time) during hand instrumentation reduced bacterial colonies significantly. There was no difference in bacterial reduction when rotary instrumentation was used with or without PUI.

INTRODUCTION

The main goals of endodontic chemo-mechanical preparation are cleaning and disinfecting the entire root canal system and eliminating bacteria and any sources of their nutrient supply such as the tissue remnants. Failure to accomplish these objectives jeopardizes the outcome of endodontic therapy. Bacteria persisting within the root canal system are the major cause of endodontic treatment failures.^{1,2} The use of ultrasonically energized instruments in conjunction with copious amounts of irrigant has been suggested to reduce bacterial content.^{3,4,5} Researchers concluded that the use of

passive ultrasonic irrigation (PUI) after canal preparation is the best way to ensure canal cleanliness.^{6,7} The use of ultrasonics as a primary cleansing and shaping technique has not been shown to result in better canal debridement when compared to hand instrumentation alone.⁸⁻¹¹

In recent years, endodontic therapy has been revolutionized by advances in rotary instruments and instrumentation techniques. These instruments reduce the time necessary to shape the root canals considerably. It takes less than 5 minutes on the average to shape one canal using rotary instruments. On the other hand,

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adequately disinfecting the root canal of microbial irritants requires considerable time. Buchanan judged that NaOCl needs 5-10 minutes to effectively clean the root canal.¹²

The combined use of rotary instrumentation and PUI in the canals might produce a better cleaned canal with less microbial content in less time. PUI of files with NaOCl irrigation for as little as 30 seconds after canal instrumentation produced canals with less debris than canals instrumented by hand files alone.⁶ However, the currently recommended time for PUI of root canal irrigant is 3 minutes after hand filing for bacterial elimination from root canals.¹³ Current reviews suggest that more research is needed to clarify the effect of irrigation time on the efficiency of PUI, and the effect of refreshing NaOCl during its ultrasonic activation.¹⁴ Also, the suggested time for PUI was not studied with differentiation of hand versus rotary instrumentation.

The aim of this study was to investigate the effectiveness of reduction of *E. faecalis* in root canals using passive ultrasonic irrigation (PUI) of 2.25% NaOCl for 1.5 minutes intermittently during hand instrumentation and continuously after rotary instrumentation.

MATERIALS AND METHODS

Forty-eight extracted human anteriors or bicuspid with a single root canal, which were free from caries, cracks and root canal treatment were used in this study. The teeth were preserved in tap water. Access preparations were made using a high speed round bur under water coolant spray. The canals were coronally enlarged with a size #3 Gates Glidden drill. A size #10 file was introduced into the canal till it extruded through the apex, then 1mm was deducted from this length to determine the working length. The canals were enlarged to size #20K file.

The canals were irrigated with tap water. To make handling and identification easier, the teeth were mounted vertically in plaster blocks with 12 roots per block totaling 4 plaster blocks, representing the 4 experimental groups. The teeth were randomly divided between the groups so that each group contained on average equal canal lengths. The 4 plaster blocks were sterilized by gamma radiation. Three random samples were taken from each group, plated on agar plates and incubated at 37°C for 48 hours to determine the absence of bacterial growth and effectiveness of the gamma radiation sterilization. This served as a negative control group.

The sterilized plaster blocks were opened in a laminar air flow cabinet (Baker Company, Maine, USA). A suspension of 1 ml of pure culture of *Enterococcus faecalis* (ATCC-29210) grown in Brain Heart Infusion broth (Oxoid, England) was prepared. Each root canal was completely filled with the *E. faecalis* suspension using sterile 1 ml tuberculin syringes. Sterile K-type #15 files were used to carry the bacterial suspension to the entire root canal length. The blocks were then placed inside sterile plastic bags and incubated at 37°C for 48 hours.

The root canals of the four experimental groups were instrumented under the laminar air flow cabinet. In Group 1, the root canals were hand-instrumented using stainless steel files (Maillefer, Ballaigues, Switzerland) in a step-back technique. The canals were prepared to an apical foramen size 40K. Ten cc of 2.25% NaOCl kept at room temperature (25°C) was used for irrigation using disposable syringes for a total period of 15 minutes. Each canal was flushed with 1 cc of NaOCl each time the file was changed till the master apical file was reached. All canals were then flooded with a final 1 cc of NaOCl and left for 5 minutes (to complete 15 minutes). In Group 2, the root canals were prepared

in a similar manner to Group 1 but the canals were flooded with 1cc of NaOCl after reaching the MAF of #40 and an ultrasonic file size 25 was introduced to the full length of each canal and oscillated for 30 seconds. This was repeated also after final preparation of the canal (step-back) and again after a change of the NaOCl for the third time after leaving the NaOCl in the canal for 5 minutes (to complete 15 minutes). In Group 3, the root canals were instrumented using Profile taper 0.04 series (Maillefer, Ballaigues, Switzerland) in a technique described by the manufacturer. Each canal was enlarged to a taper of 0.04 with an apical enlargement of size 40. Ten cc of 2.25% NaOCl kept at room temperature (25°C) was used for irrigation using a 27 gauge disposable syringe for a total period of 15 minutes. The needle was placed up to the apical third without binding. Each canal was flushed with 1cc of NaOCl each time the file was changed. The canals were then flooded with a final 1 cc of NaOCl and left for 5 minutes (to complete 15 minutes). In Group 4, the root canals were prepared in a similar manner to Group 3 but the canals were flooded with 1cc of NaOCl after final preparation and an ultrasonic file size 25 was introduced to the full length of the canal and then oscillated for 30 seconds. This was repeated again after flushing the canals with NaOCl for the third time after leaving the NaOCl in the canal for 5 minutes (to complete 15 minutes).

NaOCl was inactivated by flushing the canals with sodium thiosulphate. The canals were then filled with a brain heart infusion broth. Bacterial samples were taken by inserting a sterile paper point in the canal and rotating it. The paper point was placed on an agar plate and incubated for 48 hours. To prevent cross-contamination of the samples, the tweezers used to manipulate the paper points were sterilized by open

flame before use. The root canals were sampled before instrumentation to serve as a positive control. Immediately after instrumentation, the canals were sampled again in the same manner as before and plated on blood agar plates. The roots were then incubated at 37°C for an additional 48 hours and then sampled for the final time. The widest area of bacterial growth around the paper points were measured in mm and recorded for each sample.

Wilks' Lambda Multivariate tests of repeated measures were performed to determine differences between groups and the effect of time on these groups.

RESULTS

Negative control group of randomly plated bacterial samples showed no growth. Mean zones of bacterial growth of each group are shown in Table 1. The Wilks' Lambda Multivariate test showed that there was a significant difference between groups [$F(3,44) = 4.506$, $P < .01$]. Post Hoc comparisons utilizing Tukey's HSD test showed that there was significantly more bacterial growth in Group 1 than in Group 2 ($P = 0.046$) (Table 2). Furthermore, there was a marginally significant difference between Groups 2 and 3 ($P = 0.051$). There was no difference found due to the effect of time as shown in Figure 1.

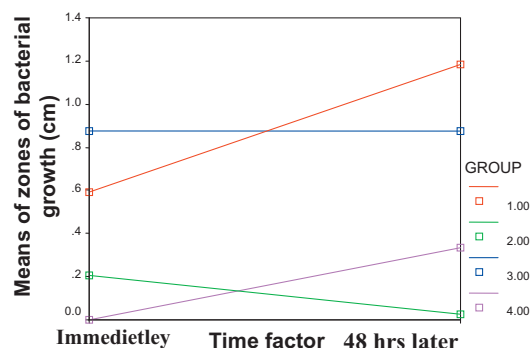


Fig 1. Difference in zones of bacterial growth immediately after instrumentation and 48 hours later.

Table 1. Mean zones of bacterial growth (mm) in each group immediately after root canal instrumentation and after 48 hours incubation.

	Group	Mean	Std. Deviation	No. of samples
Immediately	1	0.59 mm	1.08	12
	2	0.87 mm	1.19	12
	3	0.20 mm	0.72	12
	4	0.00 mm	0.00	12
	Total	0.42 mm	0.92	48
After 48 hrs	1	1.18 mm	1.60	12
	2	0.87 mm	1.46	12
	3	0.02mm	0.08	12
	4	0.33mm	1.15	12
	Total	0.60mm	1.27	48

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

(i) group	(j) group	Mean difference (i-j)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
1	2	0.7708*	.2848	0.046	1.035 E-02	1.53
	3	1.25 E-02	.2848	1.000	-.748	.77
	4	0.72	.2848	0.069	-3.96 E-02	1.48
2	1	-.7708*	.2848	.046	-1.53	-1.0354 E-02
	3	-.75	.2848	.051	-1.51	2.146 E-03
	4	-5.0000 E-02	.2848	.998	-.81	.7105
3	1	-1.2500 E-02	.2848	1.000	-.7730	.7480
	2	.7583	.2848	.051	-2.1463 E-03	1.5188
	4	.7083	.2848	.076	-5.2146 E-02	1.4688
4	1	-.7208	.2848	.069	-1.4813	3.965 E-02
	2	5.0000 E-02	.2848	.998	-.7105	.8105
	3	-.7083	.2848	.076	-1.4688	5.215 E-02

DISCUSSION

E. Faecalis was chosen for the study because of its high resistance to a wide range of microbial agents,¹⁵ its presence in association with persistent apical periodontitis,¹⁶ its difficulty of elimination from the root canal with the use of chemomechanical procedures,¹⁷ and finally for its ease in culturing and manipulation.¹⁸ Results of negative control

group showed the effectiveness of using gamma radiation as a method to achieve sterile root canals before inoculation of the canals with bacteria.

In this study, measures of bacterial growth on blood agar were used to evaluate the amount of bacteria sampled from the canals. Although this is a subjective evaluation, it could allow a comparative evaluation between different irrigation methods because the bacterial inoculation, sampling and measurement are standardized. In addition, this model enables easy handling and testing with limited cost. However, it should be borne 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 occurs *in vivo*. Thus, direct extrapolations to clinical conditions must be exercised with caution.

NaOCl has long been used as an effective irrigating solution and it remains the irrigant of choice in modern endodontic therapy. A synergistic relationship exists between 2% sodium hypochlorite and medium power ultrasound when combined with ultrasonic therapy during root canal therapy which resulted in a debris-free canal wall.¹⁹ In addition, removal of microorganisms and organic tissue from the root canal is effectively implied.

In this study, hand instrumentation with passive ultrasonication of NaOCl irrigant intermittently (IPUI) after enlarging the canal to the MAF eliminated bacteria from the canals more efficiently than hand instrumentation alone ($P < 0.05$). This is in agreement with Sjogren and Sundqvist who concluded the same with 3 minutes of ultrasonic use.¹³ In this study, significant differences occurred in bacterial reduction after only 1.5 minutes. This is half the recommended time for passive ultrasonication by previous researchers.^{6,20,21} This could be due to the number of the intermittent

use of the ultrasonication (after MAF, after step-back and after completion of canal preparation). Enlargement of the canals to the MAF before ultrasonication could also have an effect on the bacterial reduction. Senia²² and Ram²³ doubted the effectiveness of irrigation in narrow canals, where effective irrigation may not occur consistently unless the canals are enlarged to at least a size 40 instrument. This would logically apply to ultrasonic irrigation of the canals as suggested by Walmsley²⁴, as was done in the present study.

Previous studies have shown that passive ultrasonic irrigation provided cleaner canals walls free of debris and tissue remnants.^{15,25,26} On the other hand, Walker¹⁰ did not find ultrasonic irrigation to be effective in removing soft tissue from the main canal, the isthmus between the canals and multiple branches and deltas. He used ultrasonics for only 3 minutes with a No. 15 file and one minute with a No. 25 diamond file. Further studies are needed to verify debris and tissue removal with intermittent ultrasonication of canals.

In the present study, 2.25% NaOCl showed significant difference in bacterial reduction with ultrasonics than without. This was significantly less concentrated than proposed by Huque *et al.*²⁷ who stated that ultrasonic irrigation with 12% NaOCl eliminated bacteria efficiently from surface, shallow and deep layers of root dentin. Siqueira²⁸ suggested that frequent and copious irrigation with a weaker NaOCl solution may maintain a chlorine reserve sufficient to eliminate a significant number of bacterial cells. In this study, intermittent ultrasonic activation may play this role.

There was no significant difference in the bacterial growth between canals instrumented by hand or by rotary instruments when ultrasonic vibration was used. However, it took less time

to complete the cleaning and shaping of the canals with rotary instruments. Buchanan¹² stated that it was the irrigants alone that cleaned out the accessory canals and only the copious use of a tissue dissolving irrigant left in place for 5 to 10 minutes repeatedly will ensure auxillary canal cleaning. He did not use ultrasonic vibration in his study, although the use of ultrasonic vibration could effectively reduce bacterial colonization and thereby minimize the time necessary for auxillary canal cleansing.¹²

Surprisingly, there was no significant difference in bacterial reduction between canals instrumented with rotary instruments with or without the use of ultrasonic vibration. However, it has been stated that NaOCl irrigation with rotary instrumentation could not consistently render canals bacteria free. Studies showed that only 61.9% of canals were bacteria free.²⁹ It might be that 1.5 minutes was not enough time to significantly reduce bacteria colonies if only used after complete canal instrumentation, in contrast to intermittent use of ultrasonic activation of irrigant after reaching MAF, Stepback and at canal completion as in hand instrumentation. Although studies have shown that ultrasonically activated irrigation did not reduce debris nor smear layer scores of prepared root canals.³⁰ This ultrasonic activation was done after complete canal preparation with regard to rotary instrumentation. It may be that ultrasonic activation during hand preparation of the root canals in a step-back technique may loosen and eradicate debris during the preparation to effectively reach and eliminate bacterial content. It could also be that this function is more effective if done in an intermittent fashion rather than at one time only after canal preparation. Further studies are needed to reach an optimum time for ultrasonication with rotary instrumentation.

There was no significant difference regarding the effect of time on the bacteria (immediately after instrumentation and 48 hours later) within the canals after instrumentation. This is in disagreement with the findings of Sjogren and Sundqvist¹³ who reported that bacteria that survived the ultrasonic treatment increased in number in the empty root canals in the period between appointments.

CONCLUSIONS

A different methodology of intermittent ultrasonic irrigation has been proven to be an effective aid in root canal instrumentation in the form of bacterial reduction. Within the limitations of this study,

1. Intermittent use of passive ultrasonication of 2.25% NaOCl for a total of 1.5 minutes during hand instrumentation resulted in significantly less bacteria in infected root canals than in canals in which ultrasonic activation of irrigant was not used.
2. Rotary instrumentation of infected root canals with the use of ultrasonic activation of 2.25% NaOCl was as effective as hand instrumentation with intermittent passive ultrasonication of 2.25% NaOCl for a total of 1.5 minutes in bacterial reduction of microbial colonies.
3. There was no significant difference in reduction of bacterial colonies of infected root canals when rotary instrumentation was used with or without ultrasonic vibration of 2.25% NaOCl for 1.5 min after canal preparation.
4. There was no significant difference in bacterial colonies present in the infected root canals immediately after ultrasonic treatment and 48 hours later.

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