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The Effectiveness of Sonic-Activated Irrigation in Reducing Intratubular *Enterococcus faecalis*

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ABSTRACT

Introduction: The purpose of this *in vitro* study was to compare the effectiveness of sonic activation and syringe irrigation of 5.25% sodium hypochlorite in removing the *Enterococcus faecalis* (*E. faecalis*) biofilm. **Methods and Materials:** Root canals of 54 extracted human single-rooted central incisors were prepared with ProTaper S1-S2-F1-F2 and Gates Gliden burs size 1, and 2 at the working length. After sterilization, the root canals were contaminated with *E. faecalis* suspension and randomly assigned to three groups: G1, conventional syringe irrigation; G2, sonic agitation of NaOCl with Endo Activator system; and G3, no subjected to the mentioned irrigation techniques (negative control). Canals were sampled after the disinfection procedure. The colony forming units (CFU) count was evaluated. Samples were also visualized under fluorescent microscope to count viable bacteria. Data were statistically analyzed using the Kruskal-Wallis and one-way ANOVA followed by Tukey's test (P<0.05). **Results:** There was a significant reduction in the CFU count after both irrigation techniques. There was no significant difference between two techniques (P=0.874). Using bacterial viability kit, Endo Activator displayed the least viable bacteria than the other groups (P<0.001) and control group showed the greatest one (P<0.001). **Conclusion:** In this *in vitro* study, the Endo Activator system was more successful in reducing intratubular viable bacteria compared with NaOCl syringe irrigation alone.

Keywords: *Enterococcus faecalis*; Irrigation; Root Canal Disinfection; Sodium Hypochlorite; Sonic Irrigation

Introduction

Role of bacteria and their byproducts in the pulpal and periradicular disease has been well documented [1]. Apical periodontitis is the defense mechanism of the human body to the destruction of dental pulp and microbial infection of the root canal system. It is essential to eliminate remnants of pulp tissue, bacteria, and microbial toxins from the root canal system to prevent or eliminate apical periodontitis [2-4]. Studies have demonstrated that a significant portion of the root canal walls may remain untouched during manual or rotary [5, 6] instrumentation, emphasizing the importance of irrigation as an adjunct to mechanical debridement of root canals. Irrigation in combination with mechanical instrumentation can improve the removal of bacteria, necrotic pulp tissue and debris from the root canals [7].

Sodium hypochlorite (NaOCl) is widely used as an irrigant with excellent antibacterial property and strong ability to dissolve organic tissues [8-12]. Adequate distribution and frequent replenishment of the optimal concentration and amount of irrigant throughout the root canal system can achieve by an effective delivery system. Traditionally, irrigants have been delivered using a syringe and needle [13]. A limiting factor in conventional irrigation is the inadequate distribution of the irrigant throughout the root canal system because the highest streaming velocity is present only around the tip of the needle [14].

Several mechanical devices have been developed to overcome the limitations of conventional irrigation technique and improve the penetration and effectiveness of irrigants [15]. The Endo Activator (Dentsply, Tulsa Dental, Tulsa, OK, USA) is a sonic device with noncutting polymer tips producing vigorous intracanal

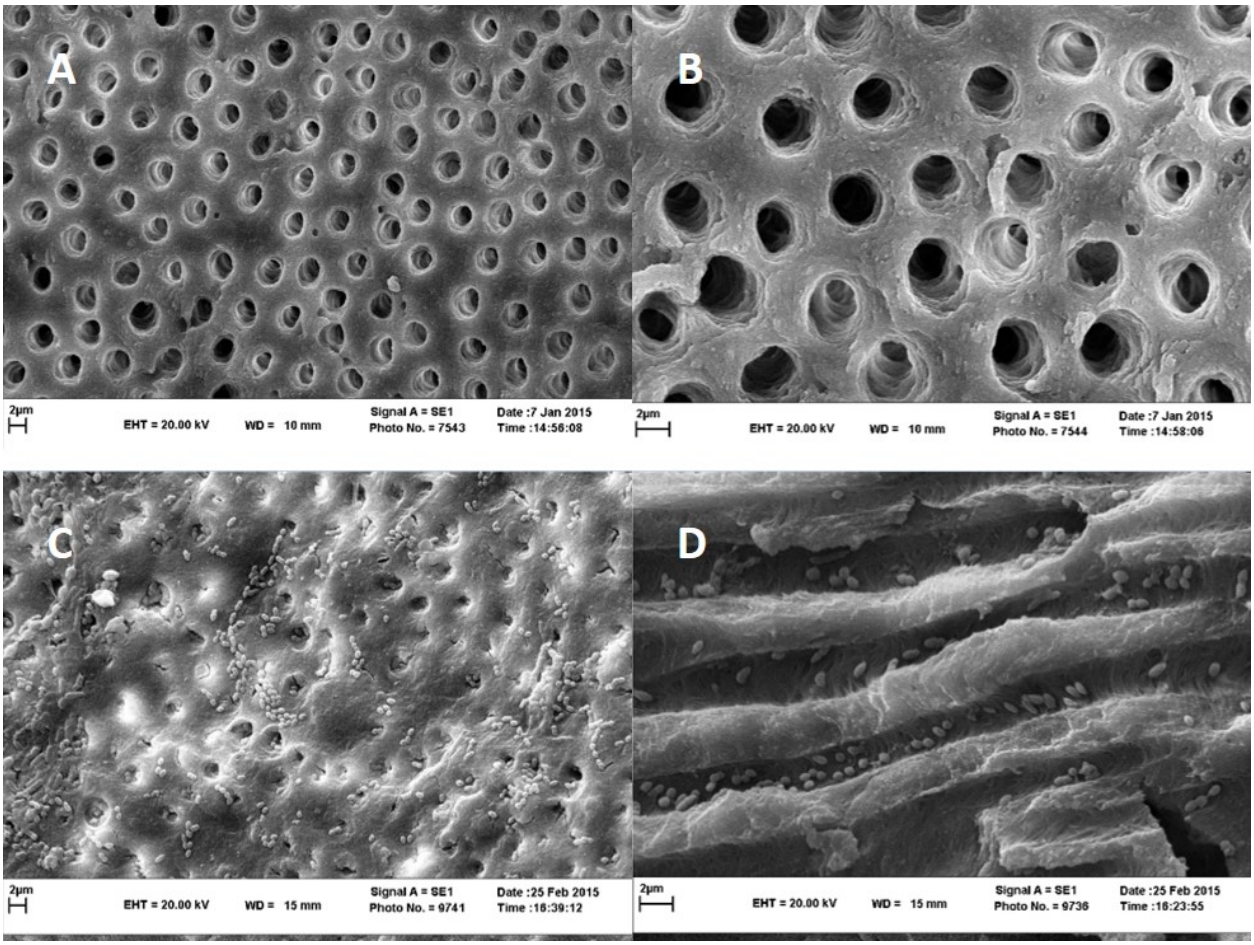

Figure 1. Scanning electron microscopic images of root canal walls. *A and B)* Open dentinal tubules after smear layer removal and sterilization; *C and D)* Colonization of bacteria on the root canal wall and in the dentinal tubules after bacterial contamination

fluid agitation by acoustic micro streaming and cavitation [16]. However, the superiority of the Endo Activator to syringe irrigation remains controversial [17-21].

The purpose of this *in vitro* study was to compare the effectiveness of Endo Activator irrigation system with conventional syringe on elimination of intratubular *Enterococcus faecalis* (*E. faecalis*) biofilm using both culture and fluorescence microscopy methods.

Materials and Methods

This *in vitro* study was approved by the ethics committee of Mashhad University of Medical Sciences (approval no 922318). Fifty four freshly extracted human maxillary central incisors with a fully formed apices and a straight single root canals were selected for this experiment. Teeth were cleaned with periodontal curettes and stored in 10% formaldehyde for 2 weeks. Then they were washed and stored in 0.9% sterile saline for no longer than 1 week.

The teeth were decoronated to standardize the root lengths to 13 mm. The patency of the canals was checked with a #10 K-file (Dentsply, Maillefer, Ballaigues, Switzerland). The canals were prepared using ProTaper S1-S2-F1-F2 and Gates Glidden burs size 1 and size 2 (Dentsply Maillefer, Ballaigues, Switzerland) to the full working length.

Irrigation with 2.5% NaOCl solution was performed during instrumentation. The smear layer was removed by irrigation with 1 mL of 17% liquid ethylenediaminetetraacetic acid (EDTA) that was left in canal for 1 min and subsequently 5 mL of 5.25% NaOCl solution. Then 3 mL of saline was used as final irrigation and paper points were used to dry the canals. Two teeth were prepared to evaluate the effectiveness of smear layer removal by scanning electron microscopy (SEM). Two coats of nail varnish were applied over the root surfaces. Then, all the specimens were sterilized at 121°C and pressure of 15 lb/in^2 for 20 min. A suspension of *E. faecalis* ATCC 29212 grown in brain heart infusion (BHI) broth was prepared to equal the turbidity of a 2.0 McFarland standard (~ 6.0×10^8 colony forming unit (CFU)/mL).

The root canals were inoculated with this suspension. The teeth were then incubated at 37°C for 7 days. The suspension was renewed every 2 days to preserve the bacterial viability. SEM evaluation was used to confirm the bacterial penetration into the dentinal tubules in two samples. According to the irrigation method, the contaminated teeth were divided into three groups as follows: Group 1 (n=20): Conventional syringe irrigation with 5 mL of 5.25% NaOCl for 1 min at 1 mm short of the working length (WL); Group 2 (n=20): Irrigation with 5 mL of 5.25% NaOCl for 30 sec followed by NaOCl agitation for another 30 sec, using the Endo Activator device. Agitation was performed 1 mm short of the WL using the Red Tip instrument size 25/0.04 ; and Group 3 (n=10): Not subjected to the mentioned irrigation techniques used as control.

The final irrigation was performed using 5 mL of sterile saline in all groups and then the root canals were dried with sterile paper points.

After the disinfection procedures, dentinal fragment samples were collected using Gates Glidden drills #3. Each bur was used for three times in the entire length of the root canal. A new sterilized bur was used for each tooth. The dentinal shavings were transferred into tubes containing 500 µL saline solution and vortexed for 1 min. A 10-fold serial dilution was prepared from each sample and 50 µ of each dilution was plated onto BHI agar. After incubation at 37°C for 24 h, colony forming units (CFU) were counted and the actual bacterial count were adjusted and reported based on the known dilution factors.

The rest of dentinal shavings in saline solution (450 µL) were stained using LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes Inc., Eugene, Oregon, USA) according to manufacturer protocol. After centrifugation of each sample tube, the samples were stained. Then the stained bacterial suspension observed by a fluorescent microscope (Carl Ziess Microscopy GmbH, Oberkochen, Germany) to evaluate the viability of bacteria. Bacterial viability was reported as the number of green bacteria.

Statistical analysis

The results are expressed as the mean (SD). Data were analyzed statistically using the Kruskal-Wallis and one-way ANOVA followed by Tukey's test. The level of significance was set at 0.05.

Results

The effectiveness of smear layer removal, sterilization procedure and *Enterococcus faecalis* contamination of dentinal tubules was confirmed by SEM. The sterile controls showed patent dentinal tubules without bacteria on the root canal walls (Figures 1A and 1B). However, bacterial colonization were observed in infected samples (Figures 1C and 1D).

The quantitative data of the remaining bacteria in each group is shown in Tables 1 and 2.

There was a significant difference in the CFU count among the groups (P=0.003). There was no significant difference between groups 1 and 2 (P=0.874).

E. faecalis viability was evaluated by fluorescence microscopy analysis. The irrigation of canals significantly reduced the viable bacteria compared with the control (P<0.001). The viable bacteria significantly reduced in the Endo Activator group compared with conventional irrigation group (P=0.001).

Discussion

Although mechanical instrumentation reduced bacteria from the root canals, potential niches may remain untreated [22]. Thus, in recent decades, improvement of root canal disinfection through innovative irrigant delivery devices and agitation techniques increasingly attract interest [23]. The aim of the present study was to compare the effectiveness of sonic energizing of sodium hypochlorite and traditional syringe irrigation. We found that Endo Activator agitation technique can improve the root canal disinfection.

E. faecalis is associated with persistent apical inflammation [24, 25], due to its capability to penetrate the dentinal tubules and escape chemomechanical disinfection of the root canals [26]. It is demonstrated that the biofilm maturity influences its resistance to antimicrobial agents [27]. A 7-day-old biofilm was chosen on the basis of previous studies which have shown the 7-day growth phase is optimal for testing the efficacy of disinfection methods [28, 29].

Table 1. Means (SD) of bacterial plate counts (CFU/mL) in each experimental group

Group (N)	Mean (SD)	*P*-value
Control (10)	1987.9 (3512.7)	0.003
Conventional (20)	6 (11.4)	
Endo Activator (20)	3 (7.3)	

Table 2. Means (SD), maximum, and minimum of viable bacterial count in different experimental groups

Group (N)	Mean (SD)	Max	Min
Control (10)	19.10 (3.63)	28	16
Conventional (20)	6.20 (3.78)	14	1
Endo Activator (20)	2.65 (1.27)	5	5

We found no significant difference in reduction of bacterial CFUs using Endo Activator agitation technique compared to traditional syringe irrigation. This is consistent with the results of two previous studies [17, 18]. It is reported that Endo Activator might not be powerful enough for complete bacterial eradication from root canal because sonic waves produces only 1 node along the activated file; if therefore the instrument touches the canal wall, the node will be diminished and subsequently, the acoustic streaming necessary to dislodge and carry away necrotic debris will significantly decrease [30]. However, this should be taken into consideration that the CFU determination method has no sufficient sensitivity for detecting the possible viable cells that their concentration is below the limit of detection of solid culture media [31] or bacteria in viable but non-culturable (VBNC) state [32]. It has been demonstrated that many bacteria after facing with adverse environmental conditions can enter the VBNC state [32]. The clinical importance of this issue is that bacteria in VBNC state are capable of resuming active growth when optimal conditions are restored [33, 34]. Various methods have been used to evaluate VBNC state. Fluorescence microscopy is used commonly with vital staining technique to determine the viability profile of bacteria [35] .

The results of fluorescence microscopy showed that the number of viable bacteria was significantly lower in the canals irrigated by Endo Activator. Since it has been reported that the Endo Activator system can provide deeper penetration of irrigant to all areas of the canal space [36], the reduction of bacterial load in the Endo Activator-treated group in our study can be due to this issue.

Conclusion

Despite some limitations of this *in vitro* study, the results show that sonic agitation of sodium hypochlorite solution is more effective than conventional syringe irrigation in elimination of *E. faecalis* from the root canal dentinal walls of extracted human teeth.

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Conflict of Interest: 'None declared'.

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