

Penetration depth of sodium hypochlorite into dentinal tubules influenced by different agitation systems

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ABSTRACT

Objectives: To compare the effect of different agitation systems on the penetration depth of sodium hypochlorite into dentinal tubules: An *in vitro* study.

Background: Studies have found the presence of bacteria in dentinal tubule up to 200 μ . Passive irrigation using NaOCl may not adequately eliminate these bacteria. Hence, it is mandatory to use agitation systems during irrigation and disinfection of root canal.

Materials and Method: Eighty extracted teeth with single canals were used. Standardized access cavity preparation and working length determination were done using visual method. All canals were instrumented till #30, 6%. All samples were immersed in crystal violet dye. The samples were randomly divided into four groups depending on the type of agitation system used ($n = 20$): Group A: manual dynamic agitation (MDA), Group B: EndoActivator (EA), Group C: EndoUltra (EU), and Group D: EndoVac (EV). Each agitation system was used with 5 ml of 5.25% NaOCl, and each tooth was sectioned into two halves. These sections were analyzed under a stereomicroscope, and NaOCl penetration depth was measured at coronal, middle, and apical third levels.

Statistical Analysis Used: Statistical analysis was performed using one-way ANOVA and Tukey's *post hoc* test.

Results: At all levels of examination, EU significantly showed a maximum penetration depth of NaOCl throughout the length of root canal, followed by EA, MDA, and EV.

Conclusion: Ultrasonic agitation using EU is the most effective method to disinfect the dentinal tubules throughout the length of root canal than EA and MDA. EV showed the least penetration depth of sodium hypochlorite.

Keywords: EndoActivator, EndoUltra, EndoVac, sodium hypochlorite

INTRODUCTION

The primary objective of chemomechanical preparation of root canal systems is elimination of pulpal tissue, inorganic and organic debris, bacteria, and their toxic by-products through the use of instruments and irrigation devices.^[1] Biomechanical preparation, independent of the technique or type of irrigation used, provides a significant reduction of the microbiota but only in the main root canal.^[2]

Even after completion of biomechanical preparation, the microorganisms remain situated in dentinal tubules,

isthmuses and apical delta may reinfect the root canal and lead to asymptomatic apical periodontitis.^[3] In fact, within oval canals, only 40% of the root canal wall can be contacted by instruments when a rotating technique is used. Research showed *Enterococcus faecalis* penetrated deeply into dentinal tubules, up to 400 μ .^[4] Therefore, irrigation is an essential part of a root canal treatment as it allows for cleaning beyond the root canal instruments.^[5]

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Lussi *et al.* stated that the generation of a smear layer is almost inevitable during root canal instrumentation which consists of dentine mud as well as remnants of odontoblastic processes, fragments of pulp tissue, and bacteria.^[6,7] The presence of smear layer limits the effective disinfection of dentinal tubules by preventing sodium hypochlorite and other intracanal medicaments from penetrating the dentinal tubules.^[8] The final rinse of root canal with 17% ethylenediaminetetraacetic acid (EDTA) and 5.25% NaOCl was confirmed as the most effective combination in the removal of smear layer.^[9] The efficacy of irrigation solution depends on the irrigation modality and its penetration depth in the main canal as well as the lateral ones.^[10]

Studies showed that efficiency of sodium hypochlorite increases when activated or agitated.^[11,12] Different agitation methods are available such as hand activation such as passive irrigation, manual dynamic agitation (MDA), and devices that include sonic, ultrasonic, passive ultrasonic, and apical negative pressure irrigation devices.^[13]

So far, literature has failed to evaluate and compare the effect of MDA, sonic activation – EndoActivator (EA), ultrasonic activation EndoUltra (EU), and apical negative pressure EndoVac (EV) systems, together. Therefore, on this background, the present study was carried out to evaluate the aforementioned systems.

MATERIALS AND METHOD

Approval for the study was obtained from the institutional ethical committee. Eighty extracted human mandibular premolar teeth with single root were used in this study. The teeth were extracted due to periodontal reasons. All the teeth were radiographed buccolingually and mesiodistally to confirm straight single root canal with mature apex and no resorptive defect. Teeth with caries, restoration, and cracks were excluded. Selected teeth were stored in distilled water, until used.

Standard access cavity preparation was carried out in all. The position of apical foramen was determined by a stainless-steel K-type hand file #15 (Dentsply, Maillefer, Ballaigues, Switzerland). After seeing the tip of file in the apical foramen, 1 mm was reduced from the length of file and that file length was considered as the final working length. The canals were prepared using Hero Gold rotary NiTi files #30, 6% (MicroMega, France) using crown-down technique. Each file set was used to prepare 4 canals. The canals were irrigated using intermittent irrigation with 1 ml of 5.25% NaOCl (Chloraxid, CerKamed, Stalowa Wola, Poland) per canal after each file. Initially, a saline flush of 1 ml/canal followed by

17% EDTA (Endo-Solution, CerKamed, Stalowa Wola, Poland) liquid 1 ml/canal and a final saline flush of 1 ml/canal was done.

The teeth were stained using crystal violet (Gram's crystal violet) dye; it was syringed into the canals using a needle and a syringe until the dye was seen flowing from the apical end of each tooth for about 5 s. The teeth were immersed in crystal violet for 5 days and then rinsed under tap water for 10 min.

The teeth were randomly assigned into four experimental groups. Each group was irrigated using 5 ml of 5.25% NaOCl at room temperature with different agitation protocols:

- Group A: MDA ($n = 20$). Initially, all the canals were irrigated with 5.25% NaOCl at the rate of 2.5 ml/15 s. A well-fitted master gutta-percha (GP) cone (#30, 6%) was used approximately 2–3 mm short of working length in rapid push-pull strokes at frequency of 100–120 strokes per min. MDA was divided into two cycles of 15 s each
- Group B: EA ($n = 20$). Initially, all the canals were irrigated with 5.25% NaOCl at the rate of 2.5 ml/15 s. Then, the tip was inserted passively 3 mm short of the apex with in-and-out motion according to manufacturer's instructions. Total two cycles of 15 s each were performed. The polymer tip #25/0.04 was used at 10 khz
- Group C: EU ($n = 20$). Initially all the canals were irrigated with 5.25% NaOCl at the rate of 2.5ml/15 seconds. Then metal tip was inserted passively 3 mm short of the apex with in and out motion according to manufacturer's instructions. Total 2 cycles of 15 seconds each were performed. The Ni-Ti tip #15 was used at 40khz.
- Group D: EV ($n = 20$). The canals were irrigated with 5.25% NaOCl at the rate of 2.5 ml/15 s using master delivery tip. Initially, macrocannula was used for 15 s, followed by microcannula for the next 15-s cycle, according to manufacturer's instructions.^[14]

All the experimental samples were prepared and irrigated by an endodontist to avoid bias. After irrigation, the teeth were then rinsed under tap water and wiped dry with paper towel. Longitudinal partial grooves were prepared on buccal and lingual sides of teeth using a diamond cutting disc. Then, teeth were divided into two halves using chisel. One half was randomly selected. The surfaces were evaluated using a stereomicroscope Model LA-SZM45-B1 (Nanjing Sunny Optical Instrument Co., Ltd, Nanjing, China) under $\times 20$ magnification, as shown in Figure 1(a-d). Images were saved in JPEG format by Dinocapture 2.0 (Dino-Lite, Almere, Netherlands) software using AM423X Dino-Eye digital eyepiece and RL-L64 LED light source LED model. The depth of the bleached zone (depth of penetration of sodium hypochlorite) was calculated in microns (μ).

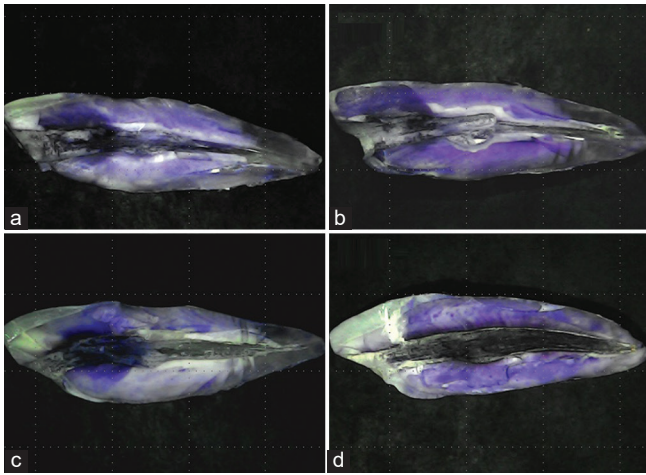


Figure 1: Representative samples from group a, b, c, d shown as 1a, 1b, 1c and 1d respectively.

Method of calculating the bleached zone

The captured images were opened with Photoshop software. The limits of bleached zone were determined by polygonal lasso tool, number of selected pixels was read in Photoshop histogram menu, and likewise, height of canal interior wall was read based on pixels in Info menu. For images with 72 dpi resolution, every 100 pixels were equivalent to 35.28 mm, and by dividing that to the magnification, the real size which is convertible to micron was obtained, and in that way, the average, minimum, and maximum penetration in the total penetrated surface was obtained, as shown in Figure 2.

RESULTS

The results of penetration depth of NaOCl were evaluated by a trained blind observer in such a way that they came to the same conclusion. Data of penetration depth of NaOCl was collected in terms of microns (μ). The results were statistically analyzed using one-way ANOVA and Tukey's Post Hoc test using Statistical Package for the Social Sciences-version 21 (SPSS 21, IBM, Armonk, New York, USA) software, which is depicted in Tables 1 and 2, respectively.

As shown in Graph 1, at the coronal third level, Group C reported a significantly higher mean value (330.4 μ) of penetration depth for NaOCl ($P < 0.001$) compared to Group B (306 μ), Group A (263.4 μ), and Group D (214.6 μ). Similarly, at the middle third level, Group C reported a significantly higher mean value (243.2 μ) of penetration depth for NaOCl ($P < 0.001$) compared to Group B (233.5 μ), Group A (146.6 μ), and Group D (122.2 μ). Furthermore, at the apical third level, Group C reported a significantly higher mean value (207.1 μ) of penetration depth for NaOCl ($P < 0.001$) compared to Group B (190.2 μ), Group A (103.1 μ), and Group D (83 μ), respectively.

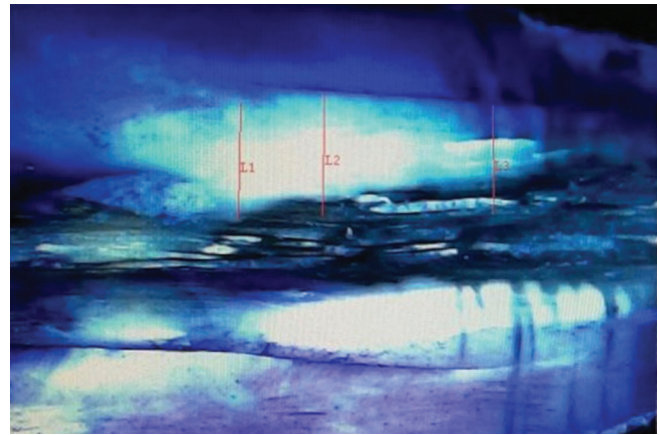


Figure 2: Showing maximum penetration depths of sodium hypochlorite at coronal, middle and apical levels as L1, L2 and L3 respectively.

DISCUSSION

A standardized study using human extracted teeth was undertaken in order to quantitatively evaluate the penetration depth of NaOCl in dentin, as it is impossible to be done *in vivo* due to ethical and practical impedances.

Nair and Reddy reported that 88% mesial root canals of mandibular molars harbored residual infection after instrumentation, irrigation with sodium hypochlorite, and obturation.^[15] Since mechanical instrumentation only eliminates 50% of bacteria from root canals, irrigating solutions are required to eliminate microbiota from the zones not accessible to instruments.^[16]

There is always a need to be in a constant pursuit of devices that can enhance the efficiency of NaOCl, during disinfection of dentinal tubules. Therefore, on similar lines, in order to enhance the efficiency of irrigating solutions, various agitation techniques are recommended to be used such as hand file agitation, MDA using GP, and sonic and ultrasonic modalities.^[17]

Now, in order to quantify the penetration depth of NaOCl in dentinal tubules using the abovementioned agitation modalities, crystal violet dye was used to stain the dentinal tubules as it provides for better visualization under stereomicroscope. Because sodium hypochlorite is a strong oxidant, it whitens the purple color of crystal violet and reveals the clear natural color of dentin. Hence, the width of the discolored zone was easily recorded and considered as the penetration depth of sodium hypochlorite.^[18]

As per the data obtained from this study, EU performed well as compared to EA, MDA, and EV with respect to penetration of sodium hypochlorite in dentin tubules at the level of

Table 1: Showing comparison of mean depth of penetration at three levels using One-way ANOVA test

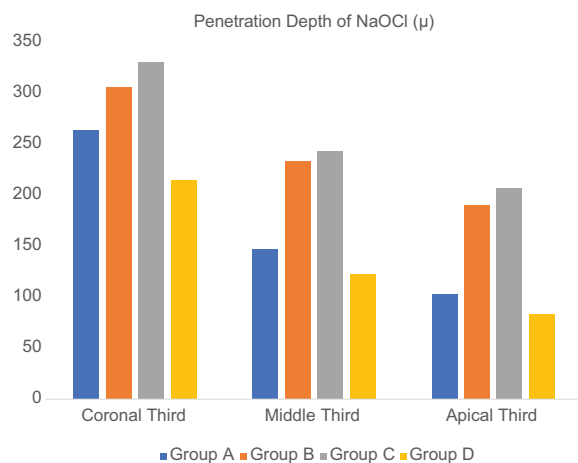
Group	Coronal third				Middle third				Apical third			
	Mean	SD	F	P	Mean	SD	F	P	Mean	SD	F	P
Group A	263.4	10.74	313.87	<0.001**	146.60	9.51	246.775	<0.001**	103.1	14.27	278.05	<0.001**
Group B	306.0	9.30			233.5	16.87			190.2	11.14		
Group C	330.4	7.61			243.2	13.24			207.1	11.93		
Group D	214.6	8.34			122.2	7.19			83.0	8.98		

Comparison of mean depth of penetration at three levels using one-way ANOVA test. $P < 0.001$. **Statistically significant. SD: Standard deviation

Table 2: Showing Intragroup comparison between experimental groups using Tukey's POST-HOC test.

Group	Coronal third			Group	Middle third			Group	Apical third		
	Comparison group	Mean difference	P		Comparison group	Mean difference	P		Comparison group	Mean difference	P
Group A	Group B	42.60	<0.001**	Group A	Group B	86.9	<0.001**	Group A	Group B	87.10	<0.001**
	Group C	67.0	<0.001**		Group C	96.6	<0.001**		Group C	104.0	<0.001**
	Group D	48.80	<0.001**		Group D	24.4	<0.001**		Group D	20.10	0.003*
Group B	Group C	24.40	<0.001**	Group B	Group C	9.70	0.305	Group B	Group C	16.90	0.014*
	Group D	91.4	<0.001**		Group D	111.3	<0.001**		Group D	107.2	<0.001**
Group C	Group D	115.8	<0.001**	Group C	Group D	121.0	<0.001**	Group C	Group D	124.1	<0.001**

Intragroup comparison between experimental groups using Tukey's *post hoc* test. $P < 0.001$. *Statistically not significant. **Statistically significant

**Graph 1: Multiple bar diagram showing extent of NaOCl penetration (in μ) in all experimental groups**

coronal, middle, and apical third. Previous studies have reported that ultrasonic irrigation system performed better than sonic irrigation system in terms of sealer and irrigant penetration in dentinal tubules.^[19]

This could be related to the fact that ultrasonic instruments operate at a higher frequency (40,000 Hz) compared to sonic instruments that use a lower frequency (1000–6000 Hz). The ultrasonic energy possesses the ability to create several nodes and antinodes throughout the length of file generating acoustic waves with the chemical action of the irrigant called as microstreaming and secondary acoustic streaming with frequency ranging from 40,000 to 45,000 Hz and along with the formation of cavitation effect.^[20,21] Furthermore, an increase in temperature of sodium hypochlorite after

ultrasonic activation increases the penetration depth and antibacterial activity of NaOCl.^[22]

However, the vibration pattern of the sonic energy is different than that of ultrasonic energy; it produces a singular node near the attachment of the file and singular antinode at the tip of the instrument over the entire length of vibrating instrument, hence the streaming velocity is less than the ultrasonic instruments, and during agitation, if the sonic polymer tip comes in contact with the root canal wall, its sideways movement disappears.^[23,24]

The current study has revealed that EA performs better compared to MDA at all three levels. This may be due to higher operating frequency and superior fluid dynamics of EA.^[23]

The results showed better performance of MDA than EV at all the three levels of assessments. This can be attributed to the rigorous hydrodynamic forces generated in the root canal system during agitation due to push and pull strokes (amplitude of 2–3 mm) applied by a well-fitted master GP cone. This generates more turbulent shear forces in the canal that promotes lateral displacement of the irrigant solution. While agitation, the strokes of the GP cone exert physical displacement, folding, and cutting of fluid.^[23,24]

The most inferior results of EV may be due to its working principle. EV only maintains a constant and fresh supply of the irrigant solution in the root canal but does not generate enough shear forces that favor lateral displacement of irrigant solution to penetrate the dentinal tubules.^[25]

CONCLUSION

Within the limitations of this study, it can be concluded that ultrasonic agitation using EU is the most effective method to disinfect the dentinal tubules throughout the length of root canal than EA and MDA. EV showed the least penetration depth of sodium hypochlorite.

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Conflicts of interest

There are no conflicts of interest.

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