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Ability of sodium hypochlorite to clean dentinal tubules by manual or sonic activation at varying temperature: a confocal laser scanning microscopy study

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Abstract

Aims: To compare the effectiveness of 2 different method of activation of sodium hypochlorite on its dentinal penetration at 2 different temperatures: A confocal laser scanning microscopic study.

Methods and Material: 40 freshly extracted mandibular premolar were collected. Routine endodontic treatment was performed in all teeth of four groups. Teeth were randomly divided into 4 group

Group1-sodium hypochlorite with hand activation

Group 2- sodium hypochlorite with sonic activation

Group 3-warm sodium hypochlorite with hand activation

Group 4-warm sodium hypochlorite with sonic activation

following irrigation, all roots were sectioned horizontally at 2mm and 5mm from the apex and examined under a

confocal laser scanning microscopy for evaluating the penetration depth of sodium hypochlorite.

Result : Warm sodium hypochlorite with sonic activation had better penetration into dentinal tubules.

Conclusion: Irrigant penetration ability was greater in the middle section then in the apical section. Sonic irrigation when used with warm sodium hypochlorite had better penetrability in the dentinal tubules of middle and apical then the conventional irrigation

Keywords: hand activation, sodium hypochlorite, sonic irrigation, ultrasonic irrigation

INTRODUCTION

The main aim of the endodontics is complete elimination of bacteria and their byproducts from the root canal system with instrumentation and chemical cleaning. This is followed by filling the root canal with an inert material in order to maintain the health of periradicular tissues.[1]Bacteria are causative factor for pulpal and periapical changes and mainly occur in areas such as lateral or accessory canals, apical deltas, isthmus, ramification and dentinal tubules. Various studies have reported 300µm of bacterial penetration into dentinal tubules.[2] Enterococcus faecalis has a penetration depth of 500µm into dentin, with a front of infection may reach 1000 µm.[3] For complete eradication of the pulpal tissue, dentinal shavings and bacteria from the root canal, mechanical preparation and shaping is done followed by irrigation. Sodium hypochlorite is considered as the potent endodontic irrigant. Also, sodium hypochloite shows no residual activity.[4] Its properties can be enhanced by thermodynamic changes within the root canal.[5] Sirtes et al. stated that 1% NaOCl solution heated to 45 °C equals the tissue dissolution capacity of a 5.25% NaOCl solution at 20°C.The tissue dissolution capacity of the 1% solution was even higher at 60 °C.[4] Traditionally, Syringes are used for effective irrigation. It can only deliver irrigant upto 1mm deeper than the needle tip,due to creation of vapor lock at the apical area.[6] Several studies have found that apical vapor lock has adverse effect.[7] Throughout the history of endodontics, modifications have been made to develop a more effective irrigant delivery and agitations technique for root canal system. These systems are divided into manual agitation and machine-assisted agitation devices.[6]Among these techniques, machine assisted agitation has been considered more effective. Machine assisted agitation is further divided into sonic and ultrasonic.Activation with sonic devices produces frequency ranging from 1 to 6KHz with mechanical oscillation.[8] The aim of this study is to evaluate the penetration depth of sodium hypochlorite in dentin using manual and sonic agitation techniques at two different temperatures. The null hypothesis states that there is no difference in the penetration of 5.25% NaOCl solution into dentinal tubules activated by manual agitation or by sonic agitation at different temperatures

Materials and Methods

Ethics institutional review board approval was obtained for this research. Forty freshly extracted single rooted human first premolar teeth with single canal extracted for orthodontic, periodontal purpose or any therapeutic purpose were selected for the study. The roots of the teeth were evaluated in a radiograph for straight, non carious, resorption or any cracks. Teeth were then stored in normal saline. The crown was cut at 15mm using diamond discs with a thickness of 1mm(D & Z, Co- logne, Germany).Apical foramen was determined by a stainless steel K-type hand file #15 (Dentsply, Maillefer, Ballaigues, Switzerland). Working length was determined radiographically. All canals were instrumented to working length with Protaper Next system (Dentsply Mallifer) to size 30. Samples were randomly further divided into four

group. Each group was subjected to different irrigation protocols.

Group IA- **sodium hypochlorite with hand activation**: 2 ml of 5.25% NaOCl and an endodontic syringe with 30 gauge needle (Max-i- Probe; Dentsply, Rinn, Elgin, IL) was used for 20 seconds for root canal irrigation. The tip of the needle was placed 1mm above the apical foramen and was moved up and down in the apical third with amplitude of 2mm. NaOCl was left in the canal for 20 seconds, canals were then irrigated again as described previously with 30 gauge needle. The irrigant was delivered at a rate of 1ml/min. A final irrigation was done with normal saline for 60 seconds.

Group IB- **sodium hypochlorite with sonic activation** : 2 ml of 5.25% NaOCl and an endodontic syringe(size ~2 ml) with 30 gauge needle (Max-i- Probe; Dentsply, Rinn, Elgin, IL) was used for 20 seconds for root canal irrigation. A sonic device. (Waterpik® Water Flosser model 660W, Water Pik, Inc., waterpik.com) was used to agitate the solution. The nozzle of the tip was inserted into the canal till the time it loosely binds in the canal. Then the canals were irrigated with 2ml NaOCl with a syringe for 20 sec. Final irrigation was done with normal saline for 60 seconds.

Group IIA- **warm sodium hypochlorite with hand activation**: Sodium hypochlorite was heated by filling 2ml of solution in disposable syringe and placing in the incubator at 37°C.A new syringe was used for each sample. Mercury barometer was used to control the laboratory temperature. The temperature of the solutions was controlled using a pH meter with a temperature sensor (AlfaKitLtda, Florianopolis, SC, Brazil).

Group IIB- **warm sodium hypochlorite with sonic activation for 10 sec.** The steps carried out were similar to that described earlier with sodium hypochlorite with sonic activation except that the sodium hypochlorite was preheated in incubator at 37°C.

Samples were rinsed with normal saline. They were then flushed with 5ml of 17% EDTA(Dental Avenue India Pvt. Ltd. Mumbai India), solution after root canal preparation for smear layer removal. Two coats of nail polish were applied around the root surface, and modelling wax was used to seal the apex. 5.25% sodium hypochlorite(Prime Dental Products Pvt. Ltd., India) was than mixed with 0.01% rhodamine B isothiocyanate dye(Merck, Darmstadt, Germany) and irrigated into the root canal for 1 minute and then was rinsed with saline solution for 1 minute.

Samples were mounted vertically in the acrylic block and sectioned horizontally at 2mm (apical) and 5mm (middle) from the apical foramen, removing 1mm of thickness with a low speed water cooled 0.3mm blade Isomet saw(Buehler, Lake Bluff, IL, USA) The coronal surfaces of slices were polished with silicon abrasive carbide paper to remove dentin debris created during the cutting procedures. The slides were then examined using a confocal laser scanning microscope at 10X magnification with a wavelength of 543nm. The dentinal tubule penetration area was measured as micrometers (µm) and converted to square millimeters (mm2) for the statistical analysis.

Statistical Analysis

Data were analysed with IBM SPSS Statistics 22 software (PASW Statistics 20; SPSS Inc., Chicago, IL, USA). Unpaired t test were used to compare the penetration depth of each group in middle and apical section. One - way ANOVA test was used to compare the depth of irrigant penetration in apical section with different irrigation techniques at different temperature at each level. Intergroup comparsion was done by Tukey's Post

hoc test. The significance level for all analyses was set at $P<0.05$.

Results

Statistical analysis indicated a significant effect on penetration depth of different groups in middle and apical sections (TABLE -1). Group IIB showed higher significance($p<0.001$) when compared to other groups. Group IA (sodium hypochlorite with hand activation) showed the lowest penetration in middle section and apical section and Group IIB (warm sodium hypochlorite with hand activation) showed highest penetration. Table 2 showed Intergroup comparison of different group in middle section. Group IA was compared to Group IB, IIA, IIB. Group IB (sodium hypochlorite with sonic activation) did not show significant difference. Table 3 compared penetration depth in apical section Group IA was compared with IB, IIA and IIB. Group IA did not show significant difference when compared to Group IB.

Discussion

The main cause of post treatment failure is the presence of microorganism within the root canal system.[9] Bacterial invasion occurs deeper within the dentinal tubules and is important for the persistence of infection. Hence the depth of penetration of irrigant is evaluated as the antibacterial property of the chemical irrigants which is critical to prevent reinfection.[10] Sodium Hypochlorite acts as a solvent in presence of organic tissue by releasing chlorine which forms chloramines by combining with the protein amino groups. The efficacy of sodium hypochlorite can be increased by increasing the concentration, contact time, volume and temperature.[11] When 1% NaOCl is raised to 45℃ can cause tissue dissolution eqvivalent to 5.25% sodium hypochlorite at 20℃.[5] Waltimo et al stated that NaOCl solution at 50 ppm can kill microorganism within 30s at 60°C.[8] It has been found that increasing the concentration will decrease the sensitivity of the experiments in invitro studies between manual activation and machine activation of the irrigant.[12] Needle irrigation being one of the most commonly employed technique in clinics.[13] It can produce adequate canal debridement but it fails to create any hydrodynamic turbulence energy within the irrigants.[6] To overcome this problem, various agitation methods are advocated. Gutta Percha master cone is used to manually agitate the irrigants within the canal.[6]

In an in vivo to study the extent of the depth of penetration of sodium hypochlorite in dentin due to practical and moral limitations. For standardization of the study, human extracted teeth were used.

Bhuva et al conducted the study comparing the conventional irrigation with passive ultrasonic unit and found that both these techniques were effective in removal of intraradicular E. faecalis biofilms[14] In this study, conventional irrigation had better cleaning efficiency in coronal third than in apical third. This may be due to depth of penetration of the irrigating needle which was limited to 2mm from the working length to avoid NaOCl accident. Sonic Activation can produce powerful hydrodynamic intracanal waves. These waves create bubbles which will oscillate within the given solution. These bubbles will expand and collapses as an implosion that can lead to radiating shockwaves that dissipates at a speed of 25,000-30,000 times per second. These shock waves detaches the biofilm from the root canal surfaces.[13]Yan shen et al reported the bioaccoustic effect of sonic irrigation where the shockwaves created by sonic agitation transports disinfecting agents deep into the biofilms by breaking its protective mechanism which leads to bacterial death. This action fails to effectively clean the particles from the middle and apical third of the root canals.[15] A study done by plotino et al found no significant difference between irrigation done by sonic and ultrasonic unit.[16]

In the present study passive sonic agitation when used with warm sodium hypochlorite has proven to achieve maximum disinfection by removing intra-radicular biofilm significantly compared to other methods in the coronal third then the apical third. The results are attributed to the fact that tubules within the coronal and middle third of the root are more permeable.[17] Tubules in the apical third are impermeable due to the presence of tubular sclerosis, smaller diameter, and a reduced number of dentinal tubules in this region.[17]

In this study manual dynamic agitation has not performed as effectively as sonic aggitation technique. The reason behind this is because of the energy created by push-pull motion of manual activaition is (3.3 Hz) much lesser than sonic energy of 1-6kHz.[13]

Numerous microscopic techniques such as stereomicroscopy, scanning electron microscopy (SEM) and CLSM have been advocated to evaluate irrigant penetration into dentinal tubules. CLSM provides detailed information about the presence and distribution of irrigants inside the dentinal tubules along the circumference of the canal walls. It provides image acquisition from several sections, which is further reconstructed to obtain final image.[10]

The current results of this study is similar to that of furkan et al.[18] There are other studies which found no significant difference in conventional syringe irrigation when compared to other methods of activation.[19] Guo X in 2014 stated that the smear layer can be removed effectively even without any activation by using combination of 60°C 3% NaOCl and 17% EDTA, Better results were obtained at middle third as compared to apical third.[20] The results are also in accordance to the study done by Kucuk.M et al in 2018. They concluded that the penetration into dentinal tubules is significantly greater in middle section than at apical section of root canal.[10]More studies that employ the smear layer model are required to confirm the findings.

Conclusion

Intracanal agitation techniques could enhance the penetration of irrigant.Within the limitations of this study, it was shown that passive sonic agitation with warm sodium hypochlorite was more effective from the root canal walls of extracted human teeth then a conventional irrigation method. Conventional irrigation was partially effective at removing the biofilm.

Future Scope / Clinical Significance

The invitro model has limitation of its own. More work remains to be done to identify and take advantage of sonic agitation and means of increasing the temperature of sodium hypochlorite within the canal**.**

List of Abbreviations

NaOCl -sodium hypochlorite

CI- conventional irrigation

MDA-manual dynamic Activation

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Legend Tables

Table 1: Comparison of penetration depth of each group in middle section as compared to apical section of root canal

	Middle Section Mean (SD)	Apical Section Mean (SD)	Unpaired t test	p value, Significance
Group IA (Sodium Hypochlorite + Hand activation)	48.75 (2.98)	32.50 (15.54)	t = 2.053	p =0.086
Group IB (Sodium Hypochlorite +Sonic activation)	429.5 (87.94)	387.5 (85.39)	t = 0.658	p = 0.519
Group IIA (Warm Sodium Hypochlorite +Hand activation)	1662.5 (268.87)	1212.5 (278)	t = 2.327	p =0.059
Group IIB (Warm Sodium Hypochlorite +Sonic activation)	2887.5 (143.61)	1800 (70.71)	t = 13.587	p <0.001**

p >0.05 – not significant *p<0.05 – significant **p<0.001 – highly significant *All measurements are done in µm.

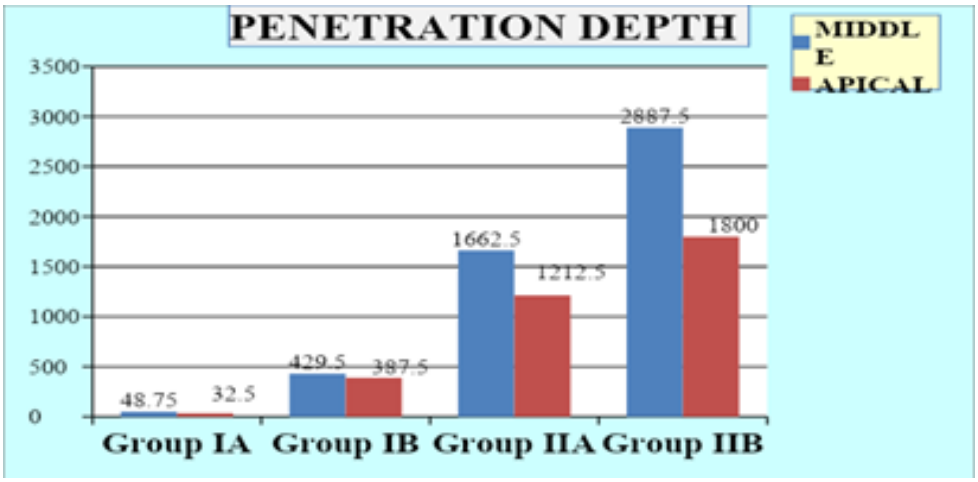


Table 2: Intergroup comparison of penetration depth among four study group in middle section of root canal

Middle Section		Mean	S.D	Anova F Test	P Value, Significance
Group IA (Sodium Hypochlorite +Hand activation)		48.75	2.98	F = 263.201	p <0.001* *
Group IB(Sodium Hypochlorite +Sonic activation)		429.5	87.94		
Group IIA(Warm Sodium Hypochlorite +Hand activation)		1662.5	268.87		
Group IIB(Warm Sodium Hypochlorite +Sonic activation)		2887.5	143.61		
Tukey's post hoc test to find pairwise comparison					
Group	Comparison Group	Mean Difference			P Value, Significance
GROUP IA	GROUP IB	380.75			p =0.024*
	GROUP IIA	1613.75			p <0.001**
	GROUP IIB	2838.0			p <0.001**
GROUP IB	GROUP IIA	1233.0			p <0.001**
	GROUP IIB	2458.0			p <0.001**
GROUP IIA	GROUP IIB	1225.0			p <0.001**

p >0.05 – not significant *p<0.05 – significant **p<0.001 – highly significant *All measurements are done in µm.

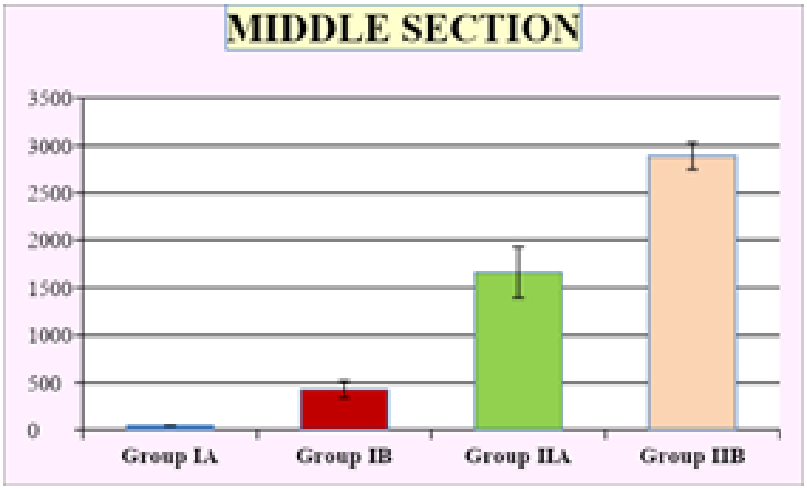
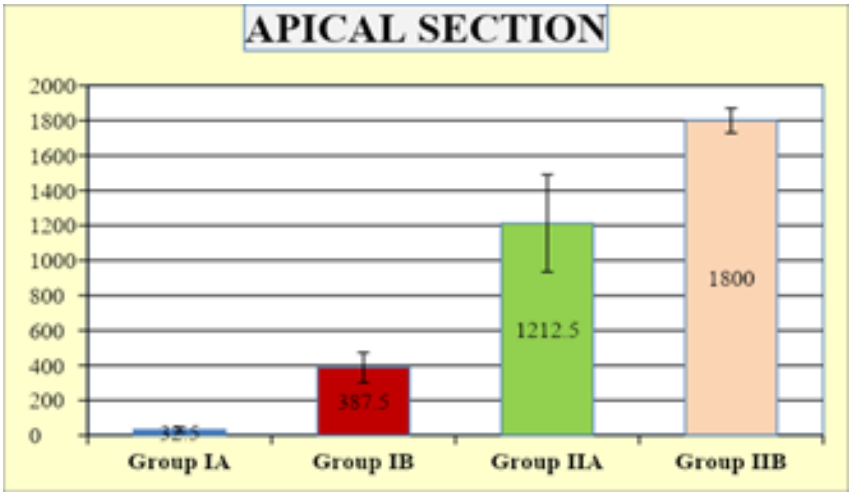
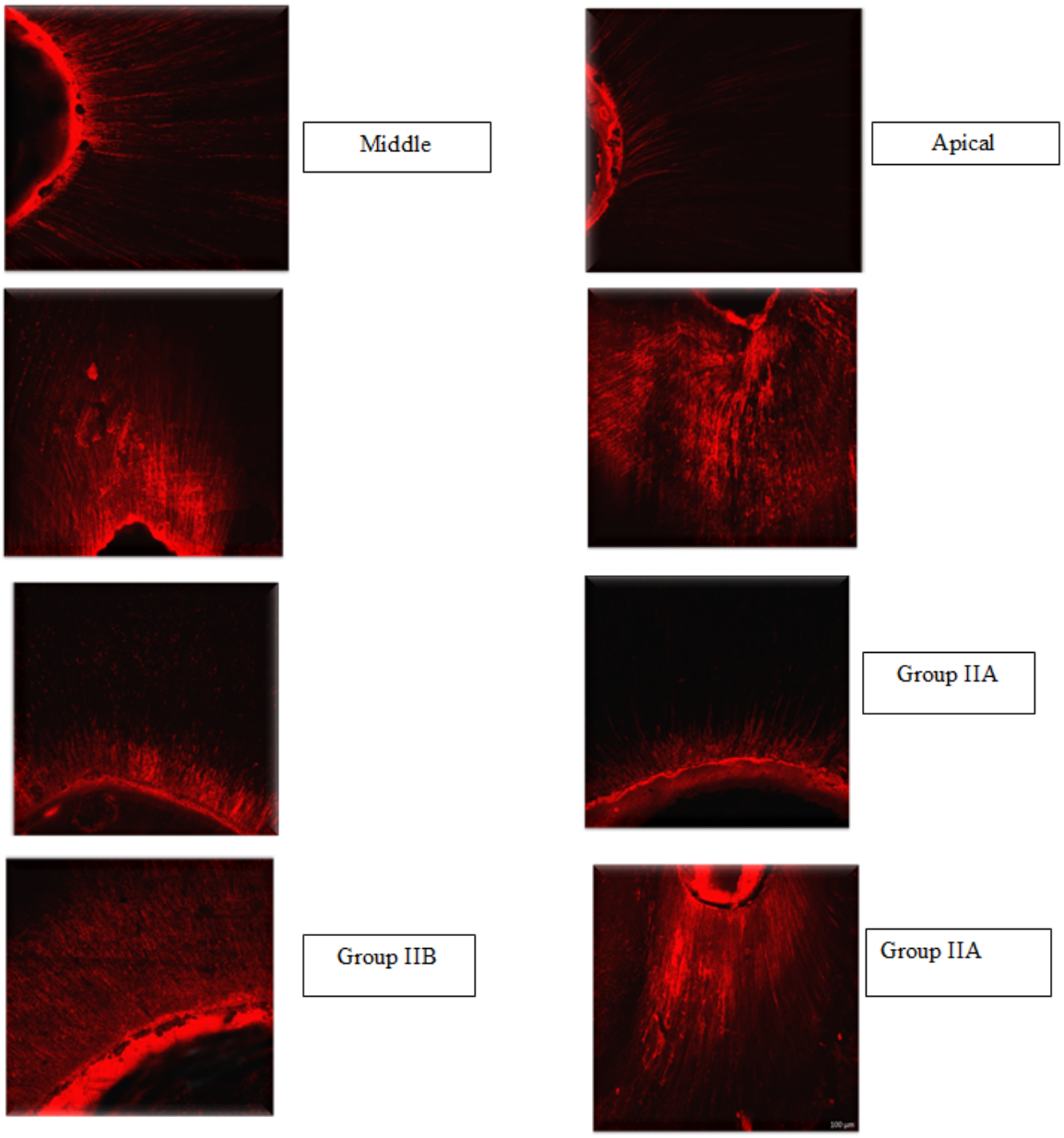


Table 3: Intergroup comparison of penetration depth among four study group in apical section of root canal

Apical Section	Mean	S.D	Anova F Test	P Value, Significance
Group IA (Sodium Hypochlorite + Hand activation)	32.5	15.54	F = 113.753	p <0.001**
Group IB(Sodium Hypochlorite + Sonic activation)	387.5	85.39		
Group IIA (Warm Sodium Hypochlorite + Hand activation)	1212.5	278.01		
Group IIB (Warm Sodium Hypochlorite + Sonic activation)	1800.0	70.71		
Tukey's post hoc test to find pairwise comparison				
Group	Comparison Group	Mean Difference	P Value, Significance	
GROUP IA	GROUP IB	355.0	p =0.026*	
	GROUP IIA	1180.0	p <0.001**	
	GROUP IIB	1767.5	p <0.001**	
GROUP IB	GROUP IIA	825.0	p <0.001**	
	GROUP IIB	1412.5	p <0.001**	
GROUP IIA	GROUP IIB	587.5	p <0.001**	

p >0.05 – not significant *p<0.05 – significant **p<0.001 – highly significant *All measurements are done in µm.





Legends of Figures

Group IA-Penetration of sodium hypochlorite with hand activation in middle section

Group IA-Penetration of sodium hypochlorite with hand activation in apical section

Group IB-Penetration of sodium hypochlorite with sonic activation in middle section

Group IB-Penetration of sodium hypochlorite with sonic activation in apical section

Group IIA-Penetration of warm sodium hypochlorite with hand activation middle section.

Group IIA-Penetration of sodium hypochlorite with warm sodium hypochlorite and hand activation apical section.

Group IIB-Penetration of warm sodium hypochlorite with sonic activation for 10 sec in middle section.

Group IIB-Penetration of warm sodium hypochlorite with sonic activation for 10 sec in apical section.