ORIGINAL RESEARCH

Comparative Evaluation of Penetration Depth of Irrigants into Root Dentin after Manual Sonic and Ultrasonic Activation using Dye Penetration Method under Light Microscope: An *In Vitro* Study

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Abstract

Aim: To evaluate the penetration depth of irrigants into root dentin after manual, ultrasonic, and sonic activation using the dye penetration method under light microscope.

Materials and methods: Ninety-two extracted single-rooted human teeth were used in the age range of 14–18 years. After access opening, root canals were instrumented. Canals were irrigated with preheated 5% sodium hypochlorite (NaOCl). Teeth were divided into four groups—group I, control group without activation; group II, manual dynamic activation; group III, ultrasonic activation; and group IV, sonic activation. About 1% methylene blue dye was used, which was activated for 30 seconds using the respective activation method. Cross sections of apical 1 mm were prepared from the apical third. Each section was examined under a light microscope to check the penetration depth of dye in a micrometer at the apical third level.

Results: There was a statistically highly significant difference seen for the values between the groups ($p < 0.01$) with higher values in group IV and least in group I, revealing that group IV had higher penetration depth compared to other groups.

Conclusion: The use of needle irrigation with the use of EndoActivator (sonic agitation method) has enhanced irrigation in the apical third.

Clinical significance: As during biomechanical preparation and irrigation with the traditional method of needle and syringe, canals remain inadequately disinfected at the apical third level. So, this study was done to assess whether the advanced methods of activation are more effective than commonly used techniques in their ability to penetrate dentinal tubules.

Keywords: 1% methylene blue, Manual dynamic irrigation, Passive ultrasonic irrigation, Sonic activation.

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Introduction

Due to oral commensal microbes, the opportunistic infections of root canals and dental pulp in pulpitis and subsequent periradicular lesions. The main objective of endotherapy is to effectively remove microorganisms from the system.[1]

Biomechanical preparation is an essential step in root canal system and is only effective when used along with proper irrigating materials. Thus, it will help in reduction of various bacterium and viruses from the system.[2,3]

Traditionally used irrigation system such as syringes and needle does not reach the depth of dentinal tubules, so other activation methods will help in gaining proper effect of irrigants.[4]

For adequate disinfection, various activation methods and devices are there, such as manual dynamic irrigation (MDI), passive ultrasonic irrigation (PUI), and sonic irrigation (EndoActivator).

Manual Dynamic Irrigation (MDI)

Manual dynamic irrigation (MDI) (needle irrigation) utilizes systems having different gauges and needles to administer irrigant solution into the system. Some needles are designed in such a way that irrigants will reach the depth of dentinal tubules through side vents present at the tip. Irrigation can be performed passively or with needle agitation *via* up and down movement to allow the needle, which will not engage into the canal, and hence, prevent extrusion of avoid irrigants toward the apex. Continuous agitation and consistent introduction of irrigant significantly improve the role of irrigants in endotherapy. In cases where irrigation needle cannot easily access the apical canal, it is recommended to use a gutta-percha point or a K file that matches the prepared canal system. This helps facilitate the exchange of solutions in that specific region, ensuring thorough cleaning and disinfection.[5]

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Passive Ultrasonic Irrigation (PUI)

Ultrasonically activated solutions are utilized in endodontic treatment to effectively clean and disinfect the system. The efficacy of activation depends on the following factors—(1) cavitation and (2) acoustic streaming. When ultrasonic energy is applied, it creates bubbles that grow and collapse, generating a pressure vacuum effect known as cavitation. This irrigation system involves use of vibrating instrument in the root canal after preparation for proper microstreaming at frequencies above 25 kHz. The rapid motion enables the file to penetrate in the unprepared areas and, hence, improves removal of remaining tissues and smear layer.[6]

Sonic Activation

Use of sonic or subsonic activation system improves the cleanliness of root canal system. These devices typically work at frequencies ranging from 20 to 20,000 Hz. One commonly used system for sonic/subsonic agitation is the EndoActivator® system, which includes attached polymer tips from Dentsply Tulsa Dental Specialties. These polymer tips are designed to produce sonic or subsonic vibrations when placed in the canal, improving the effectiveness of irrigation and, hence, cleaning the root canal system properly.[7]

Sonic activation system such as EndoActivator® which is a portable device that consists of three tips made of polymer in different sizes, namely 15/0.02, 25/0.04, and 35/0.04. These tips are smooth and disposable. The EndoActivator works at frequencies of 160, 175, and 190 Hz, as reported by the manufacturer, but also at frequencies of 33, 100, and 167 Hz. The agitation of irrigants is achieved by placing the tips into the root canal in conjunction with needle irrigation using appropriate needles, and its use improves the penetration in the lateral canals. Additionally, when used with demineralizing agents like ethylenediaminetetraacetic acid (EDTA), the EndoActivator eliminates the smear layer and the biofilm effectively.[8]

So, the study aims to see the effectiveness of these methods and their ability to go into the dentinal tubules and their structure.

Materials and Methods

Sample Collection and Storage

A total of 92 single-rooted premolars were collected (age range 14–18 years) and were cleaned using ultrasonics and kept in distilled water until usage.

Grouping of the Specimens

Group I: ($n = 23$)
- Control group irrigation without activation.

Group II: ($n = 23$)
- Included irrigation with manual dynamic activation.

Group III: ($n = 23$)
- Included irrigation with ultrasonic activation.

Group IV: ($n = 23$)
- Included irrigation with sonic activation.

Preparation of Teeth

After proper endodontic access cavities, the location of the canal orifice was identified. Using a size 10 hand file with a 0.02 taper glide path was achieved, followed by determination of working length by a number 10 K file.

After the confirmation of working length, preparation of root canals was done by using rotary files, ProTaper S1-F1. With increase in number of file sizes, irrigation was done by using disposable syringe with a 24-gauge needle (Dispo van syringe, 5 mL, Hindustan Syringes & Medical Devices Ltd, Faridabad, Haryana, India). Biochemical preparation was completed along with the use of preheated (60°C) solution of 5% sodium hypochlorite (NaOCl Ramagundam Fertilizers and Chemicals Limited, Faridabad, Haryana, India), using a volume of 1 mL of solution between each file. Normal saline and EDTA (17%) (Glyde, Dentsply Maillefer) and irrigation steps used for this study were as follows:

- Sodium hypochlorite (NaOCl) (5 mL, 1 minute).
- Around 5 mL normal saline (1 minute).
- Around 5 mL EDTA (1 minute, 30 seconds activation).
- Around 5 mL normal saline (1 minute).
- Around 5 mL NaOCl (1 minute, activation for 30 seconds, resting phase for 30 seconds, activation for 30 seconds).
- Normal saline.
- Dye.

Group I (control group): After completing biomechanical preparation, 5 mL irrigation with NaOCl was done with the side vent needle inside the canal for 1 minute, then normal saline was used 5 mL for 1 minute to remove the remaining NaOCl, then for 1 minute, 5 mL of EDTA was agitated after which 5 mL of normal saline for 1 minute was flushed to remove the remaining EDTA, where irrigants activation was not done in this group. Later, canals were filled with 1% methylene blue (Indian Platinum Pvt Ltd.).

Group II (manual irrigation): After completing biomechanical preparation of the canals, 5 mL irrigation with NaOCl was done with the side vent needle (Neoendo Orikam Healthcare India Pvt Ltd), in manual up- and down-motion inside the canal for 1 minute, then normal saline was used 5 mL for 1 minute to remove the remaining NaOCl, then for 1 minute, 5 mL of EDTA was agitated and 30 seconds activation was done after which 5 mL of normal saline for 1 minute was flushed to remove the remaining EDTA. During final irrigation, 5 mL of NaOCl was activated for 30 seconds, then resting phase for 30 seconds, and again activation was done for 30 seconds, in which NaOCl was agitated manually with hand files number 10 K file (MANI Inc., Utsunomiya, Tochigi, Japan). Later, canals were filled with 1% methylene blue (Indian Platinum Pvt Ltd.), which was activated for 30 seconds.

Group III (ultrasonic irrigation): After completing biomechanical preparation of the canals, 5 mL irrigation with NaOCl was done with the side vent needle (Neoendo Orikam Healthcare India Pvt Ltd), in manual up- and down-motion inside the canal for 1 minute, then normal saline was used 5 mL for 1 minute to remove the remaining NaOCl, then for 1 minute, 5 mL of EDTA was agitated and 30 seconds activation was done after which 5 mL of normal saline for 1 minute was flushed to remove the remaining EDTA, During final irrigation 5 mL of NaOCl was activated for 30 seconds, then resting phase for 30 seconds and again activation was done for 30 seconds, in which NaOCl was agitated using an ultrasonic irrigation system (Guilin Woodpecker DTE, Medical Instrument, China), with number 15 and 20 ultrasonic files (Guilin Woodpecker DTE, Medical Instrument, China). Later, canals were filled with 1% methylene blue (Indian Platinum Pvt Ltd.), which was activated for 30 seconds.

Group IV (sonic irrigation): After completing biomechanical preparation of the canals, 5 mL irrigation with NaOCl was done with the side vent needle (Neoendo Orikam Healthcare India Pvt Ltd), in manual up- and down-motion inside the canal for 1 minute, then normal saline was used 5 mL for 1 minute to remove the remaining NaOCl, then for 1 minute, 5 mL of EDTA was agitated and 30 seconds activation was done after which 5 mL of normal saline for 1 minute was flushed to remove the remaining EDTA. During final irrigation, 5 mL of NaOCl was activated for 30 seconds, then resting phase for 30 seconds, and again activation was done for 30 seconds, in which NaOCl was agitated using a sonic irrigation system, that is, an EndoActivator (Dentsply Maillefer, Switzerland) with number 15/0.02 and 25/0.04 sonic tips (Dentsply Maillefer, Switzerland). Later, canals were filled with 1% methylene blue (Indian Platinum Pvt Ltd.), which was activated for 30 seconds.

Sectioning and Microscopy

Cross sections of apical 1 mm of each were prepared using a diamond disk from the apical third. Then, sections were numbered and placed under a microscope (Zeiss Axio vert. A1) at 5 × 10x magnification under AxioVision release 4.9.1 SP1(08-2013) version 15.0. Each section was examined under a light microscope to check the penetration depth of dye in a micrometer (Fig. 1).

Data Analysis

Kruskal–Wallis analysis of variance (ANOVA) test was used in intergroup comparison (more than two groups) of penetration depth and Mann–Whitney *U* test for pairwise comparison using Statistical Package for the Social Sciences (SPSS) v 26.0. p-value < 0.05 indicated a significant difference.

Results

Analysis of data was assessed using the Shapiro–Wilk test. As a result, nonparametric tests were employed for making comparisons.

This graph shows that group I has lower penetration depth, that is, 594.38 μm compared to the other groups. Samples that were activated with sonic irrigation (group IV) have higher penetration depth, that is, 2277.358 μm. Samples with manual irrigation (group II) and ultrasonic irrigation (group II) have penetration depth, that is, 649.11 and 1947.051 μm (Fig. 2).

On statistics analysis, a significant difference was observed among the groups ($p < 0.01$), indicating that there were notable variations in the values. Group IV had the highest values, while group I had the lowest values (Table 1).

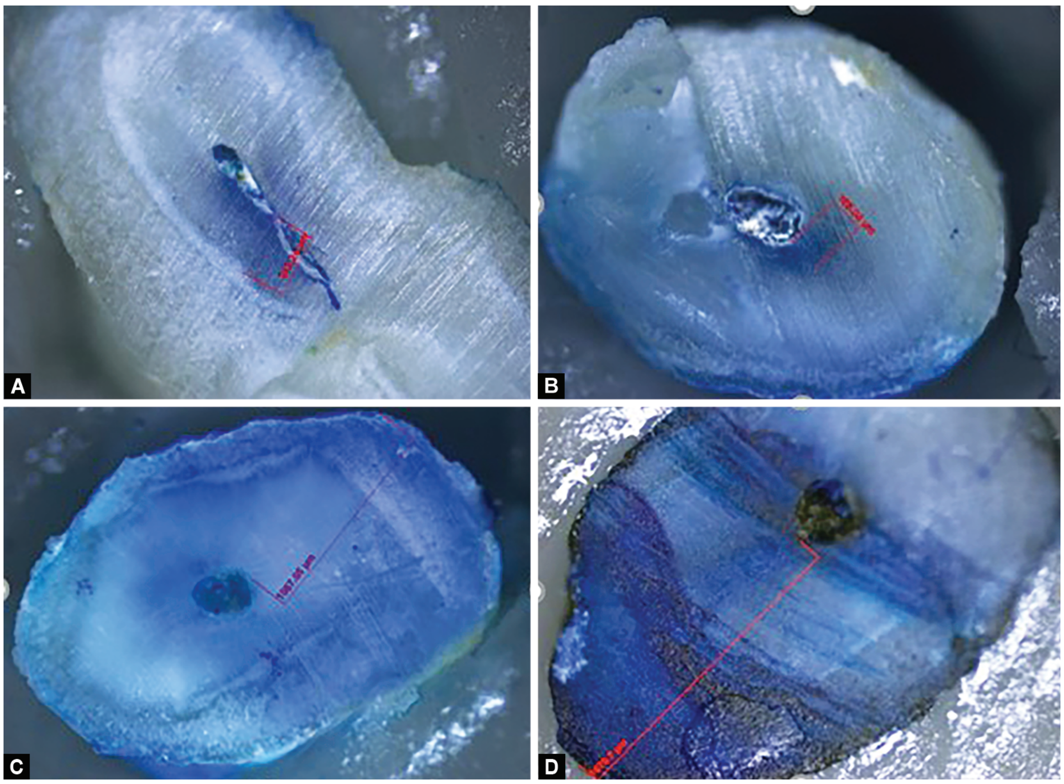
A highly significant difference was seen statistically for the values between all the pairs of groups ($p < 0.01, 0.05$) except for group I vs II and group III vs IV, where there was a statistically nonsignificant difference seen ($p > 0.05$) (Table 2).

Discussion

Biomechanical preparation is an important step in the success of endodontic therapy, and inadequate biochemical preparation can lead to the presence of smear layer and biofilm, which leads to periapical inflammation, hinders tissue repair, and ultimately leads to treatment failure, particularly in nonvital and infected teeth.[9]

This study aimed to assess the penetration of irrigants into dentinal tubules at a microscopic level. This study compared different activation methods, including manual irrigation, ultrasonic irrigation, and sonic irrigation, using the dye penetration method.

For this study, single-rooted teeth with round and oval canals with uncomplicated canal anatomy were selected. This choice was made to uniformity across different teeth as they had similar root canal anatomies. Additionally, collecting teeth from patients within a limited age range allowed for standardization of factors such as sclerotic dentin.[10]



Figs 1A to D: Evaluation of penetration depth into root dentin. (A) Conventional method in apical section; (B) Manual activation method in apical section; (C) Ultrasonic activation method in apical section; (D) Sonic activation method in apical section

Table 1: Intergroup comparison of penetration depth

	N	*Mean*	*Standard deviation*	*Standard error*	*95% confidence interval for mean*		*Minimum*	*Maximum*	*Chi-squared value*	*The p-value of Kruskal–Wallis ANOVA*
					Lower bound	*Upper bound*				
1	23	594.38913	220.954815	46.072264	498.84110	689.93716	234.770	987.920	17.069	0.001**
2	23	649.11000	396.571751	82.690926	477.61951	820.60049	126.000	1691.920		
3	23	1947.05087	2658.081071	554.248217	797.61042	3096.49132	303.880	7876.980		
4	23	2277.35826	2786.956819	581.120668	1072.18776	3482.52876	485.040	8799.000		

Table 2: Pairwise comparison using Mann–Whitney *U* test

Group	*Vs group*	*Mann–Whitney U value*	*Z value*	*A p-value of Mann–Whitney U test*
I	II	264.00	–0.011	0.991#
I	III	102.00	–3.570	0.000**
I	IV	160.500	–2.285	0.022*
II	III	121.00	–3.153	0.002**
II	IV	161.500	–2.263	0.024*
III	IV	223.00	–0.912	0.362#

*Statistically significant difference ($p < 0.05$); **statistically highly significant difference ($p < 0.01$); #non-significant difference ($p > 0.05$)

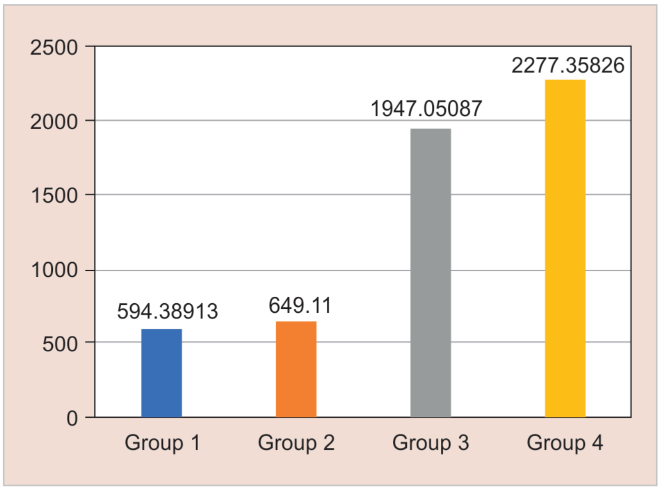


Fig. 2: Intergroup comparison of penetration depth

In the present study, chemomechanical preparation included using ProTaper instruments up to size F1. Previous research has demonstrated that these instruments effectively remove smear layer and dentin shavings compared to manual instrumentation. However, it is important to note that the efficacy of irrigation delivery systems can also be affected by the size and tip taper of the instrument used for preparation.[11]

In the current research, a solution of NaOCl at a concentration of 5.25% and a temperature of 60°C was employed, which suggests that increase in its concentration and raising its temperature can boost its disinfection capabilities and tissue dissolution efficiency. The warming of NaOCl solutions seemed to enhance their capacity to dissolve necrotic pulp tissue and improve their effectiveness.[12]

In this study, methylene blue dye was selected due to its favorable characteristics, such as its low molecular weight and excellent penetration capabilities when compared to other dyes. Using a blue dye to penetrate dentinal tubules provides a straightforward yet dependable means of evaluating the diffusion of molecules within dentin.[13]

A highly significant difference was seen statistically for the values between all the pairs of groups ($p < 0.05$).

Group I (control group) showed lower penetration depth, that is, 594.38 µm in comparison with other groups.

As per Rossi-Fedele et al., the conventional approach for canal irrigation involves the use of a syringe and needle, which can be quite effective when used carefully. To reach the apical canal, it is advisable to utilize small-sized needles, preferably needles with finer gauges (27- or 30-gauge needles). While needles with side vents at the tip may offer a better and safer means of irrigation, they might not effectively reach certain areas, such as fins, isthmuses, and lateral canals of root canal system.[14]

Group II (manual dynamic activation) showed minimal penetration depth, that is, 649.11 µm compared to groups III and IV.

Properly preparing the canals is crucial for enhancing the efficiency of irrigants. When using a size 28 gauge with safety-ended needle increases its effectiveness when the canal has been instrumented to a size smaller than 40 at the root tip. The significance of apical irrigation is directly linked to how deeply the needle is inserted. For optimal results, the needle should fit loosely in the root canal, which allows the solution to flow back and remove debris in a coronal direction. To achieve deeper and more effective placement, it is advisable to use a smaller gauge needle.

Alternatively, MDI can be performed by gently moving a well-fitting gutta-percha master cone up and down in short 2–3 mm strokes. This technique creates an effective hydrodynamic effect and enhances the exchange of the irrigant.[15]

According to Goodman et al., PUI and manual agitation techniques, when combined with NaOCl, are more effective than conventional hand irrigation in removing dentin debris from the root canal.[16]

Group III (ultrasonic irrigation) showed significant penetration depth, that is, 1947.051 µm. Fabricius et al. conducted a study and found that PUI significantly improves the removal of the smear layer in the apical portion of the root canals when compared to the control group ($p < 0.05$). This study concluded that the use of ultrasonic agitation effectively improved the final rinse procedure's effectiveness in cleaning the apical portion of the canal walls.

Another technique used was PUI, which uses acoustic energy from a rotating file to an irrigant solution within the root canal. These ultrasonic waves facilitate the transmission of energy and induces acoustic streaming of the irrigant solution, resulting in improved penetration and increased irrigant volume. PUI also serves as a valuable alternative that enhances the efficacy of irrigating solutions in eliminating both organic and inorganic debris from the walls of root canals.[17]

Group IV (sonic irrigation) has an advanced penetration depth, that is, 2277.358 µm compared with all three groups.

Niu et al. examined a study in which they investigated the impact of various sonic activation protocols using the EndoActivator® on the cleaning of root canals. In this study, 48 single-rooted teeth were prepared and irrigated with NaOCl, chlorhexidine (CHX), and EDTA. Scanning electron microscopy was used to evaluate and assign scores for smear and debris removal from the canals. They conclude that, regardless of the specific sonic activation sequence used, activating the irrigant for 30 seconds during a 60-second application of CHX and EDTA appears to optimize the removal of the smear layer and debris when utilizing the EndoActivator® system.[18]

Finally, it can be summed up that out of all the four groups, the sonic activation group showed higher penetration depth and enhanced better irrigation even in the deepest part of the canals when compared with ultrasonic and manual irrigation, where the apical third of the canal system typically has an unusual anatomy.

Conclusion

Within the limitations of the present study, it was concluded that use of needle irrigation with the use of the EndoActivator (sonic agitation method) has increased the penetration of irrigants in apical third, which also shows effectiveness in cleaning the root canals from isthmuses, canal ramifications, and dentinal tubules. The results concluded that the order for evaluating the penetration depth of irrigants was—sonic irrigation > ultrasonic irrigation > manual irrigation. In conclusion, we can say that machine-assisted irrigation devices and activation of irrigants resulted in better irrigation at the apical third level in both quality and quantity; thus, increasing efficiency in eliminating the smear layer was observed when manual irrigation resulted in reduced irrigant penetration into the apical third.

Clinical Significance

This study demonstrates the clinical application of both sonic and ultrasonic activation, showing a significant ability to remove dentin, particularly in the apical third of tooth. These advanced techniques have highlighted that new devices relying on distinct mechanisms for delivering irrigants, even to the deepest part of the root canal, can achieve superior removal and debridement of the smear layer. This research paves the way for further exploration of the practical implications of these irrigation techniques in clinical practice.

References

1. Paque F, Balmer M, Attin T, et al. Preparation of oval-shaped root canals in mandibular molars using nickel-titanium rotary instruments: a micro-computed tomography study. J Endod 2010;36(4):703–707. DOI: 10.1016/j.joen.2009.12.020
2. Möller AJ, Fabricius L, Dahlén G, et al. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89(6):475–484. DOI: 10.1111/j.1600-0722.1981.tb01711.x
3. Grande NM, Plotino G, Butti A, et al. Cross-sectional analysis of root canals prepared with NiTi rotary instruments and stainless steel reciprocating files. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103(1):120–126. DOI: 10.1016/j.tripleo.2005.12.022
4. Ram Z. Effectiveness of root canal irrigation. Oral Surg Oral Med Oral Pathol 1977;44(2):306–312. DOI: 10.1016/0030-4220(77)90285-7
5. Siqueira JF Jr, Lima KC, Magalhaes FA, et al. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod 1999;25(5):332–335. DOI: 10.1016/S0099-2399(06)81166-0
6. Zehnder M. Root canal irrigants. J Endod 2006;32(5):389–398. DOI: 10.1016/j.joen.2005.09.014
7. Jiang LM, Verhaagen B, Versluis M, et al. Influence of the oscillation direction of an ultrasonic file on the cleaning efficacy of passive ultrasonic irrigation. J Endod 2010;36(8):1372–1376. DOI: 10.1016/j.joen.2010.03.019
8. Sabins RA, Johnson JD, Hellstein JW. A comparison of the cleaning efficacy of short-term sonic and ultrasonic passive irrigation after hand instrumentation in molar root canals. J Endod 2003;29(10):674–678. DOI: 10.1097/00004770-200310000-00016
9. Boutsioukis C, Gogos C, Verhaagen B, et al. The effect of apical preparation size on irrigant flow in root canals evaluated using an unsteady computational fluid dynamics model. Int Endod J 2010;43(10):874–881. DOI: 10.1111/j.1365-2591.2010.01761.x
10. Gulabivala K, Ng YL, Gilbertson M, et al. The fluid mechanics of root canal irrigation. Physiol Meas 2010;31(12):R49–R84. DOI: 10.1088/0967-3334/31/12/R01
11. Stewart GG. The importance of chemomechanical preparation of the root canal. Oral Surg Oral Med Oral Pathol 1955;8(9):993–997. DOI: 10.1016/0030-4220(55)90303-0
12. de Gregorio C, Estevez R, Cisneros R. Efficacy of different irrigation and activation systems on the penetration of sodium hypochlorite into simulated lateral canals and up to working length: an in vitro study. J Endod 2010;36(7):1216–1221. DOI: 10.1016/j.joen.2010.02.019
13. Haikel Y, Wittenmeyer W, Bateman G, et al. A new method for the quantitative analysis of endodontic microleakage. J Endod 1999;25(3):172–177. DOI: 10.1016/S0099-2399(99)80136-8
14. Rossi-Fedele G, Guastalli AR, Doğramacı EJ, et al. Influence of pH changes on chlorine-containing endodontic irrigating solutions. Int Endod J 2011;44(9):792–799. DOI: 10.1111/j.1365-2591.2011.01911.x
15. Grossman LI. Irrigation of root canals. J Am Dent Assoc 1943;30(23):1915–1917. DOI: 10.14219/jada.archive.1943.0397
16. Goodman A, Reader A, Beck M, et al. An in vitro comparison of the efficacy of the step-back technique versus a step-back/ultrasonic technique in human mandibular molars. J Endod 1985;11(6):249–256. DOI: 10.1016/S0099-2399(85)80180-1
17. Fabricius L, Dahlén G, Sundqvist G, et al. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth. Euro J Oral Sci 2006;114(4):278–285. DOI: 10.1111/j.1600-0722.2006.00380.x
18. Niu LN, Luo XJ, Li GH, et al. Effects of different sonic activation protocols on debridement efficacy in teeth with single-rooted canals. J Dent 2014;42(8):1001–1009. DOI: 10.1016/j.jdent.2014.05.007