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Comparative evaluation of depth of sealer penetration into radicular dentinal tubules following the use of Endoactivator, Irrisafe, Endoirrigator plus and Endovac: An *in-vitro* confocal laser scanning microscopic study

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Abstract
The aim of this in-vitro study was to compare the depth of sealer penetration into radicular dentinal tubules after irrigation activation with endoactivator, irrisafe, endoirrigator plus and endovac. Seventy-five tooth samples were instrumented with the protaper universal rotary system and randomly divided into five groups based on the irrigant activation technique used. Obturation was performed using AH plus sealer labeled with fluorescent dye. Two transverse sections were taken corresponding to 2mm and 4mm from the apex and analyzed under confocal laser scanning microscope for measurement of the depth of sealer penetration. The results revealed that the depth of sealer penetration was found to be superior in Endoaoirrigator plus and Irrisafe groups compared to Endoactivator and Endovac groups. An increasing trend of sealer penetration from the apical to the coronal third was observed in all the groups.

Keywords: Confocal laser scanning microscope, Endoactivator, Endoirrigator plus, Irrisafe, Endovac, Sealer penetration

1. Introduction
One of the main purposes of root canal treatment is to facilitate complete debridement of the root canals using a combination of mechanical instrumentation and chemical irrigation. Regardless of the instrumentation technique used, 35% or more of the root canal surfaces have been observed to remain uninstrumented [1].
Hence, irrigation is an essential part of root canal debridement, which for its effective action must come in direct contact with the root canal walls. This can be made possible by activation of the irrigants in the canal which initiate fluid hydrodynamics and thus holds significant promise to improve disinfection. This activation and exchange of intracanal irrigants can be done with the use of various irrigant activation systems such as Sonic, Ultrasonic, Apical negative pressure irrigation and Continuous warm activated irrigation and evacuation system (CWAIS).
The aim of the present study, therefore, was to compare the effect of Sonic (Endoactivator), Ultrasonic (Irrisafe), CWAIS (Endoirrigator plus) and Apical Negative Pressure Irrigation system (Endovac) on sealer penetration into dentinal tubules using Confocal laser scanning microscope. The null hypothesis tested in the present study was that there will be no difference in the mean depth of sealer penetration for all the irrigant activation systems at both the levels that is level 1- two millimeters and level 2- four millimeters from the apex.

2. Materials and Methods
Seventy-five single-rooted freshly extracted human premolar teeth were selected and stored in 0.1% thymol. A dental operating microscope (Carl Zeiss, Pico OPMI; Germany) was used to verify that the selected teeth were free of cracks or apical resorption. Radiographs from facial and proximal views were used to ensure the presence of a single canal. The specimens were decoronated using a water-cooled, high-speed diamond bur (MANI Dia-Bur, SF-41), and the

root canal length was standardized at 12 mm.

A size ten K-file (Dentsply Maillefer, Switzerland) was introduced into each canal until it could be seen through the apical foramen and the length was measured. Working length was established by subtracting 0.5 mm from that length and confirmed radiographically. The apical ends were sealed with nail paint. Canal preparation was done using the Protaper Universal system (Dentsply Maillefer) upto a master apical file size – F4. Instrumentation was performed with intermittent irrigation with 6% sodium hypochlorite(NaOCl) for one minute followed by sterile distilled water using a five millilitres syringe with a 30 gauge side vented needle (Dentsply Rinn, Elgin, II)

2.1 Experimental Groups

The specimens were broadly divided into five experimental groups based on the final irrigation technique used.

Group I [Control]

Irrigation was performed with Normal saline for one minute using Conventional needle irrigation by placing the needle two millimetres short of the working length.

Group II [Sonic Irrigation]-

Irrigating solutions (in the sequence of 6% NaOCl, 17% EDTA, 6% NaOCl with intermittent saline irrigation) were delivered using a 30 gauge side vented needle and sonically activated for one minute using Endoactivator (Dentsply Maillefer) size 25/04 tip (red) placed two millimetres short of the working length

Group III [Passive Ultrasonic Irrigation (PUI)]-

Ultrasonic activation was performed using a stainless steel ultrasonic Irrisafe file (Satelec Acteon) of size 20 following the same sequence of irrigation as followed in group II keeping the device two millimetres short of the working length.

Group IV [Continuous Warm Activated Irrigation and Evacuation system (CWAIS)]

Irrigating solutions (in the same sequence as group II) were delivered and simultaneously activated for one minute using negative tips of Endoirrigator plus (K Dent Dental systems) placed two millimetres short of the working length.

Group V [Apical Negative Pressure Irrigation system (ANP)]

Irrigating solutions (in the same sequence as group II) were delivered using the Master delivery tip of Endovac Irrigation system (SybroneEndo) and negative pressure irrigation was performed for one minute using the microcannula of Endovac, placed two millimetres short of the working length.

All the canals were dried with absorbent paper points and obturation was done using Protaper Universal gutta-percha cones (Dentsply Maillefer, Switzerland) of size F4 and AH plus sealer (Dentsply, DeTrey, Germany). The sealer was mixed with 0.1% Rhodamine Isothiocyanate dye for fluorescence and placed in the canal using gutta-percha cones. Heated plugger (Touch ‘n heat, System B cordless obturation, Sybronendo) was activated and placed four to five millimetres short of the working length to compact the apical third of gutta-percha and remaining canal was filled with injectable gutta percha system (System B cordless obturation, SybronEndo). A coronal filling was obtained with a temporary material (Cavit; 3M ESPE, Seefeld, Germany). Finally, the teeth were stored in an incubator at 37-degree centigrade and 100% humidity for 24 hours to allow the sealer to set.

All the specimens were sectioned perpendicular to the long axis using water cooled diamond disc mounted on a Micromotor with straight handpiece (Marathon-3). Two 1mm sections were obtained at two millimetres and four millimetres from the root apex. All sections were polished using silicon carbide abrasive papers and mounted onto glass slides.

2.2 Confocal laser scanning microscope investigation-

Sections were imaged using a confocal laser scanning microscope at 10x magnification. Evaluation of results was completed using FV 10-ASW 4.1 Imaging software with a calibrated measuring tool. The point of deepest penetration of sealer was calculated from the canal wall to the point of maximum sealer penetration, as shown in figure 3(b).

3. Results

The results of the current comparative, observational in-vitro study revealed that there was a significant difference in the mean depth of sealer penetration for all the irrigant activation systems at two millimeters and four millimeters from the apex and thus, null hypothesis was rejected.

The numerical data from which results were derived is presented in figure 1. One way ANOVA was applied to find out the comparison of depth of penetration in various irrigation systems at Level one and two followed by Post-hocTukey test to find out the pairwise comparisons.

There was a statistically significant difference in mean depth of sealer penetration among the various irrigation systems at level one as well as level two (P=0.000) (Figure 2). The highest depth of sealer penetration was observed with the Endoirrigator Plus and Irrisafe while lowest was seen with the Control group at both the levels. No statistically significant difference was found between Irrisafe-Endoirrigator plus groups as well as Endovac-Endoactivator groups at both the levels. There was a significant difference between level one and two, with level two showing greater penetration depth, with all the irrigation systems.

3.1: Tables and figures:

Table 1: Mean depth of sealer penetration

Level one	Two millimetres from the apex	Endoirrigator Plus ~Irrisafe >Endovac~Endoactivator> Control
Level two	Four millimetres from the apex	Irrisafe~ Endoirrigator Plus >Endovac ~Endoactivator~Control

Fig 1: Numerical data showing depth of sealer penetration/total tubule length in micrometer for all the samples in the group

	Sealer penetration /Total tubule length onilln)									
S. No.	Group I		Group II		Group III		Group IV		Group V	
	Control Group		Endo-Activator		Irrisafe		Endoirrigator Plus		Endovac	
Level 1		Level 2	Level 1	Level 2	Level 1	Level2	Level 1	Level 2	Level 1	Level 2
1600/680		1300/1300	360/530	440/730	710/920	1040/1090	830/830	1030/1160	620V810	990/1010

2110/460	760/880	930/1080	1060/1270	850V850	1030/1030	810/810	1540/1540	550/600	890/950
3290/480	690/1010	560/950	1250/1250	970/970	1360/1390	710/850	980/980	770/770	900V990
4390/780	790/1380	940/1070	780/1050	840/840	1590/1590	940/980	1170/1270	810/1010	1310/1410
5650/890	990/1000	490/940	730/990	830V1080	1430/1430	840/840	1070/1070	570/980	740V910
6580/910	890/1020	210/620	1030/1350	810V850	1420/1490	1070/1070	1360/1360	730V990	980/1000
7410/970	1210/1430	630/810	740/740	550/550	970/970	960/960	1490/1490	510/560	870/990
8260/890	830/990	440/750	740/930	730/730	970/1000	840/840	1070/1070	820/820	1390/1410
9550/910	990/1000	550/760	890/990	910V950	1220/1290	820/970	1280/1310	880/1120	1470/1590
10300/930	790/1300	950/1090	1100/1390	920/920	1230/1260	1110/1110	1450/1450	580/590	940/990
11290/910	880/1300	775/890	870/980	830/830	1410/1410	790/790	1180/1180	410/930	760/1020
12180/790	590/990	390/570	450/770	890/890	1330/1330	870/870	1540/1540	330/700	490/980
13660V980	1000/1310	920/1010	990/1110	860V860	1430/1430	910/1090	1190/1190	940/1090	1010/1300
14420/980	1010/1400	290/610	980/1010	560/870	860/890	990/990	1020/1120	360/540	450/720
15210/690	560/1080	530/890	890/990	810/990	1220/1220	840/880	1070/1270	270/690	960/1200

Level 1- 2mm from apex
Level 2- 4mm from apex
Group I- Control group (Conventional needle irrigation) Group II- Endoactivator
Group II l- Irrrisafe
Group IV- Endirrigator Plus
Group V - Endovac

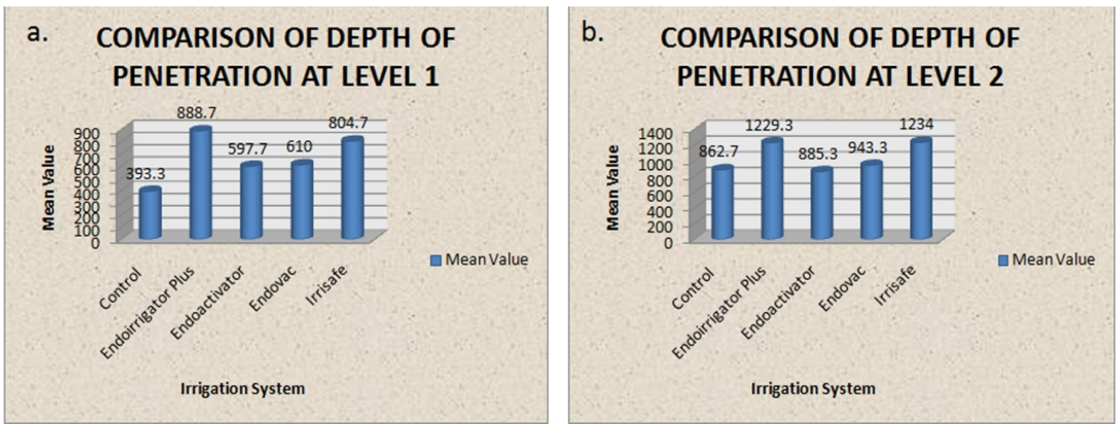

Fig 2: Bar diagram showing the comparison of depth of penetration of sealer at Level 1 and Level 2 using various irrigation systems

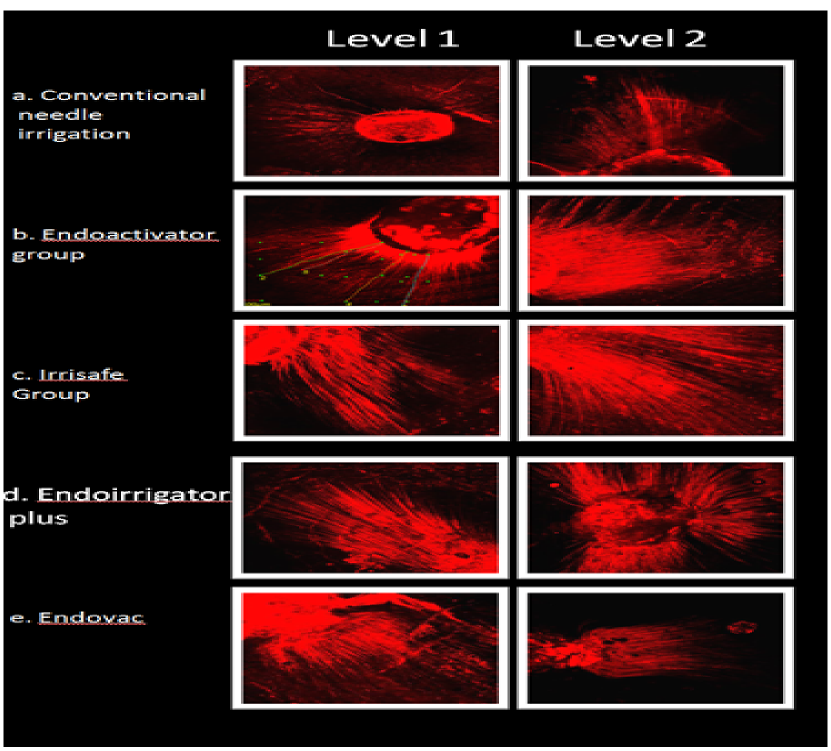

Fig 3: Confocal laser scanning microscopic image of sealer penetration seen at 2mm from the apex (Level 1) and at 4mm from the apex (Level 2) with different irrigation activation systems. a-Control group, b-Endoactivator, c-Irrisafe, d-Endoirrigator plus, e-Endovac

4. Discussion

Complete fluid tight obturation of the root canals is facilitated by adequate sealer penetration within dentinal tubules which require effective smear layer removal [2]. The irrigants used and the irrigation protocol followed during cleaning and shaping have a major role in ensuring the long-term success of root canal therapy.

The complex anatomic variations of the root canals such as accessory canals and isthmuses make the penetration of irrigants a challenging task, especially in the apical third.[3] There are multiple factors which influence the penetration of irrigants into the apical third of the root canal, like final apical preparation size, vapor lock effect in the apical third of the canal and effective activation of irrigating solutions.

Keeping these above factors in mind, in the present study, canal preparation was done using the Protaper Universal system upto a master apical file size- F_4, in accordance with the study conducted by de Gregorio *et al*. [4-6]

Another factor which affects the direct contact of the irrigant with the root canal wall, especially in the apical portion, is the presence of vapor lock phenomenon [7]. To overcome this challenge and to improve the efficacy and distribution of irrigants, different irrigation techniques and devices for activation of the irrigant have been developed.

In the present study, an ex-vivo closed-end model was used to closely simulate clinical conditions [8]. Two horizontal sections 1mm each, corresponding to 2mm and 4mm from the apex were used for the study. The presence of accessory canals in the apical portion of the root canals, as well as the presence of a Perio-Endo pathway for potential bacterial transfer, makes a fluid-tight obturation of this area important [9, 10].

In the present study, the depth of sealer penetration was found to be greater at 4mm than at 2mm from the apex which is in accordance with the study conducted by Generali *et al*. [11] This can be attributed to the presence of more sclerotic dentin, fewer number of dentinal tubules, and lower tubule density in the apical area [12]. Also, the primary dentinal tubules in this region are irregular in direction.[13] Furthermore, the apical portion of the roots shows pronounced variations in anatomy, for example, the presence of accessory canals and isthmuses.

In the present study, at two millimetres from the apex, the use of Endoirrigator plus facilitated superior sealer penetration when compared all the other irrigation systems; however, the difference between Endoirrigator plus and Irrisafe was not found to be statistically significant. The superior sealer penetration could be attributed to various reasons. One of them could be the continuous replenishment of warm NaOCl at 50 °C in contrast to other irrigation systems, where the solution is delivered at room temperature (29 °C), thus increasing the available chlorine concentration, resulting in superior dissolution of collagen [14, 15]. The other reason could be the negative pressure delivery which is responsible for overcoming the issue of vapor lock as well as excluding the risk of irrigating solution being extruded apically.

At four millimetres from the apex, the use of Irrisafe showed most effective sealer penetration into the dentinal tubules; however, the results were comparable to Endoirrigator plus. Irrisafe is an ultrasonically driven instrument which creates acoustic microstreaming inside the root canal. The superior results obtained with irrisafe may be attributed to the production of multiple nodes and internodes [16]. The wide lumen present in the coronal third allows the free movement of the ultrasonic tip resulting in a strong current.

The relatively inadequate sealer penetration at two millimetres from the apex, with both Irrisafe (ultrasonic) as well as Endoactivator (sonic), compared to four millimetres from the apex, may be attributed to the narrow lumen of the canal impeding the movement of the instrument.

In the present study, the relatively less effective Endoactivator and Endovac allowed a comparable depth of sealer penetration at both the levels which were significantly lower as compared to both Irrisafe and Endoirrigator plus.

The acceptable results obtained with sonically driven Endoactivator irrigation system which is based on the hydrodynamic action can be attributed to the Tsunami effect where the random waves break, creating bubbles that oscillate within any given solution [17]. The implosion of oscillating bubbles within the irrigating solution radiates miniature tsunamis or shockwaves that dissipate at 25,000 to 30,000 times per second thus effectively cleaning the root canals.

The comparable results obtained with Endovac irrigation system, which works on the principle of Apical Negative Pressure irrigation, can be attributed to the ability to place the needle close to the apex without the risk of the solution being extruded apically and also overcoming the issue of apical vapor lock, thus offering good apical cleaning [18].

Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including Stereomicroscopy, SEM (Scanning electron microscope), TEM (Transmission electron microscope) and CLSM. CLSM was preferred in the present study as it has certain advantages over the other techniques. It provides detailed information regarding the presence and distribution of sealers or dental adhesives inside dentinal tubules along the entire circumference of the root canal walls at relatively low magnification with the use of fluorescent Rhodamine-marked sealers and artifacts are practically excluded [19, 20].

5. Conclusion

Within the limitation of this study, it may be concluded that the use of Endoactivator, Irrisafe and Endoirrigator plus and Endovac facilitate superior sealer penetration as compared to conventional needle irrigation. Greater sealer penetration was observed with Endoirrigator plus and Irrisafe both at two millimetres and four millimetres from the apex. Thus, with the results of the present study, it may be proposed that a combination of Endoirrigator plus and Irrisafe may show promising results with removal of smear layer from the confines of the root canal thus ensuring a fluid tight three dimensional obturation of the root canals.

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