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Comparative evaluation of penetration of root canal irrigant after using four different activation techniques under confocal laser scanning microscopy: An *in vitro* study

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Abstract
Aim: The aim of this study was to compare and evaluate the penetration depth of root canal irrigant after using Manual, EndoActivator, Ultra X, and Diode Laser-Assisted Irrigant activation technique.
Materials and Methodology: Forty extracted single-rooted human mandibular premolars (N=40) were prepared using Neo Endo Flex Files up to size 25/6%, with saline irrigation during instrumentation. After chemomechanical preparation, canals were filled with Rhodamine B-labelled Twin Kleen dissolved in 10 mL of 3% sodium hypochlorite. Samples were randomly assigned to four groups (N=10) based on the activation technique:
Group 1: Manual activation with a 25/6% Gutta Percha cone
Group 2: Sonic activation using EndoActivator
Group 3: Ultrasonic activation with Ultra X
Group 4: Laser activation with a Diode Laser

Transverse sections at 2, 5, and 8 mm from the apex were examined using confocal laser scanning microscopy to assess maximum irrigant penetration depth, which was statistically analyzed.
Results: Laser activation achieved the greatest penetration in the coronal and apical thirds of root dentin, while Ultra X demonstrated superior penetration in the middle third.
Conclusion: Diode laser-assisted irrigant activation showed greater penetration depth in the coronal and apical thirds of root dentin.

Keywords: Comparative evaluation, penetration, root dentin, scanning microscopy, activation techniques

Introduction
Successful endodontic treatment depends on the thorough removal of necrotic tissue, microbes, endotoxins, debris, and the smear layer formed during instrumentation. Complete disinfection of the root canal system remains a clinical challenge, requiring effective techniques to eliminate residual tissue and microbial contaminants [1-3].
Due to the complex anatomy of the root canal system, complete cleaning and shaping are not achievable with instrumentation alone. Nickel-titanium files primarily affect the central canal, leaving areas such as fins, isthmuses, and cul-de-sacs untouched. These uninstrumented regions may harbor residual tissue, bacteria, and toxins, compromising Obturation and contributing to persistent periradicular inflammation. Therefore, effective irrigation is essential to debride areas beyond the reach of mechanical preparation [4-6].
Sodium hypochlorite (NaOCl), commonly used at concentrations of 0.5-6%, is valued for its antimicrobial, anti-biofilm, and tissue-dissolving properties. However, it cannot remove the smear layer or prevent accumulation of hard tissue debris, which can hinder its penetration and reduce its antibacterial effectiveness. Therefore, a chelating agent such as 15-17% EDTA is often used following NaOCl to eliminate inorganic residues and enhance canal cleanliness [7, 11, 12]. Chelating agents reduces the tissue-dissolving efficacy of NaOCl by diminishing its available chlorine content. To minimize this interaction, a "sequential protocol" is commonly used, where NaOCl is applied during instrumentation for its antimicrobial action, followed by EDTA at the final stage to remove the smear layer effectively [7-9].

The sequential use of NaOCl and EDTA can lead to dentinal erosion, characterized by intertubular tunneling and widening of dentinal tubules. This protocol may decalcify up to 20 µm of tubular walls and 1-5 µm of intertubular dentine, significantly weakening the dentine's flexural strength and potentially increasing the risk of vertical root fractures [7, 10].

The concept of "continuous chelation", introduced in 2005, offers an alternative to the drawbacks of the sequential NaOCl/EDTA protocol. This technique combines NaOCl with a soft chelator, such as tetra-sodium HEDP (Etidronate), which not only removes the smear layer but also provides antibacterial and proteolytic effects. Continuous chelation is favored for its simplicity, enhanced debris removal, compatibility with dental materials, and preservation of NaOCl's antibacterial and tissue-dissolving properties. Additionally, chelators help eliminate metal ions essential for bacterial nutrition and promote biofilm removal from canal walls [7, 13].

Baker *et al*. emphasized that the flushing effect of irrigants plays a more significant role in debris removal than their tissue-dissolving ability, suggesting that the effectiveness of sodium hypochlorite may depend on its application method [14, 15]. Endodontic irrigants can be activated manually or using various devices, including sonic, ultrasonic, apical negative pressure systems, and plastic rotary files. Literature indicates that ultrasonic agitation enhances irrigant penetration, even in the apical third of the root canal. Additionally, dental lasers offer unique photochemical, photoacoustic, and photothermal effects that may further improve irrigant activation [16]. Thus, the aim of this study was to compare and evaluate the penetration depth of root canal irrigants after using different irrigant activation techniques.

Materials and Methods

Forty single-rooted human mandibular premolars (N=40) were selected with ethical approval from Navodaya Dental College, Raichur, India. Teeth with caries, restorations, root resorption, severe angulation, or calcified canals were excluded. After ultrasonic cleaning, teeth were stored in distilled water and radiographically confirmed to have non-calcified canals. The working length was determined using an ISO size 10 K-file, with 1 mm subtracted. Canals were shaped using Neo Endo Flex Files up to size 25/6%. A 1 mL application of 17% EDTA was used for smear layer removal, followed by saline irrigation. Canals were dried with absorbent points. After preparation, canals were filled with an irrigant solution (Twin Kleen capsule in 3% sodium hypochlorite and 0.1 wt% Rhodamine B), delivered via a syringe.

The samples were randomly divided into four groups according to irrigation activation protocol

- Group 1 (N=10) manual irrigant activation was performed for 60 seconds with Master cone gutta-percha in gentle up and down motion. (Figure A)
- Group 2(N=10) irrigant activation was performed using EndoActivator for 60 seconds. (Figure B)
- Group 3 (N=10) irrigant activation was performed using Ultra X for 60 seconds. (Figure C)
- Group 4 (N=10) laser activation was performed using diode laser for 60 seconds with short interval between every cycle. (Figure D)
- After irrigant activation, transverse sections were made using slow-speed diamond disk at 2mm (Apical), 5mm (Middle), and 8 mm (Coronal) from root apex (Figure E).

These sections were examined using a confocal laser scanning microscope (CLSM)

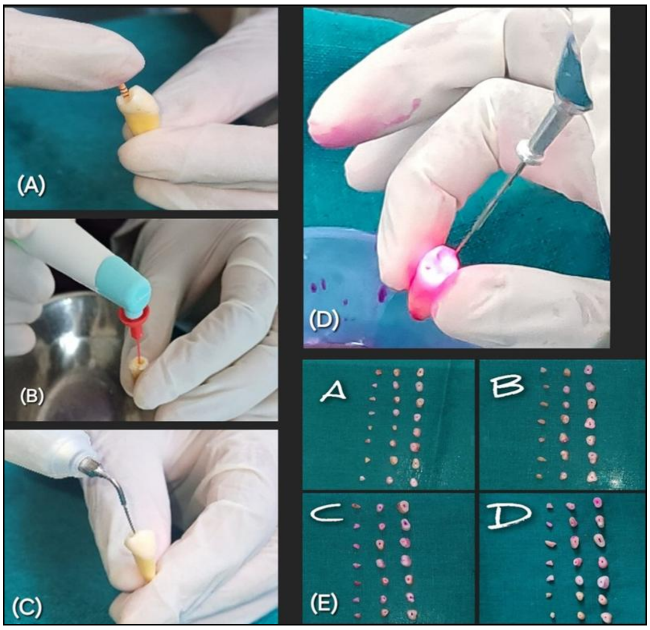


Fig 1: Illustrations of the four irrigant activation methods manual, EndoActivator, Ultra X ultrasonic, and diode laser along with transverse root canal sections for CLSM analysis.

The image was divided into four equal parts and the penetration depth was measured at each part. The average value was recorded as the penetration depth of irrigant. These specimens were evaluated by a single operator, who was blinded to the groups.

Table 1: Tests of Normality

Tests of Normality							
	Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	p value	Statistic	df	p value
Coronal	Manual	.108	10	.200*	.965	10	.838
	EndoActivator	.164	10	.200*	.914	10	.312
	Ultra X	.156	10	.200*	.912	10	.294
	Laser	.160	10	.200*	.966	10	.855
Middle	Manual	.217	10	.198	.891	10	.173
	EndoActivator	.179	10	.200*	.891	10	.174
	Ultra X	.180	10	.200*	.903	10	.237
	Laser	.192	10	.200*	.912	10	.297
Apical	Manual	.207	10	.200*	.950	10	.663
	EndoActivator	.198	10	.200*	.849	10	.056
	Ultra X	.148	10	.200*	.983	10	.980
	Laser	.196	10	.200*	.925	10	.400

Statistical test applied: Kolmogorov Smirnov and Shapiro wilk test, all p-values are non-significant indicating Normal distribution of data.

Statistical Analysis

Statistical analysis was performed using Kolmogorov Smirnov and Shapiro wilk test & one-way analysis of variance (ANOVA) to compare the coronal, middle, and apical values. The significance level was set at $p<0.05$.

Table 2A: Mean penetration depths and statistical analysis

		N	Mean	Std. Deviation	F value	P-Value
Coronal	Manual	10	116.6986	8.52294	48.661	.000 HS
	EndoActivator	10	143.1503	18.06209		
	Ultra X	10	236.7572	56.33241		
	Laser	10	275.9875	33.40053		
Middle	Manual	10	189.3094	33.62155	4.75	0.007 HS
	EndoActivator	10	170.0786	42.31135		
	Ultra X	10	226.8040	34.53752		
	Laser	10	201.5337	25.25220		
Apical	Manual	10	135.1437	17.50657	17.840	.000 HS
	EndoActivator	10	194.1954	32.22583		
	Ultra X	10	171.3846	16.51807		
	Laser	10	196.4902	14.07931		

Statistical test applied: One Way ANOVA; HS-Highly Significant at $p<0.01$

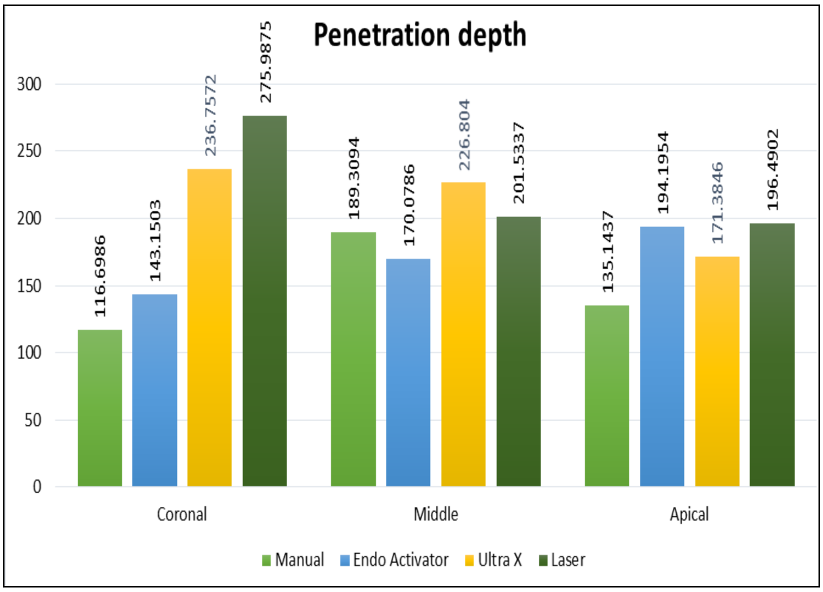
Results

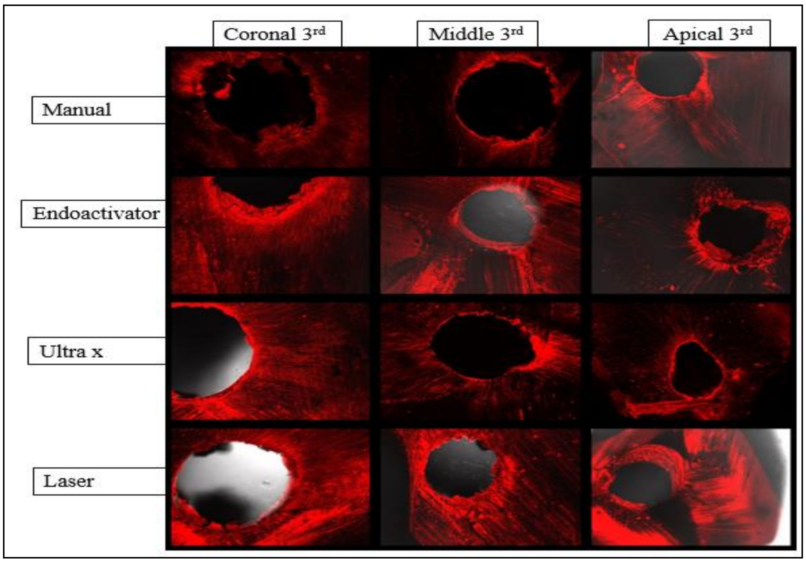
At the coronal (at 8mm), Laser activation group showed the highest penetration depth, followed by Ultra X, Endoactivator and Manual Irrigant Activation.

At the middle (at 5mm) Ultra X group showed the highest penetration depth followed by Laser, Manual and Endoactivator Irrigant Activation.

At the apical (at 2mm), Laser activation group showed the highest penetration depth, followed by Endoactivator, Ultra X and Manual Irrigant Activation.

One-way ANOVA and Turkey test showed there were significantly high differences among all the three groups and also at all three levels ($p<0.05$).





Discussion

Successful root canal therapy requires effective mechanical preparation, irrigation, microbial control, and complete Obturation [17, 18]. Historically, rotary instruments were considered the primary means of cleaning and debriding the root canal system, with less emphasis on the role of irrigants (Schilder, 1974).

Over time, it became evident that many areas of the root canal system are inaccessible to instrumentation (Peters, 2004), leading to a paradigm shift in which the importance of irrigation has grown significantly in recent decades [24]. For successful endodontic treatment, ideal irrigants must meet several key criteria. However, despite efforts to enhance their effectiveness such as increasing temperature, lowering pH, and adding surfactants no irrigant currently fulfills all of these requirements [4]. Sodium hypochlorite remains the preferred primary irrigant, though it requires the addition of a chelator.

The most common irrigation techniques are syringe and needle delivery of the irrigants and ultrasonic file activation [24]. Apical periodontitis is thought to be mostly caused by bacteria in the dentinal tubules following initial root canal therapy. Therefore, to achieve optimal disinfection, efficient irrigation is essential [19, 20]. In this *in vitro* study, results showed that laser activation achieved significantly higher penetration in both the coronal and apical thirds, while Ultra X was most effective in the middle third.

Manual dynamic agitation involves moving a well-fitting gutta-percha cone in short 2-3 mm strokes within the canal (Gu *et al*., 2009). Though simple and cost-effective, its labor-intensive nature makes automated methods more desirable [21].

The EndoActivator is a cordless handpiece with a three-speed sonic motor that activates flexible polymer tips. The vibrating tip is moved vertically, remaining 2 mm short of the working length. Gu *et al*. (2009) reported that its hydrodynamic activation enhances irrigant penetration and flow into hard-to-reach areas of the root canal [21].

Weller *et al*. first introduced the term passive ultrasonic irrigation (PUI) to describe an irrigation technique that avoids contact with canal walls and involves no cutting or shaping. This non-instrumentation approach reduces the risk of altering the canal anatomy. In PUI, ultrasonic energy is transmitted from a smooth or oscillating file to the irrigant, inducing acoustic streaming and cavitation [4]. The Ultra X Ultrasonic Activator (Eighteeth, China) is a lightweight, cordless device with flexible, autoclavable tips and a contra-angle design. Operating at 45 kHz, it utilizes acoustic streaming, agitation, and cavitation to enhance irrigant effectiveness.

Laser-activated irrigation (LAI) enhances root canal cleaning by using visible or near-infrared lasers transmitted through flexible glass fibers, allowing access to narrow canals (Blanken & Verdaasdonk 2007; George & Walsh 2008). The laser induces transient cavitation via optical breakdown of the irrigant, improving smear layer removal and potentially facilitating irrigant extrusion through the apex [23].

The commercially available etidronic acid product Twin KleenTM (Maarc Dental Innovations Endo, India) contains 9% HEDP. It is safe to use with NaOCl and has been recommended for continuous gentle chelation of the root canals[22].These findings align with earlier studies by Zehnder *et al*. and Kamin *et al*., which reported that the continuous presence of etidronic acid (HEDP) inhibits smear layer formation along dentin walls. Similarly, Paqué *et al*. found that combining HEDP with NaOCl significantly reduced hard tissue debris compared to using 2.5% NaOCl alone. However, Sunanda *et al*. reported contrasting results, observing greater irrigant penetration with a chitosan-NaOCl combination than with HEDP-NaOCl [22].

Fluorescent dyes used in confocal laser scanning microscopy (CLSM) are a standard for assessing tubular penetration, as they avoid artifacts from sectioning, dehydration, or polishing. However, CLSM cannot visualize non-fluorescent materials, which necessitated the addition of a fluorescent dye to the irrigant in this study [22].

Recent studies show that Rhodamine B at 0.1% concentration has minimal impact on sealer setting, maintaining flow properties within ADA standards. Its smaller particle size and greater surface activity compared to methylene blue make it a suitable dye. Therefore, 0.1% Rhodamine B was used in this study [22].

Conclusion

Within the limitations of this *in vitro* study using a continuous chelation protocol with etidronic acid and NaOCl, diode laser-assisted irrigant activation demonstrated greater penetration in the coronal and apical thirds of root dentin, while Ultra X showed superior penetration in the middle third.

Conflict of Interest

Not available

Financial Support

Not available

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