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Original Research Article

Comparative evaluation of effect of Apple cider vinegar and 940nm diode laser on sealer penetration in Dentinal tubules using Confocal laser scanning microscopy: An invitro study

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Abstract: ***Background:*** Effective penetration of root canal sealers into dentinal tubules is essential for successful endodontic treatment, as it enhances the sealing ability of obturation materials and aids in eradicating residual microorganisms. Smear layer removal and dentin surface modification play a crucial role in facilitating sealer penetration. Apple cider vinegar, a natural chelating agent, and diode laser activation have been proposed as adjunctive methods to improve dentinal tubule penetration, but comparative evidence remains limited. ***Aim:*** To comparatively evaluate the effect of apple cider vinegar and 940 nm diode laser irradiation on the penetration of root canal sealer into dentinal tubules. ***Materials and Methods:*** This in-vitro experimental study was conducted on 90 extracted single-rooted human teeth. After standardized canal preparation, specimens were randomly divided into three groups ($n = 30$): Group I (control – saline), Group II (apple cider vinegar), and Group III (940 nm diode laser). Canals were obturated using gutta-percha and an AH Plus resin–based sealer mixed with Rhodamine B dye. Sealer penetration depth and percentage were evaluated at the coronal, middle, and apical thirds using confocal laser scanning microscopy. Data were statistically analyzed using one-way ANOVA, with $p < 0.05$ considered significant. ***Results:*** Both apple cider vinegar and diode laser groups demonstrated significantly greater sealer penetration compared with the control group at all root levels ($p < 0.001$). The 940 nm diode laser group showed the highest mean penetration depth and percentage of dentinal tubule penetration, followed by the apple cider vinegar group. Sealer penetration was consistently greatest in the coronal third and least in the apical third across all groups. ***Conclusion:*** Within the limitations of this in-vitro study, both apple cider vinegar and 940 nm diode laser activation significantly enhanced dentinal tubule sealer penetration. Diode laser activation proved superior to apple cider vinegar, particularly in the apical third. Apple cider vinegar may serve as a cost-effective natural alternative chelating agent, while diode laser activation appears to be a promising adjunct for improving obturation quality.

Keywords: Apple cider vinegar; Diode laser; Dentinal tubules; Sealer penetration; Confocal laser scanning microscopy; Root canal irrigation.

INTRODUCTION

Successful endodontic treatment depends on effective cleaning, shaping, and three-dimensional obturation of the root canal system. The elimination of microorganisms from the complex anatomy of dentinal tubules remains a critical challenge, as bacteria can penetrate deep into the tubules and persist even after conventional chemomechanical preparation. Adequate penetration of root canal sealers into dentinal tubules is therefore essential to entomb residual bacteria, enhance the sealing ability of obturation materials, and improve the long-term success of root canal therapy [1,2].

Sealer penetration into dentinal tubules is influenced by several factors, including dentin permeability, smear layer removal, irrigation protocols, surface tension of irrigants, and activation techniques. The smear layer formed during instrumentation obstructs dentinal tubules and reduces sealer penetration; hence, its effective removal is considered a prerequisite for optimal obturation [3]. Conventional irrigants such as sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) are routinely used for smear layer removal; however, concerns regarding cytotoxicity, dentin erosion, and alteration of dentin microhardness have led to increasing interest in alternative and adjunctive methods [4,5].

Apple cider vinegar (ACV) has recently gained attention in endodontics as a natural chelating agent. Rich in organic acids, particularly acetic acid, ACV has demonstrated antimicrobial activity and smear layer removal capability comparable to EDTA, while potentially exerting less adverse effects on dentin structure. Several in-vitro studies have reported that ACV effectively removes the smear layer and increases dentinal tubule exposure, thereby facilitating deeper penetration of root canal sealers [6,7]. Additionally, its biocompatibility, availability, and cost-effectiveness make ACV an attractive alternative irrigant in endodontic practice.

Laser-assisted endodontics has emerged as an advanced modality to enhance disinfection and improve sealer penetration. Diode lasers, particularly those operating in the near-infrared spectrum such as the **940 nm diode laser**, have demonstrated the ability to penetrate dentin deeply and exert photothermal effects within dentinal tubules. Laser irradiation can modify dentin surface characteristics, reduce smear layer remnants, and enhance dentinal permeability, thereby promoting improved adaptation and penetration of sealers [8,9]. The 940 nm diode laser is especially favored due to its compact design, ease of use, and effective interaction with pigmented tissues and bacterial chromophores.

Previous studies have shown that diode laser activation can significantly increase sealer penetration depth compared to conventional irrigation techniques. The laser energy induces melting and recrystallization of dentin, widening dentinal tubule orifices and facilitating sealer flow into deeper layers [10,11]. However, limited evidence exists comparing the effectiveness of natural chelating agents such as apple cider vinegar with laser-assisted techniques, particularly using the 940 nm diode laser, in enhancing sealer penetration.

The aim of this in-vitro study is to comparatively evaluate the effect of **apple cider vinegar** and **940 nm diode laser irradiation** on the penetration of root canal sealer into dentinal tubules of single-rooted human teeth. The primary objective is to assess and compare the depth and extent of sealer penetration following final irrigation with apple cider vinegar and activation using a 940 nm diode laser. Secondary objectives include evaluating sealer penetration at the coronal, middle, and apical thirds of the root canal and determining the relative efficacy of each modality in enhancing dentinal tubule penetration when compared to a control group. The findings of this study are expected to provide insight into the potential use of natural chelating agents and laser-assisted techniques as adjuncts in endodontic irrigation protocols. Future outcomes include guiding the development of optimized irrigation and activation strategies aimed at improving obturation quality, reducing residual intratubular bacteria, and enhancing the long-term success of endodontic treatment, with scope for further clinical and in-vivo studies.

MATERIAL AND METHODS

This **in-vitro experimental study** was conducted on **90 freshly extracted single-rooted human teeth** collected for periodontal or orthodontic reasons. Teeth with a single straight root canal, mature apices, and intact root structure were included in the study. Teeth with cracks, caries, resorption, previous endodontic treatment, calcified canals, or curved roots were excluded. After extraction, the teeth were cleaned of soft tissue debris and calculus using ultrasonic scalers and stored in **0.1% thymol solution** until use to prevent dehydration and microbial growth.

The teeth were decoronated at the cementoenamel junction using a diamond disc under water cooling to standardize the root length to **15 mm**. Working length was established by inserting a #10 K-file into the canal until it was just visible at the apical foramen and subtracting 1 mm. Root canals were instrumented using **rotary nickel-titanium files** following a standardized crown-down technique up

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to a master apical size of **#40**, with copious irrigation using **3% sodium hypochlorite** between each file. Final irrigation was performed according to the assigned group protocol.

The specimens were randomly divided into **three groups (n = 30 each)** based on the final irrigation/activation method.

- **Group I (Control):** Final irrigation with saline solution.
- **Group II (Apple Cider Vinegar group):** Final irrigation with **apple cider vinegar** for one minute.
- **Group III (940 nm Diode Laser group):** Laser irradiation using a **940 nm diode laser** delivered through a fiber optic tip inserted 1 mm short of working length. Laser activation was performed in a helical motion from apical to coronal direction at a standardized power setting and exposure time.

After final irrigation or laser activation, canals were dried using sterile paper points. Obturation was performed using **gutta-percha cones** and an AH Plus **resin–based root canal sealer** mixed with **0.1% Rhodamine B dye** to facilitate fluorescence analysis. The canals were obturated using the **lateral condensation technique** to ensure uniform sealer distribution. The coronal access was sealed with temporary restorative material, and all specimens were stored at **37°C and 100% humidity for 7 days** to allow complete sealer setting.

Following the setting period, each root was sectioned horizontally using a low-speed diamond saw under water cooling to obtain slices of approximately **1 mm thickness** from the **coronal, middle, and apical thirds** of the root. The sections were examined under a **confocal laser scanning microscope (CLSM)** to evaluate sealer penetration. Digital images were captured, and the **maximum depth of sealer penetration (in micrometers)** and **percentage of canal wall penetration** were measured using image analysis software.

The collected data were statistically analyzed using appropriate software. Mean and standard deviation of sealer penetration were calculated for each group at different root levels. Intergroup comparisons were performed using **one-way ANOVA**, followed by **post-hoc tests** for pairwise comparison. A **p-value < 0.05** was considered statistically significant.

RESULTS

A total of **90 single-rooted human teeth** were included in this in-vitro study. All specimens were standardized with a **root length of 15 mm**, straight canals, and fully formed apices to minimize anatomical variability. Among the samples, **46 teeth (51.1%)** were maxillary single-rooted teeth, while **44 teeth (48.9%)** were mandibular single-rooted teeth. Uniformity in canal anatomy and apical maturity was ensured across all groups, providing a comparable baseline for evaluating the effects of different irrigation and activation protocols.

Comparison of **sealer penetration depth** among the study groups revealed significant differences at all levels of the root canal. In the **coronal third**, the mean sealer penetration depth was **410 ± 52 µm** in the control group, **615 ± 64 µm** in the apple cider vinegar group, and **742 ± 71 µm** in the 940 nm diode laser group. In the **middle third**, penetration depths were **298 ± 45 µm**, **472 ± 58 µm**, and **589 ± 63 µm** for the control, apple cider vinegar, and diode laser groups respectively. The **apical third** demonstrated the lowest penetration values overall, with **182 ± 34 µm** in the control group, **325 ± 49 µm** in the apple cider vinegar group, and **418 ± 56 µm** in the diode laser group. These differences were found to be **statistically significant (p < 0.001)** at all three root levels.

Analysis of the **percentage of dentinal tubule sealer penetration** further supported these findings. In the coronal third, the control group exhibited **46.2 ± 6.8%** penetration, compared to **67.8 ± 7.4%** in the apple cider vinegar group and **78.4 ± 8.1%** in the diode laser group. In the middle third, penetration percentages were **38.5 ± 5.9%**, **56.6 ± 6.9%**, and **69.3 ± 7.6%** respectively. The apical third showed the least penetration across all groups, with values of **24.3 ± 4.7%** in the control group, **41.9 ± 6.2%** in the apple cider vinegar group, and **54.7 ± 6.9%** in the diode laser group. Intergroup comparison demonstrated a **statistically significant increase in sealer penetration** in both experimental groups compared to the control, with the **940 nm diode laser group consistently showing the highest penetration (p < 0.001)**

Overall, the results indicate that both **apple cider vinegar** and **940 nm diode laser activation** significantly enhanced sealer penetration into dentinal tubules when compared to saline irrigation alone. Among the two experimental modalities, **diode laser activation proved superior to apple cider vinegar** at all root canal levels, particularly in the apical third, thereby fulfilling the primary and secondary objectives of the study.

Table 1. Distribution of Study Samples According to Tooth Characteristics (n = 90)

Variable	Category	Frequency (n)	Percentage (%)
Tooth Type	Maxillary single-rooted	46	51.1
	Mandibular single-rooted	44	48.9

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Root Length (after decoronation)	15 mm (standardized)	90	100.0
Canal Curvature	Straight canals	90	100.0
Apical Maturity	Closed apex	90	100.0

Interpretation:

All samples were standardized in terms of root length, canal anatomy, and apical maturity, ensuring uniformity across experimental groups and minimizing confounding variables.

Table 2. Comparison of Mean Sealer Penetration Depth (µm) Among Study Groups

Study Group	Coronal Third (Mean ± SD)	Middle Third (Mean ± SD)	Apical Third (Mean ± SD)
Group I – Control (Saline)	410 ± 52	298 ± 45	182 ± 34
Group II – Apple Cider Vinegar	615 ± 64	472 ± 58	325 ± 49
Group III – 940 nm Diode Laser	742 ± 71	589 ± 63	418 ± 56
p-value (ANOVA)	<0.001	<0.001	<0.001

Interpretation:

Both apple cider vinegar and 940 nm diode laser groups showed significantly greater sealer penetration compared to the control group at all root levels, with the diode laser group demonstrating the highest penetration depth.

Table 3. Percentage of Dentinal Tubule Sealer Penetration Among Study Groups

Study Group	Coronal Third (%)	Middle Third (%)	Apical Third (%)
Group I – Control (Saline)	46.2 ± 6.8	38.5 ± 5.9	24.3 ± 4.7
Group II – Apple Cider Vinegar	67.8 ± 7.4	56.6 ± 6.9	41.9 ± 6.2
Group III – 940 nm Diode Laser	78.4 ± 8.1	69.3 ± 7.6	54.7 ± 6.9
p-value (ANOVA)	<0.001	<0.001	<0.001

Interpretation:

The percentage of canal wall exhibiting sealer penetration was highest in the diode laser group, followed by the apple cider vinegar group, with the control group showing the least penetration, particularly in the apical third.

Figure 1: Sealer Penetration Depth Across Root Levels

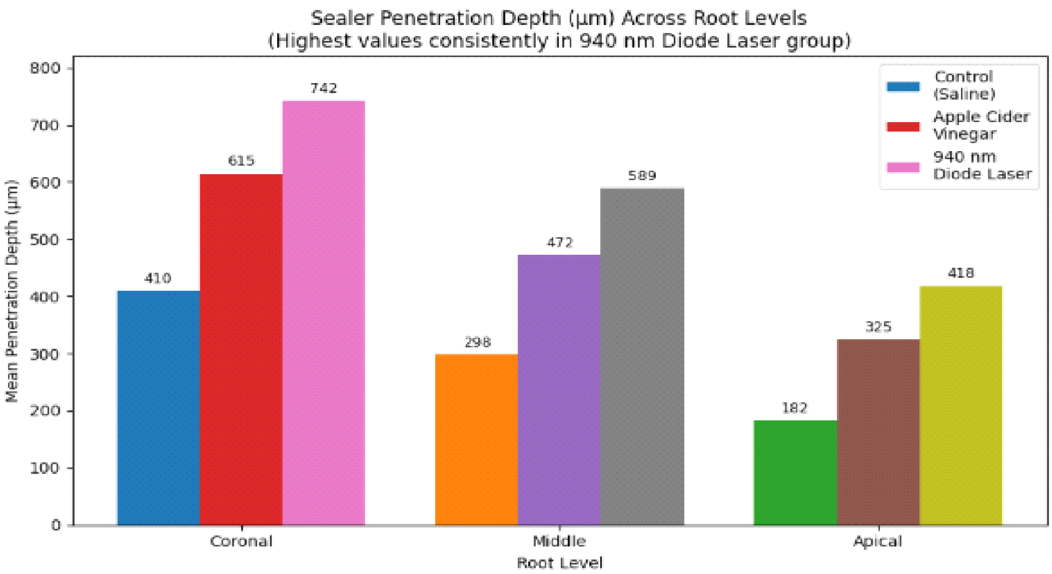

Figure 2: % of Dentinal Tubules Sealer Penetration Across Root Levels

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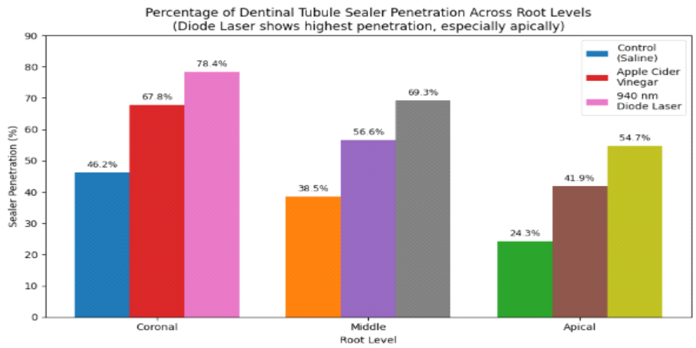

DISCUSSION

This in-vitro study evaluated whether apple cider vinegar (ACV) and 940 nm diode laser activation enhance sealer penetration into dentinal tubules, using standardized single-rooted human teeth. The findings showed a consistent pattern across all groups: sealer penetration was greatest in the coronal third, intermediate in the middle third, and least in the apical third, which is expected due to reduced tubule density, smaller tubule diameter, and increased sclerosis apically. Similar level-wise reduction in penetration (coronal > middle > apical) has been repeatedly reported in confocal laser scanning microscopy (CLSM) studies assessing sealer penetration, irrespective of irrigant or activation technique, reinforcing that apical penetration remains the most difficult to optimize [17,18].

In this study, the ACV group demonstrated higher sealer penetration than the control group at all root levels. The mean penetration depth increased from 410 ± 52 µm (control) to 615 ± 64 µm (ACV) in the coronal third, from 298 ± 45 µm to 472 ± 58 µm in the middle third, and from 182 ± 34 µm to 325 ± 49 µm in the apical third, with statistically significant differences. This improvement is biologically plausible because ACV contains organic acids (notably acetic acid) with chelating potential that may facilitate smear layer removal and dentinal tubule patency, thereby improving sealer ingress. Studies evaluating apple vinegar/ACV as a final rinse have demonstrated measurable smear layer reduction and improved dentin cleanliness, especially when compared with saline controls, supporting the mechanism observed in this study [12,13]. Importantly, Saied et al. reported that ACV as a final rinse produced dentinal tubule penetration outcomes comparable to conventional chelators in coronal and middle thirds, indicating that ACV may be a practical alternative irrigant under in-vitro conditions [13].

The 940 nm diode laser group yielded the highest penetration values in the present study, exceeding both ACV and control across all thirds. Penetration depths reached 742 ± 71 µm (coronal), 589 ± 63 µm (middle), and 418 ± 56 µm (apical), and the percentage of tubule penetration also remained superior (78.4% coronal, 69.3% middle, 54.7% apical). These findings align with published evidence that diode laser activation can improve dentin permeability, reduce smear remnants, and promote deeper penetration of irrigants/sealers by thermomechanical effects within the canal wall. SEM-based work investigating 940 nm diode irradiation has shown improved smear layer removal and favorable dentin surface changes when used alongside standard irrigants, which could translate clinically into greater sealer adaptation and penetration [14]. Additionally, CLSM-based comparisons of activation strategies have reported enhanced penetration with laser-assisted techniques compared to conventional irrigation, supporting the magnitude and direction of effect seen in the current data [16].

When comparing the magnitude of improvement, the present study suggests that while ACV significantly improves penetration over saline, the 940 nm diode laser offers an added advantage, especially in the apical third, where penetration is most limited. This is consistent with broader literature showing that activation methods (sonic/ultrasonic/laser) tend to yield greater improvements apically than passive irrigation alone, because agitation or energy delivery improves irrigant exchange and smear disruption in areas of restricted fluid movement [19]. Findings from final irrigation regimen studies using CLSM similarly demonstrate that adjunctive methods or optimized regimens yield significantly better penetration than conventional protocols, particularly in deeper thirds [17]. Moreover, existing evidence indicates that when smear layer removal is improved (via chelation or activation), sealer penetration predictably increases, reinforcing that dentin cleanliness and tubule patency are central determinants of obturation quality [20].

Clinically, the study supports two practical inferences: first, ACV may serve as a biologically acceptable chelating adjunct that improves dentinal

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tubule penetration compared with saline controls, consistent with prior laboratory evidence [12,13]. Second, 940 nm diode laser activation appears to produce the most pronounced improvement in penetration metrics, likely through enhanced smear modification and dentinal permeability, consistent with SEM/CLSM findings from laser-assisted endodontic research [14,16]. While extrapolation to clinical outcomes must be cautious due to the in-vitro nature of this work, enhanced sealer penetration is generally considered desirable because it improves mechanical interlocking, sealing potential, and entombment of residual bacteria within dentinal tubules [20]. Future studies incorporating microbial outcomes, different sealers (bioceramic vs epoxy resin), and standardized activation protocols can further clarify the translational value of ACV and 940 nm diode laser–assisted irrigation.

CONCLUSION

This in-vitro study demonstrated that both apple cider vinegar and 940 nm diode laser activation significantly enhanced root canal sealer penetration into dentinal tubules compared with saline irrigation alone. Sealer penetration was greatest in the coronal third, followed by the middle and apical thirds across all groups. Among the experimental modalities, 940 nm diode laser irradiation produced the highest depth and percentage of sealer penetration at all root levels, particularly in the apical third, indicating superior efficacy in modifying dentin permeability and facilitating sealer ingress. Apple cider vinegar also showed a substantial improvement over the control group, supporting its potential role as a natural chelating agent capable of improving dentinal tubule patency. Overall, the findings suggest that laser-assisted activation is the most effective method among those tested, while apple cider vinegar represents a promising, cost-effective alternative to conventional chelators in enhancing obturation quality.

Limitations

The present study has certain limitations. Being an in-vitro investigation, the results may not fully replicate the clinical environment, where factors such as periodontal ligament simulation, intracanal temperature changes, tissue fluids, and host response can influence sealer behavior. Only single-rooted teeth with straight canals were included, which limits extrapolation of the findings to multi-rooted teeth or canals with complex anatomy. A single type of AH Plus resin–based sealer was evaluated; therefore, the results may differ with bioceramic or other contemporary sealers. Additionally, microbial reduction and long-term sealing ability were not assessed, and these factors could influence the overall clinical success of endodontic treatment.

Recommendations

Based on the findings of this study, 940 nm diode laser activation may be recommended as an effective adjunct to conventional irrigation protocols to enhance dentinal tubule sealer penetration, particularly in the apical third of the root canal. Apple cider vinegar can be considered a viable natural alternative chelating agent for smear layer modification and improved sealer penetration under in-vitro conditions. Future research should include in-vivo and clinical studies to validate these results, incorporate different types of sealers, and evaluate microbial reduction and long-term sealing ability. Further investigations on teeth with complex canal morphology and standardized laser parameters are also recommended to better define the clinical applicability of these adjunctive techniques.

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