Original Article

Analysis of the depth of penetration of an epoxy resin-based sealer following a final rinse of irrigants and use of activation systems: An *in vitro* study

Aishwarya Sanjay Awati, Neha S. Dhaded, Siddhesh Mokal, Preeti Kore Doddwad

Department of Conservative Dentistry and Endodontics, KLE VK Institute of Dental Sciences, Belagavi, Karnataka, India

Abstract

Objective: The objective of the study was to compare and evaluate the depth of penetration of an epoxy resin-based sealer following a final rinse of 17% ethylenediaminetetraacetic acid (EDTA) and 18% 1-hydroxyethylidene 1, 1-diphosphonate (HEDP), with diode laser and passive ultrasonic activation (PUI): an *in vitro* confocal laser scanning microscopy study.

Materials and Methods: Fifty-two extracted human mandibular premolar teeth with single root and single canal were selected. They were disinfected in 0.1% thymol solution, cleaned of calculus and soft tissues, and stored in 0.1% thymol solution till use. All teeth were radiographed and selected as per the inclusion and exclusion criteria. The teeth were decoronated using a diamond disk under copious water spray to acquire a standardized root length of 14 mm. Working length was established by inserting a size 10-K file into each root canal until it is visible at the apical foramen and by subtracting 1 mm from the recorded length. Instrumentation of the root canal was done till master apical file size of F3 using ProTaper universal, rotary instruments. The canals were irrigated with 2 mL of 3% sodium hypochlorite between successive files. Teeth were randomly divided into four subgroups $n = 12$ according to the intervention. Passive ultrasonic irrigation and diode laser were used to activate the irrigants. Final irrigation was performed with distilled water. These specimens were examined using confocal laser scanning microscope (OLYMPUS FLUOVIEW FV 3000) for dentinal tubule penetration of the sealer. Two-way ANOVA test and Tukey's multiple *post hoc* test were used for statistical analysis.

Results: Highly significant difference was seen between the groups with EDTA and HEDP, with HEDP demonstrating the highest penetration. Among the activation techniques used in this study, PUI showed the highest penetration of the sealer. The least penetration was seen with diode laser activation and EDTA.

Conclusions: The irrigation activation techniques significantly influence the penetration of sealer into root dentinal tubules. When penetration of sealer with different irrigation techniques and irrigants was evaluated, significant greater level of sealer penetration was attained with PUI activation of HEDP.

Keywords: 1-hydroxyethylidene 1; 1-diphosphonate; AH plus; confocal laser scanning microscope; dentinal tubule penetration; ethylenediaminetetraacetic acid; rhodamine dye

INTRODUCTION

A successful root canal therapy depends on the method and the quality of instrumentation, irrigation, sterilization, and three-dimensional (3D) obturation of the root canals.[1]

Mechanical preparation of the canal was traditionally carried out using stainless steel hand files which saw a revolutionary change with the introduction of rotary nickel–titanium files within the past 2 decades.[2,3] Manual

Address for correspondence:
Dr. Aishwarya Sanjay Awati,
Department of Conservative Dentistry and Endodontics, KLE VK Institute of Dental Sciences, Belagavi - 590 010, Karnataka, India.
E-mail: draishwaryaawati@gmail.com

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or mechanized techniques of endodontic instrumentation produce a smear layer. Calcified and necrotic tissue debris, odontoblastic processes and microbes in the tubules of the dentin are the constituents of the smear layer.[1] This smear layer formed during biomechanical preparation gets smeared on the wall of the root canal, thereby inhibiting the complete locking and adhesion of the canal-filling materials into the tubules of dentin[1,3,4]

Hence, complete biomechanical preparation aims to achieve a sterile and debris-free canal. Irrigants play a very important role as it facilitates mechanical debridement by cleaning out debris, dissolving tissue, and sterilizing the root canal.

Among the various root canal irrigants, sodium hypochlorite (NaOCl) is the most favored due to its excellent organic tissue solvent properties. It has its own limitations while dealing with inorganic component of the smear layer.[4] Literature supports that a canal, in which the apical third is covered with a smear layer and contains an abundance of inorganic debris which can be a constraint for NaOCl.[5] It has been suggested that to remove the smear layer, a final flush with 17% ethylenediaminetetraacetic acid (EDTA) may be helpful.[5]

Since both EDTA and citric acid not only remove the smear layer but also erode the dentin and thus exposing the collagen, their clinical use is not always passable.[6]

To prevail over the drawbacks of EDTA, a new endodontic chelating irrigating solution called 1-hydroxyethylidene 1, 1-diphosphonate (HEDP) was introduced in 2005.[4] When compared with the administration of EDTA, HEDP has less detrimental effects on the root dentin. The smear layer treatment efficacy of HEDP is comparable to EDTA, without affecting the organic tissue dissolution potential of NaOCl.[4] The unique short-term compatibility of HEDP with NaOCl makes it possible to use a combined NaOCl/HEDP irrigant during chemomehanical root canal preparation and for the final irrigation. The smear layer can be effectively removed by the combined application of HEDP and NaOCl with minimal effect on the root dentin wall. However, HEDP does not affect the proteolytic and antimicrobial properties of the NaOCl.[7] This is the concept of continuous chelation which has gained momentum in endodontics over recent years.[8]

Needles and syringes of different sizes and tip designs have popularly been used to administer irrigants in the canal space. However, according to research, ineffective irrigation has been a typical result while using this classic approach, especially in area of the periphery where anastomoses between canals and fins are present. The organic and inorganic components of the smear layer which gets compacted in the complex apical anatomy remain untouched by conventional irrigation method.[9] Activation is a contributing factor for the amplification of the efficiency of an irrigant in the complex root canal spaces[7,10] These irrigants must be activated to achieve better results. This can be done with a sonic, ultrasonic, apical negative pressure irrigation system, plastic rotary files, and lasers which unanimously improve cleaning compared to needle irrigation and conventional syringe.[11]

According to various articles, sonics, ultrasonics, and lasers are widely researched as activation methods for irrigating solutions. The research is in favor that passive ultrasonic activation (PUI) has greater affinity in flushing the organic and inorganic debris as compared to conventional irrigation.

Diode laser has shown optimistic results in the removal of the smear layer and disinfection of the root canals in endodontics.[12,13] The formation of vapor-containing cavitations inside the fluid has been induced by laser. Impactful implosions, surface deformation, and removal of surface material are caused by the force generated by the collapse of bubbles. 940 nm and 980 nm diode laser wavelength are of keen interest because these are similar to wavelength for water absorption and are much better absorbed, unlike the other available near-infrared wavelengths such as 810, 830, and 1064 nm.[14]

Although HEDP has proven to be effective in the removal of smear layer, no literature is available so far investigating the efficacy of HEDP when agitated by diode lasers and ultrasonics.

Hence, the current study aims at comparative assessment of the efficiency of smear layer removal by various irrigants using diode laser and ultrasonics at the apical third of the root canal space.

MATERIALS AND METHODS

Fifty-two extracted single-rooted, single-canal mandibular premolar teeth were selected. They were disinfected in thymol solution of 0.1%, cleaned of calcified debris and soft tissues, and stored in thymol solution of 0.1% concentration till use. All teeth were selected as per the inclusion and exclusion criteria after being radiographed. Decoronation of the teeth was done using a diamond disk under continuous water spray to acquire a standardized root length of 14 mm. Working length was established by inserting a size 10 K file until it was visible at the apical foramen. 1 mm was subtracted from the recorded length. Instrumentation of the root canal was carried out till the master apical file size of F3 was obtained using ProTaper universal rotary instruments depending on apical gauging. The canals were irrigated thoroughly with 2 mL of 3% NaOCl between successive files. Teeth were randomly divided into four subgroups $n = 13$ according to the intervention.

- SUBGROUP 1A: 17% EDTA + Diode laser
- SUBGROUP 1B: 17% EDTA + PUI
- SUBGROUP 2A: 1:1 mixture of 3% NaOCl + 18% HEDP + Diode laser
- SUBGROUP 2B: 1:1 mixture of 3% NaOCl + 18% HEDP + PUI.

Irrigating solution preparation

Eighteen percent HEDP was prepared from commercially available HEDP in 3% NaOCl solution. Two capsules were mixed in 10 mL of NaOCl solution. The solution was freshly prepared before use.

Activation of the irrigating solution

Diode lasers activation

Diode laser activation of 17% EDTA – Irrigation of the canals was initially done with 0.8 mL of 17% EDTA and the remainder 0.2 mL was activated with the help of Diode Laser (Siro laser blue, 970 nm ± 10, peak power 2 watts (CW), 200 µm diameter fiber tips) for 20 s cycle.

Diode laser activation of 1:1 mixture of 3% NaOCl + 18% HEDP – irrigation of the canals was initially done with 0.8 mL of 1:1 mixture of 3% NaOCl + 18% HEDP and the remainder 0.2 mL was activated with the help of diode laser (Siro laser blue, 970 nm ± 10, peak power 2 watts (CW), 200 µm diameter fiber tips) for 20 s cycle.

Ultrasonic activation

Ultrasonic activation of 17% EDTA – canals was irrigated with 1 mL of 17% EDTA with PUI using number 25 ultrasonic tip (Aceton, Satelec) for 30 s cycle.

Ultrasonic activation of 1:1 mixture of 3% NaOCl + 18% HEDP – irrigation of the canals was initially done with 1 mL of 1:1 mixture of 3% NaOCl + 18% HEDP with PUI using number 25 ultrasonic tip for 30 s cycle.

Irrigation of canals was done with 3 mL of saline to terminate the action of irrigating solutions. The root canals were dried with paper points and prepared for obturation.

Preparation of the sealers

The sealer was mixed with Rhodamine B dye during manipulation in an approximate ratio of 0.1% (wt). This was done to help create fluoresce under the confocal laser scanning microscope (CLSM). The manufacturer's instructions were followed to prepare the sealer and then with the help of the master cone gutta-percha, it was coated on the root canal walls. Obturation followed this step and any excess gutta-percha at the orifice was removed, and the remaining was condensed with a plugger. Caviton was used to seal the teeth and then they were incubated at 37°C under humidified conditions for 7 days.

Preparation of samples

Sectioning was carried out at apical third (3 mm from the apex) of each root to obtain 1 mm thick sections. These specimens were examined using CLSM (OLYMPUS FLUOVIEW FV 3000) for dentinal tubule penetration of the sealer. Excitation and emission wavelengths of 514–561 nm were used to acquire epifluorescence for rhodamine dye.

Calculation of "dentinal tubule penetration"

Fiji Image J software was used to analyze images, and the longest penetration depth of sealer was measured. This depth was measured using the measuring tool in the "Image J software" (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, LOCI, University of Wisconsin USA) and was calculated from the wall of the canal to the point of deepest penetration of the sealer. Measurement analysis was done by a single operator, and each measurement was repeated twice to ensure reproducibility and consistency.

Statistical analysis

"Two-way ANOVA test" was used for statistical analysis for maximum "tubular penetration" depth, and Tukey's multiple *post hoc* test was used for pairwise comparison among the four groups.

RESULTS

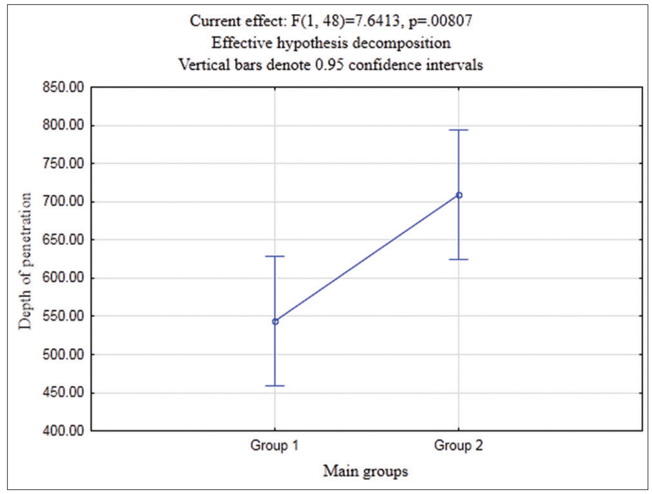
In all, 13 samples in each subgroup were analyzed for dentinal tubular penetration of sealer in "apical" third sections.

The mean depth of penetration, standard deviation, and standard error of the four categories, consisting of 13 specimens per group by one-way ANOVA, in which the highest mean depth of penetration in the apical section was seen with "HEDP" after Passive Ultrasonic Irrigation (Group 2B), being 998.26 µm, and the results were shown to be significant statistically ($P = 0.0001$). Samples filled with "AH Plus" sealer after EDTA + DIODE laser activation (Group 2A) showed the least penetration, i.e. 420.28 µm.

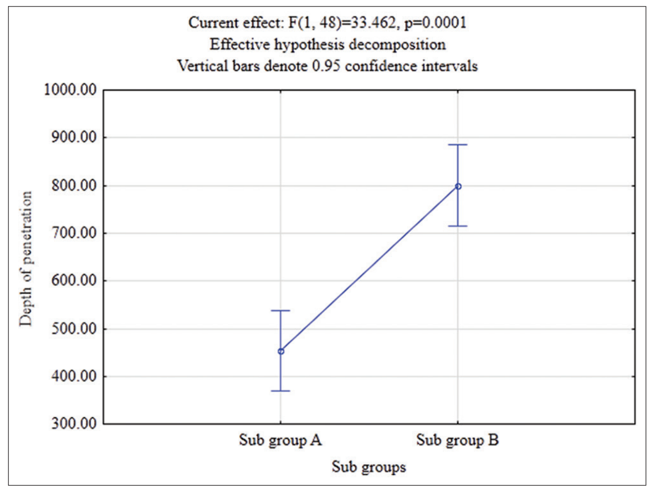
Tukey's multiple *post hoc* test was used for the comparison of the two main groups in the apical section, and highly significant difference was seen between "EDTA" (Group 1) and "HEDP" (Group 2). A significant difference ($P = 0.0082$) was seen statistically between the two main groups, thereby indicating that HEDP showed a positive influence on the sealer penetration [Graph 1].

Tukey's multiple *post hoc* test was done for the comparison of the two subgroups in the apical section, and highly significant difference was seen between "DIODE" (Subgroup A) and "PUI" (Subgroup B). Statistically significant difference ($P = 0.0001$) was seen between the two subgroups, thereby indicating that "PUI" showed a positive influence on the sealer penetration [Graph 2].

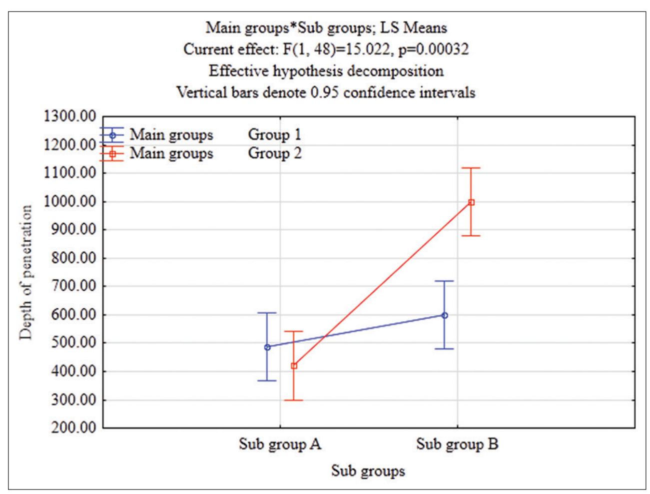
Comparison of interactions of two main categories (Group 1 and Group 2) and two subgroups (Subgroup A and Subgroup B) [Graph 3] with the depth of penetration by Tukey's multiple *post hoc* procedures. When EDTA with diode laser activation was compared with EDTA with PUI activation, EDTA with PUI showed a better penetration of sealer. HEDP with diode laser activation was compared to EDTA with diode laser activation, and showed similar depth of penetration (not significant). EDTA with PUI was compared with HEDP with diode, the sealer penetration was comparable (not significant). HEDP with PUI activation was compared with EDTA with diode laser activation, sealer penetration was highest in HEDP with PUI activation. Comparison between HEDP with PUI activation was compared with EDTA with PUI activation, better sealer penetration was seen with HEDP with PUI activation. When intergroup comparison was done between HEDP with PUI activation and HEDP with diode laser activation, the highest sealer penetration was seen with HEDP with PUI activation.



Graph 1: Comparison of two main groups (Group 1 and Group 2) with depth of penetration



Graph 2: Comparison of two subgroups (Group 1 and Group 2) with depth of penetration



Graph 3: Comparison of interactions of two main groups (Group 1 and Group 2) and two sub groups (subgroup A and subgroup B) with depth of penetration

DISCUSSION

An important objective of root canal therapy is the sterilization of the internal anatomy of the root canal. During instrumentation, a layer of debris comprising dentin, traces of pulp tissue, odontoblastic processes, and sometimes microorganisms, forms on the canal walls. Even after thorough chemomechanical debridement, there remain traces of microbial colonies. The main root canal and also the dentinal tubules are invaded by microorganisms. This smear layer formed during biomechanical preparation gets smeared on the internal wall of the canal and the filling materials, thereby inhibiting the total interlocking and adhesion of the canal obturating materials in the tubules of dentin.[1,3]

Currently, multiple methods have been introduced to clear the smear layer which includes chemical, ultrasonic, and laser techniques.

The preparation of root canal is followed by the use of irrigating solutions for the removal of the formed smear layer. NaOCl and EDTA solution are the most popularly used irrigants. NaOCl has the ability to dissolve organic tissues and is bactericidal but not effective in removing the formed smear layer. The effective penetrability of NaOCl into the dentinal tubules is dependent on time, concentration, temperature, and activation time.[15,16]

Other irrigants introduced such as Mixture of Tetracycline, Acid and Detergen (MTAD), containing 3% doxycycline, 4.25% citric acid, and detergent (Tween 80), can be used along with NaOCl to effectively remove the smear layer.[1,17]

Multiple studies have stated that the effective removal of organic and inorganic debris is done with the help of the combination of NaOCl (2.5%–5%) and EDTA (10%–17%). EDTA is capable of removing smear layer because it is a Ca2+ chelating agent. The number of lateral canals to be filled increases as dentinal tubules open up after a final flush of EDTA.[18-20]

Research reports that these chelators cause the conversion of dentinal structure chemically as well as change the ratio of calcium and phosphorus in the root canal dentin. Changes in the diffusibility and soluble properties of human dentine are caused by any changes in Ca: P ratio which further affects the quality of root canal sealer adhesion. This results in sequelae of significant apical microleakage due to compromised sealer penetration. Moreover, EDTA and citric acid (a component of MTAD) highly react with NaOCl, thereby making the agent incapacitated. Hence, it is essential to search for an updated, biocompatible, and efficient irrigating protocol during root canal procedures.[1]

Chelating agents, such as HEDP, can be mixed with NaOCl. The ability to use both agents together clinically is time-saving while sterilizing the root canal surfaces and clearing the smear layer, during the chemomechanical preparation of the root canals.[7] When compared with EDTA, HEDP is less harmful with respect to its effects on the root dentin. Since HEDP removes less Ca++ ions than EDTA, it is attributed to the basic pH (11.5) of this irrigant.[7] The smear layer removal efficacy of HEDP is comparable to that of EDTA and it does not affect the property of NaOCl to dissolve the organic tissue.[4] HEDP can be used in different concentrations such as 9% and 18%. The researcher concluded that the lowest concentration used should be 18% to guarantee optimal removal of smear layer.[4] This irrigant is suggested to be used in combination with NaOCl as an alternative to the popularly used EDTA solution.

Difficulties in NaOCl reaching the inaccessible areas can be attributed to the inadequate administration of irrigating solution, using a syringe in the root canal system having the highest streaming velocity which is only present in the lumen and around the tip of the needle. The direct contact of irrigant with the dentinal walls of anatomical complexities is prevented by the high surface tension of NaOCl. Literature shows that the irrigating solution has an effect beyond the needle's tip only to a certain extent due to the dead-water zone or air bubbles in the apical region of the root canal, which prevents the penetration of the solution apically.[21]

To achieve better results, manual activation of endodontic irrigants as well as machine-assisted devices such as sonic, ultrasonic, apical negative pressure irrigant system, plastic rotary files, and lasers should be done. Improvement in canal cleaning is apparent with these methods when compared to conventional syringe and needle irrigation.[11]

Due to their challenging anatomic variations and frequent extraction for orthodontic treatment, human mandibular premolars were chosen for the study. To prevent fungal growth, 0.1% thymol solution was used to store the specimens. To obtain a uniform root length of 14 ± 1 mm with a flat coronal surface, specimens were decoronated. This was done to combat the discrepancies due to variations in access cavity preparations and to create an equivocal reference for evaluation. ProTaper Universal files were used for biomechanical preparation as they possessed improved cutting efficiency and reduced torsional loading.[22,23]

The irrigation protocol followed was – 3% NaOCl between successive instrumentation because of its potential to dissolve tissue and antimicrobial characteristics, followed by a final flush with 17% EDTA after the use of NaOCl for smear layer elimination. In another group, the final flush was done with 1:1 mixture of 3% NaOCl + 18% HEDP.

Passive ultrasonic irrigation and diode laser were used to activate the irrigants. Final irrigation was performed with saline to banish the effect of the remnant oxygen from NaOCl on the polymerization of the sealers.[2,24] To prevent microleakage, caviton was used over the gutta-percha as a temporary restorative material.[25] To guarantee sealer's complete setting and polymerization and to simulate the oral environment, all the samples were stored at 37°C in humidified conditions for 1 week.

To evaluate the tubular penetration depth in the study, CLSM was the method of choice over scanning electron microscope, due to its ability to create a 3D image, visualize sections at different levels, and make depth measurement more precise. The integrity of the dentin was preserved, thereby allowing the samples to be used again with the help of CLSM, as it eliminated added steps of sample preparation such as gold spluttering or dehydration. Another advantage of CLSM is that it prevents artifacts. This is due to the analysis being performed from the surface to 20–30 μ depth. Minute amounts (0.1%) of the rhodamine dye were needed to obtain the fluorescence and hence the sealer's properties remained unchanged[26,27] [Figures 1-4].

The present study showed that Apexit Plus sealer had the deepest penetration in the apical third of the canal when the final flush was performed with HEDP and activated with passive ultrasonic irrigation. Specimens filled with AH Plus sealer after HEDP + DIODE laser activation showed the least penetration. Intragroup comparison was done between the two subgroups in the apical section, and highly significant difference was seen between DIODE (subgroup A) and PUI (subgroup B). In the present study, statistically

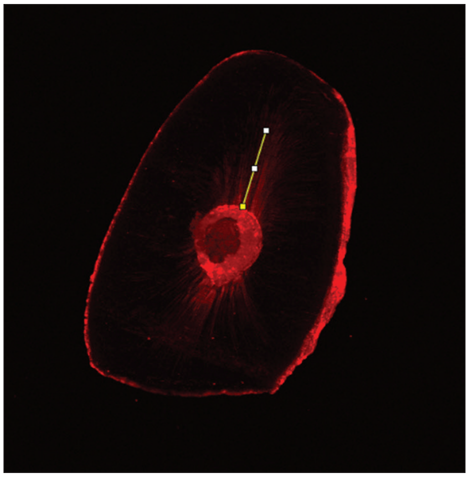


Figure 1: Confocal laser scanning microscope image representing the depth of penetration of AH Plus sealer after ethylenediaminetetraacetic acid and DIODE LASER activation

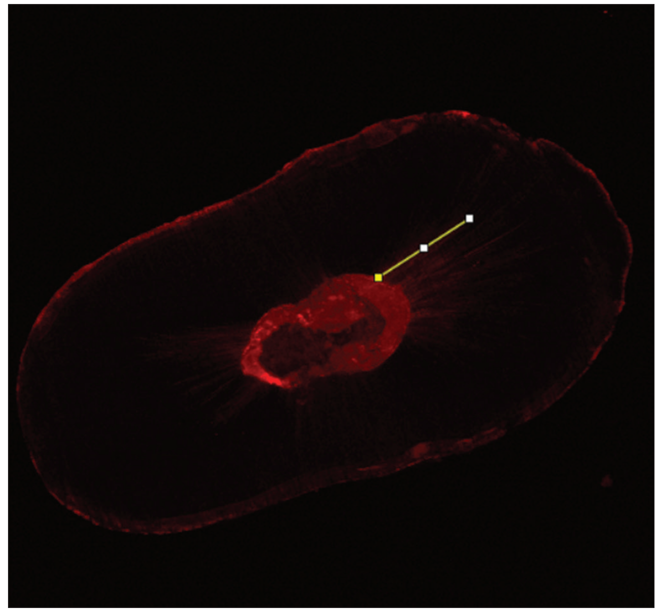


Figure 2: Confocal laser scanning microscope image representing the depth of penetration of AH Plus sealer after ethylenediaminetetraacetic acid and passive ultrasonic activation activation

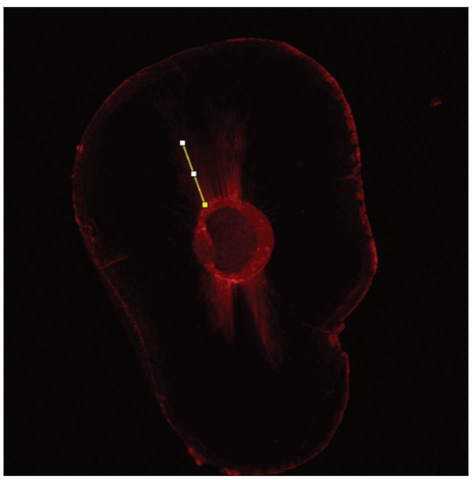


Figure 3: Confocal laser scanning microscope image representing the depth of penetration of AH Plus sealer after 1-hydroxyethylidene 1, 1-diphosphonate and DIODE LASER activation

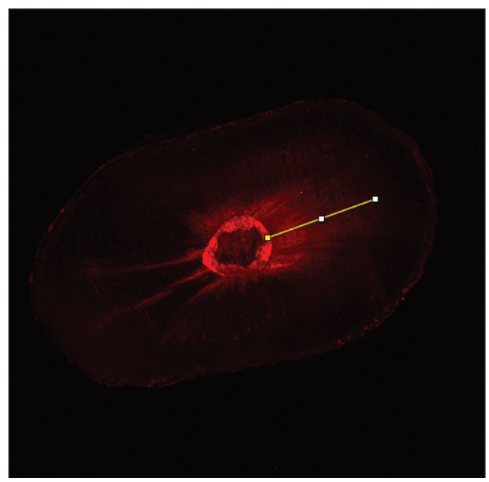


Figure 4: Confocal laser scanning microscope image representing the depth of penetration of AH Plus sealer after 1-hydroxyethylidene 1, 1-diphosphonate and passive ultrasonic activation activation

significant difference (P = 0.0001) was seen between the two subgroups, thereby indicating that PUI has a positive influence on the sealer penetration which was also conferred by Amin *et al*.[16] [Graphs 2 and 3]. Literature supports that the apical penetrability of irrigating solution can increase ultrasonic agitation.[15,28,29] As opposed to manual irrigation, it is the high speed and flow volume created due to ultrasonics, which causes decreased apical accumulation of debris and increased availability of the solution to the inaccessible parts of the apex. Limitation of debris, reduced apical packing, enhanced access of the product to the accessory canals, and flush effect caused by the ultrasound but not manual irrigation can be due to the fact that ultrasound creates a higher speed and flow volume of the irrigating solution.[11]

The results of the past studies show that diode laser gave optimistic results in the activation of the irrigant. This might be due to unique properties of diode lasers such as photochemical, photoacoustic, and photothermal effects.[13,15] Laser induces the formation of cavitation-creating vapor inside the fluid. A force created by the collapse of these bubbles creates implosions that impact the root surfaces and cause surface deformation and surface material removal.[14] However, in the present study, diode laser activation showed the least penetration of sealer into the tubules of the dentin, which might be due to low peak power (2 watts) used in this study. Emissions of laser from the tip of the optical fiber make it impossible to obtain uniform coverage of the canal surface owing to the fact that it was not lateral onto the root canal walls and hence caused failure.[21]

In this study, intergroup comparison revealed HEDP irrigant performing better than EDTA irrigant with a statistically significant difference, thereby indicating that "HEDP" showed a positive influence on the sealer penetration. This is contrary to studies done by Lottanti *et al*. and Kfir *et al*.[5,30] [Graph 1]. The results of this study confirm the improved efficiency of HEDP over EDTA.

The administration of HEDP has fewer negative effects on the root dentin than does EDTA. Without compromising NaOCl's ability to dissolve organic tissues, HEDP's smear layer treatment effectiveness is on par with that of EDTA.[4] It is feasible to utilize a combined NaOCl/HEDP irrigant for both the final irrigation and chemomechanical root canal preparation due to the special short-term compatibility of HEDP with NaOCl.

In this study, the highest sealer penetration was seen when HEDP was activated with PUI, followed by the group which was treated with EDTA and activated by PUI. Decreased efficacy was seen with EDTA activated by diode laser followed by the least efficiency seen when HEDP was activated by diode laser.

CONCLUSIONS

The irrigating solution activation techniques prominently influence the penetrability of the sealer in the dentinal tubules of the root. When the penetrability of the sealer with various irrigation techniques and irrigants was evaluated, significantly greater levels of sealer penetration were attained with PUI activation of HEDP. To conclude, HEDP can be used as a substitute for EDTA for smear layer removal. Taking into account, the drawbacks of EDTA, an *in vivo* study with various concentrations of HEDP, and different activation techniques should be carried out to support the results of the present study.

Author contributions

Conceptualization: Awati AS, Dhaded NS, Data curation: Awati AS. Formal analysis: Awati AS, Dhaded NS. Funding acquisition: name. Investigation: Awati AS. Methodology: Awati AS. Supervision: Dhaded NS, Dodwad. P. Writing-original draft: Awati AS, Dhaded NS. Writing-review and editing: Awati AS, Dhaded NS, Mokal S.

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Conflicts of interest

There are no conflicts of interest.

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