"COMPARATIVE EVALUATION OF THE DEPTH OF PENETRATION OF AN EPOXY RESIN BASED SEALER FOLLOWING A FINAL RINSE OF 17% EDTA AND 18% HEDP, WITH DIODE LASER AND PASSIVE ULTRASONIC ACTIVATION: AN IN-VITRO CONFOCAL LASER SCANNING MICROSCOPY STUDY"

By

REG.NO. IE0220002

Dissertation

Submitted to the

KLE Academy of Higher Education & Research Belagavi, Karnataka

In partial fulfillment of the requirements for the degree of

MASTER OF DENTAL SURGERY

In

CONSERVATIVE DENTISTRY AND ENDODONTICS

(BRANCH – IV)

DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS

KAHER VK INSTITUTE OF DENTAL SCIENCES, BELAGAVI, KARNATAKA

2020-2023

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH

BELAGAVI, KARNATAKA

ENDORSEMENT BY THE HEAD OF DEPARTMENT, PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Comparative evaluation of the depth of penetration of an Epoxy Resin based sealer following a final rinse of 17% EDTA and 18% HEDP, with Diode Laser and Passive Ultrasonic Activation: An in-vitro Confocal Laser Scanning Microscopy study"** *is a bonafide research work done by* **REG.NO. IE0220002.**

Head of Department

Dr. PREETI DODDWAD M.D.S
Professor & Head,
Department of Conservative Dentistry and Endodontics,
KAHER V.K. Institute of Dental Sciences,
Belagavi.

Date: 27/12/2022

Place: Belagavi

Professor and Head
Dept. of Conservative Dentistry
KLE V. K. Institute of Dental Sciences,
Belagavi

Principal

Dr. ALKA KALE M.D.S
Principal,
KAHER V. K. Institute of Dental Sciences,
Belagavi

Date : 28/12/22

Place : Belagavi

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar, BELAGAVI-590010.

UNDERTAKING

I, **REG. NO. IE0220002** hereby declare that the information and data mentioned in my thesis entitled **"Comparative evaluation of the depth of penetration of an Epoxy Resin based sealer following a final rinse of 17% EDTA and 18% HEDP, with Diode Laser and Passive Ultrasonic Activation: An in-vitro Confocal Laser Scanning Microscopy study"** belongs to me and is original.

I am aware of the definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the thesis prepared by me is original and does not involve plagiarism anywhere. In case at later stage it is found that I have indulged in plagiarism, then I am solely responsible for the same and the Institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the University.

Place: Belagavi

Date: ______________

LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	EDTA	Ethylene diamine tetra acetic acid
2	HEDP	1-hydroxyethylidene 1,1-diphosphonate
3	CLSM	Confocal Laser scanning microscope
4	ANOVA	Analysis of variance
5	μm	Micrometre
6	SD	Standard Deviation
7	et al	Additional persons involved in the same study
8	NaOCl	Sodium Hypochlorite
9	SEM	Scanning Electron Microscope
10	mL	Milliliter
11	° C	Degrees Celsius
12	mm	Millimeter
13	hrs	Hours
14	min	Minutes
15	n	Number of specimens
16	p-value	Probability value
17	i.e.	That is
18	nm	Nanometer

19	<	Less than
20	>	Greater than
21	SE	Standard error
22	CI	Central incisor
23	PM	Premolar
24	PUI	Passive ultrasonic irrigation
25	WL	Working Length
26	SC	Single Cone
27	NS	Not significant
28	EA	Etidronic Acid
29	PA	Peracetic Acid
30	DW	Distilled Water

ABSTRACT

Aim- To compare and evaluate of the depth of penetration of an Epoxy Resin based sealer following a final rinse of 17% EDTA and 18% HEDP, with Diode Laser and Passive ultrasonic activation: An in-vitro Confocal Laser Scanning Microscopy study

Methodology: 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 1mm 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 irrigate with 2 mL of 3% sodium hypochlorite between successive files. Teeth were randomly divided in 4 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.

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.

Conclusion- The irrigation activation techniques significantly influence the penetration of sealer into root dentinal tubules. When penetration of sealer with different irrigation techniques and irrigants were evaluated significant greater level of sealer penetration were attained with PUI activation of HEDP.

Keywords: HEDP, EDTA, AH Plus, Dentinal tubule penetration, Rhodamine dye, Confocal Laser Scanning Microscope.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF ANNEXURES

INTRODUCTION

A successful root canal therapy depends on the method and the quality of instrumentation, irrigation, sterilization, and three-dimensional 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 (NiTi) files within the past 2 decades. [2,3] Manual or mechanized techniques of Endodontic instrumentation produces a smear layer. Calcified tissue, necrotic tissue debris, odontoblastic processes, microbes such as bacteria, and blood cells in the tubules of the dentin are the factors present in the smear layer.[1] This smear layer formed during biomechanical preparation gets smeared on the wall of the root canal and the obturating materials thereby inhibiting the complete locking and adhesion of the canal filling materials into the tubules of dentin [1, 3, 14]

Hence complete biomechanical preparation aims 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.

Amongst 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 apical third is covered with a smear layer and contains abundance of debris can be a constraint for Sodium hypochlorite. It has been suggested that in order to remove the smear layer a final flush with 17% EDTA may be helpful. [5]

Since both EDTA and citric acid not only remove the smear layer but also erode the dentin and thus expose 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 sodium hypochlorite (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 size and tip design 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 periphery where anastomoses between canals and fins are present. Organic and inorganic components comprise the material that remained untouched or compacted into the root canal anatomy. [9] Efficiency of the root canal irrigating solutions has been proven to be amplified within the root canal space as well as in root canal systems with complex anatomy and dentinal tubules, upon activation of said irrigant.[7] These irrigants must be activated to achieve better results. This can be done with as sonic, ultrasonic, apical negative pressure irrigant system, plastic rotary files and lasers which unanimously improve cleaning compared to needle irrigation and conventional syringe. [10]

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

Diode Laser has shown optimistic results in the removal of smear layer and disinfection of the root canals in endodontics.[11] 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 on 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 1,064 nm.[12]

Though 1-hydroxyethylidene 1,1-diphosphonate (HEDP) has proven to be effective in removal of smear layer, no literature is available so far investigating the efficacy of HEDP when agitated by diode lasers and ultrasonics.

Hence current study aims at – Comparative assessment of efficiency of smear layer removal by various irrigants using Diode laser and Ultrasonics at the apical third of the root canal space.

AIM AND OBJECTIVES

AIM OF THE STUDY:

- "Comparative evaluation of the smear layer removal efficacy of irrigating solutions using Diode Laser and Ultrasonics and its effect on the 'dentinal tubule penetration' of Epoxy resin-based root canal sealer, AH Plus root canal sealer, using Confocal Laser Scanning Microscopy (CLSM)"

OBJECTIVES OF THE STUDY:

- "To evaluate the effect of 17% EDTA using Diode Laser and using Ultrasonics, on 'dentinal tubule penetrability' of an Epoxy Resin-based root canal sealer, AH Plus, using Confocal Laser Scanning Microscopy (CLSM)"
- "To evaluate the effect of 1:1 mixture of 3% NaOCl+18% HEDP for smear layer removal using Diode Laser and Ultrasonics"
- "To compare the effect of 17% EDTA and 1:1 mixture of 3% NaOCl+18% HEDP on removal of smear layer at the apical third region using Diode Laser and Ultrasonics"

HYPOTHESIS:

NULL HYPOTHESIS:

"There is no difference in the dentinal tubule penetration of the Epoxy Resin-based root canal sealer, AH Plus, after use of 17% EDTA and 18% HEDP for smear layer removal when activated with Diode Laser and Passive Ultrasonic Irrigation"

ALTERNATIVE HYPOTHESIS:

"There is a difference in the dentinal tubule penetration of the Epoxy Resin-based root canal sealer, AH Plus, after use of 17% EDTA and 18% HEDP for smear layer removal when activated with Diode Laser and Passive Ultrasonic Irrigation"

REVIEW OF LITERATURE

1. An in vitro study was conducted by Dineshkumar MK et al., to evaluate the result of “17% EDTA, MTAD and 18% HEBP” irrigants on the microhardness of canal dentine using the “Vickers” “microhardness” test. In this study, forty single-rooted premolars had their crowns removed at the CEJ and cut in vertical sections. 80 specimens were categorized into 4 groups (n=20). Category 1 was treated with water, category 2, 3 and 4 were treated with “1.3% NaOCl” as a working solution for 20 min, followed by “17% EDTA”, “MTAD” and “18% HEBP”, respectively. The surface microhardness of the dentine was calculated with a “Vicker's” “hardness” test. This study concludes that “HEBP” as a final flush is promising irrigation regime with least effect on the mineral content of root dentine. [1]
2. An in vitro study was conducted by Emre Erik C et al., to assess the capacity of removal of the smear layer by different “etidronate” treatments. Seventy eight roots were prepared up to size X4 apically and categorized at random into five categories and a control group (13). Categories were: 5ml of sterile saline as irrigant (control) for 3 minutes, 5ml of 17% EDTA for 3 minutes, 5ml of 9% HEBP for 3 minutes, 5ml of 18% HEBP for 3 minutes, irrigation with 5ml of 1% NaOCl + 9% HEBP for 3 minutes and 5ml of 2% NaOCl + 18% HEBP for 3 minutes. The roots were observed using SEM. The smear layer scores in the 2% NaOCl + 18% HEBP group was the least. There were no variation among the treatment categories in the “smear layer” scores of the cervical and middle thirds. However, the “smear layer” scores in the 2% NaOCl + 18% HEBP treatment group were lower than those in the 9% HEBP and 18% HEBP treatment groups in the apical 3rd (p <05). The concentration of HEBP of 18% is

recommended clinically. Use of an oxidizing agent ensures optimal removal of smear layer. [4]

3. An in vitro study was conducted by Kfir A et al., to weigh the difference in the cleanness and effect on canal walls after the administration of “HEDP” based irrigating solution with that accomplished by irrigating with “NaOCl” and “EDTA”. In this study, 40 extracted premolar teeth were instrumented till size X3, using HEDP (3% NaOCl) or 3% sodium hypochlorite followed by a final flush with 17%EDTA, which was delivered with a syringe and needle. 10 were negative controls. Vertical sections of teeth were observed under SEM. “Smear layer”, “debris” and effect on the root canal wall was studied. The apical third of the root canal had more smear layer occurrence than in the coronal third in both groups. This study concludes that, the HEDP-based irrigation solution has no difference with 3% NaOCl followed by “EDTA” in terms of cleanliness or the incidence of erosion of the canal wall. [5]
4. A study was carried out by JOUR Kaki et al., to evaluate the results of “Dual Rinse HEDP” solution on dentinal adhesion. 40 teeth were sectioned to obtain a flat bonding surface which was later polished and then categorized at random into 4 groups of 10 samples each. GA: NaOCl (5%) +DW, GB: NaOCl (5%) + EDTA (17%), GC: NaOCl (5%) + Dual Rinse HEDP, GD: NaOCl (5%) + CA (20%). Clear SE Bond was used to prepare the surfaces. “Clearfill Posterior Composite Resin blocks” were placed on the buccal and palatal area of the tooth. The bond strength was tested with a UTM at the speed of 1.0 mm/min and calculated. Data was studied with “one-way ANOVA and Duncan’s tests”. “Dual Rinse HEDP” solution does not interfere with coronal dentin adherence

and can be recommended as an option to the popularly used EDTA irrigant when used in combination with NaOCl. [7]

5. A study was conducted by Zollinger A et al., to evaluate the effect of combining novel product (HEDP) with NaOCl solutions on their stability, clinically**.** The combination of NaOCl with "Dual Rinse HEDP" initially contained 1.0%, 2.5% or 5.0% NaOCl and always 9.0% of HEDP. Controls were stand-alone NaOCl solutions. Borosilicate glass bottles were used to assess the stability of these solutions at 23°C temperature. The results of warming (60°C) or preserving the solutions at 5°C were studied in PP syringes. Concentrations of NaOCl were measured by iodometric titration (free available chlorine). Results acquired in the bottles were comparable to those in the syringes. Warming of "NaOCl/Dual Rinse HEDP" combination had a negative effect on free chlorine, with a complete loss after 1 hour. On the contrary, storing the mixtures at 5°C kept the free chlorine at a higher lever for 7 hours. Initial concentration and temperature of NaOCl had an effect on short-term storage stability of the mixtures. [8]

6. An in vitro study by Abraham S et al., was done to compare the difference in the outcome of "Diode Laser", "EndoActivator" and "passive ultrasonics" for "smear layer removal" at the apical 3rd of the canals with "0.2% Chitosan". In this study, forty premolar teeth were taken and WL of 12 mm was established and prepared till size F3. Category A, irrigating solution used was 1ml of "0.2% chitosan". Category B, irrigating solution used was 0.8ml of "0.2% chitosan" and the remainder 0.2 ml was activated with "Diode Laser". Category C, canals were irrigated with 1ml of "0.2% chitosan" which was activated with "endoActivator". Category D, irrigating solution used was "0.2% chitosan" and

activated with PUI. All specimens were rinsed with 3ml of distilled water. The evaluation of samples was done with a SEM at 1000x and 3000x. This study concludes that, **“**Diode laser” and “endoActivator” with “0.2%chitosan” was better in the “removal of the smear layer” when compared to PUI.[11]

7. A study was conducted by Hmud R et al., to study the pressure waves generated by laser and their use for the removal of debris and smear layer from the canals. Previously, middle infrared erbium laser was used. Presently, examination (Biolase, Ezlase and Sirona Sirolaser, respectively) was done to see if cavitations could be induced in aqueous media with the help of 940 and 980 nm diode lasers. Formation of cavitations were observed under a microscope after a capillary tube was exposed to the laser energy using a 200 µm fiber. An array of laser criteria was followed to establish conditions that create cavitations within 5 seconds of the laser irradiation commencement. A variety of solutions were compared using a “setting of 2.5 W/25 Hz for the Sirolaser and 4 W/10 Hz for the Ezlase” to study cavitation in distilled water, aerated tap water, degassed distilled water, ozonated water, 3 and 6% hydrogen peroxide. Cavitations in water-based media were formed by the implosion of water vapour with the help of both “diode laser systems”. Role of pulse frequency or pulse interval was not as important as laser power. When weak (3%) peroxide solution was used as the target irrigant, instead of water, optimal laser-initiated cavitation occurred. This study concludes that, this phenomenon has potential for enhancing debridement in endodontics. [12]

8. The aim of this review article by S, Llena C et al., was to sum up and debate the "available information concerning ultrasonic irrigation in endodontics". This review covers ultrasonic irrigating methods and their efficacy in debridement. This review covers the relevant literature on PUI. Information from original scientific papers or reviews listed in "MEDLINE" and "Cochrane" were included in the review. This study concluded that, enhanced canal cleanness, superior irrigant solution transfer to the canal, debridement of the soft tissue debris, smear layer and bacterial elimination are a result of the use of ultrasonic in irrigating procedures. [13]

9. A study was conducted by Amin K et al., to assess the effect of Ultrasonics and Diode Laser in the presence and absence of EDTA on the removal of smear layer from canals. 120 mandibular premolars roots with WL of 12 mm were instrumented upto files F3. First Group used 1 ml of 3% NaOCl as irrigant, followed by 3ml NaOCl (3%). The second group used 1 ml of 17% EDTA as irrigant, succeeded by 3 ml NaOCl (3%). Third group used Diode Laser. Fourth group was first irrigated with 0.8 ml EDTA (17%). The remainder 0.2 ml filled the root canals and Diode Laser was used after that. The fifth group was irrigated with 1 ml DW with PUI activation, followed by 3 ml NaOCl (3%). The last group was irrigated with 1 ml EDTA with PUI, succeeded by 3 ml of NaOCl (3%). SEM was used to examine the canals for the remaining smear layer at the cervical middle and apical 1/3rd. **L**east smear layer scores were seen with Ultrasonics with EDTA. Significantly better performance than Ultrasonics was seen with Diode Laser alone. [15]

10. A study was performed by Lottanti S et al., to measure the result of EDTA, EA and PA when used in addition to NaOCl as root canal irrigating solutions on calcium discharged from the smear layer, canals and root dentine demineralization after preparation. Premolars were irrigated were irrigated with: (1) 1% NaOCl while instrumentation, DW post instrumentation, (2) 1% NaOCl while, 17% EDTA post instrumentation, (3) 1: 1 of 2% NaOCl and 18% EA while and post instrumentation, and (4) 1% NaOCl while, 2.25% PA post instrumentation. The eluted calcium was analyzed by "Atomic Absorption Spectroscopy". Areas covered by smear and apparent canal wall decalcifications were evaluated by SEM. Sclerotic dentine was also considered for the smear layer analysis. The data revealed calcium elution of protocol (1) < (3) < (2) < (4). Smear layer was hardly present on the prepared canal walls post treatment with the decalcifying agents. Protocols (1) and (3) showed no detrimental effect on root dentine. Protocol (2) and (4) showed demineralization patterns. The solutions in this study removed or prevented smear layer formation and affected the dentine differently. [17]

11. An in vitro study performed by Lagemann M et al., to evaluate the effectiveness of activation of EDTAC with the help of 940 nm laser administered by fibre tips into EDTAC (15%) or hydrogen peroxide (3%). Middle-infrared Erbium Laser activation of EDTA- cetrimide (EDTAC) has improved removal of smear layer. 40 single roots were instrumented using rotary files, with control group, for the evaluation of the smear layer. Post laser activation, roots were sectioned vertically. Apical, middle and cervical thirds were assessed using SEM. Activation of EDTAC with laser, enhanced the removal of smear layer, while in peroxide there was minimal removal of smear layer. This protocol was better for

the removal of the smear layer than the 'gold standard. Photothermal disinfection is also an added benefit of lasers. [18]

12. A study was done by Kamble AB et al., to compare the removal of the smear layer from the root canal dentinal tubules after they were exposed to 2 canal irrigating solutions – “17% EDTA” and a new irrigating solution chitosan using SEM. Decoronation of 40 premolars was done. This was succeeded by preparation with Race files and intermittent irrigation with “3% sodium hypochlorite”. It was ultrasonically activated after this. The specimens were divided horizontally and placed in their solutions as per their categories for a period of 5 minutes. This was followed by evaluation under Scanning Electron Microscopic (SEM). The present study had results which indicated that the Chitosan proved to be effective in removing the smear layer. A moderate concentration of 0.2% chitosan proved to remove the smear layer with greater efficiency. [21]
13. A study was conducted by Wang X et al., to measure the temperature increase of root surfaces while and after “Diode Laser application”, to check the difference morphologically of the canal wall after exposure and to measure the leakage apically post irradiation and obturation. 66 single-rooted teeth after extraction were prepared up to size 60 K-file and then categorized into 3 sets. Each group had 22 teeth. The first two categories were exposed to a 5 W “Diode Laser” using fibers of diameters 550 and 365 µm, respectively for 7 sec. Group 3 served as a control and was not irradiated. Thermography was used to measure the temperature rise on root surfaces of the teeth in the first and second groups. Six teeth in each group were sectioned longitudinally and observed microscopically. Other teeth were immersed in Rhodamine B solution after

being obturated and the degree of apical leakage was calculated. A maximum rise in temperature of 8.1°C was recorded in the first group. Clean root canal walls were a result due to evaporation and removal of the smear layer after laser treatment. This was markedly better than the control group ($p < 0.05$). The laser-treated categories had markedly less leakage apically than the parent group ($p < 0.05$) after obturation. Results inferred that the "Diode Laser" was useful for eliminating the smear layer and debris from the walls and decreasing leakage apically after obturation. This suggests that it could be advantageous for root canal treatment in clinic. [22]

14. Study was performed by Ciucchi B et al., to compare the efficacy of various irrigants on the smear layer removal in 40 canals. These canals were prepared under laboratory conditions with 3% NaOCl as irrigant. Controls consisted of ten canals. The other thirty canals were divided in 3 categories and further irrigated with: NaOCl; EDTA+ ultrasound; EDTA + ultrasound. The appearance of the smear layer was scored over the number of dentinal tubule openings for the three sections of the canals, under the Scanning Electron Microscope (SEM). NaOCl as an irrigant produced consistently smeared surfaces. The smear layer was removed moderately on ultrasonic stirring of NaOCl. However, EDTA resulted in smear free surfaces. Ultrasound + EDTA did not increase its dissolvability. A decrease in the efficacy of the irrigant was seen at the apex of these canals. [23]

15. A review paper by Hülsmann M et al., presents a review of the chemical and pharmacological properties of EDTA preparations and their clinical use. Chelating agents were introduced into endodontics to aid in the negotiation of canals that are narrow and have calcific obstructions, by Nygaard-Østby. EDTA solution was hypothesized to soften the canal dentine and remove the smear layer along with increasing the permeability of dentin. Though the efficacy of EDTA in softening root dentine is questioned, chelators have gained favor in recent times. Their use as lubricant during rotary root canal instrumentation has been recommended by manufactures of NiTi instruments. It is recommended that a final flush of the canal with "15–17% EDTA" is required to help dissolve the smear layer. [24]

MATERIALS AND METHODOLOGY

STUDY DESIGN: In-vitro study

SOURCE OF DATA: The study was carried out in the "Department of Conservative Dentistry and Endodontics, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi" and the laboratory procedures were performed at Dr. Prabhakar Kore's Basic Science Research Laboratory, KLE University, Belagavi.

Specimens were examined with the help of confocal laser scanning microscope at "Birla Institute of Technology and Science- Pilani, K. K Birla Goa campus".

Collection of extracted human mandibular premolar teeth was done from "Department of Oral and Maxillofacial Surgery, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi".

INCLUSION CRITERIA:

- Extracted single rooted human mandibular premolars
- Single straight canals
- Teeth with apical width corresponding to 20 K file or less

EXCLUSION CRITERIA:

- Teeth with root resorption, cracks or fracture line
- Root canal treated teeth
- Teeth with calcified canals
- Teeth with root caries

- Teeth with multiple canals/Anatomic variation
- Teeth with curved roots, more than 5^0 curvatures
- Teeth with apical width more than 20 K file size

SAMPLE SIZE ESTIMATION:

S1= 0.42

S2= 0.42

d=0.38 (acceptable error)

Z_α=1.96 at 5% α error

Z_β = 1.282 at 90% power

$$n = \frac{2S^2 (Z_\alpha + Z_\beta)^2}{d^2} = 26 \text{ in each group}$$

SAMPLING PROCEDURE: Samples were categorized into groups according to Simple random sampling

MATERIALS AND ARMAMENTARIUM:

MATERIALS:

- Human mandibular premolar teeth
- 0.1% thymol
- 3% Sodium Hypochlorite
- 17% Ethylene Diamine Tetraacetic Acid (EDTA) (CANALARGE)
- 1-HydroxyEthylidene-1,1-Di Phosphonate (TWIN KLEEN)
- Paper points (Diadent Group International)
- Distilled Water
- 0.9% Saline

- Gutta-Percha points (Diadent Group International)
- AH Plus Sealer
- Rhodamine dye
- EDTA gel
- Cavit (Caviton)

ARMAMENTARIUM:

- Micromotor
- Airotor (NSK, Japan)
- K Files (10-40)
- ProTaper Universal
- Endomotor (Dentsply Maillefer, Switzerland)
- 27-gauge syringe (Dispovan)
- Passive Ultrasonic Irrigation Tips (Aceton, Satelec)
- Ultrasonic unit (Satelec)
- Diode Laser: (Siro Laser Blue, Dentsply Sirona)
- Diamond disks (KWALITY DIAMOND TOOLS, Mumbai)
- Confocal Laser Scanning Microscope (OLYMPUS FLUOVIEW FV3000)

METHODOLOGY WITH FLOWCHART:

Fifty two extracted human mandibular premolar teeth were cleaned of calculus and soft tissue and handled according to OSHA guidelines 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 was visible at the major diameter and by subtracting 1mm from the recorded length

↓

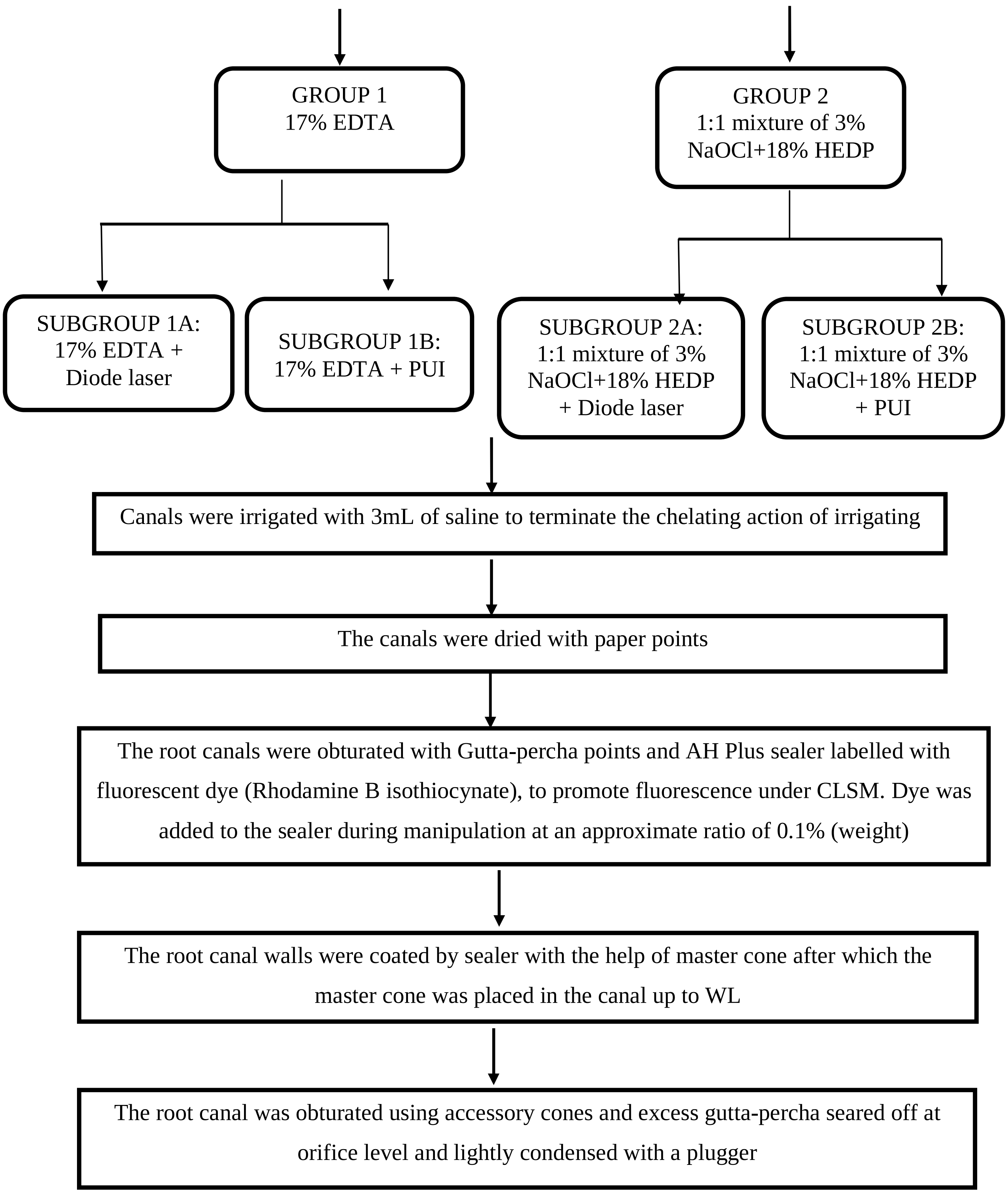
The root canals were be prepared using ProTaper universal rotary instruments up to F3 and F4 Depending on apical gauging

↓

Irrigation was done with 2 mL of 3% sodium hypochlorite (NaOCl) between successive instrumentation

↓

Teeth were categorized at random divided in 4 subgroups n= 13 according to the intervention


GROUP 1
17% EDTA
GROUP 2
1:1 mixture of 3% NaOCl+18% HEDP
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
Canals were irrigated with 3mL of saline to terminate the chelating action of irrigating
The canals were dried with paper points
The root canals were obturated with Gutta-percha points and AH Plus sealer labelled with fluorescent dye (Rhodamine B isothiocynate), to promote fluorescence under CLSM. Dye was added to the sealer during manipulation at an approximate ratio of 0.1% (weight)
The root canal walls were coated by sealer with the help of master cone after which the master cone was placed in the canal up to WL
The root canal was obturated using accessory cones and excess gutta-percha seared off at orifice level and lightly condensed with a plugger

Teeth were sealed with Caviton (3M, ESPE) and incubated at 37°C and 100% humidified conditions for a week to simulate clinical conditions

↓

Specimens were prepared by sectioning teeth horizontally with a diamond disc at apical third (2mm from apex) of each root and these slices were of 1mm thickness each

↓

All sections were polished with silicon carbide abrasive paper

↓

After mounting the specimens on glass slides, they were examined under Confocal Laser Scanning Microscopy (CLSM)

DETAILS OF THE PROCEDURES CONDUCTED DURING THE RESEARCH:

Fifty-two extracted single rooted with 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. Establishing of working length of each root canal was done by inserting a size 10 K file until it is visible at the apical foramen. 1mm was subtracted from the recorded length. Instrumentation of the root canal was carried out till master apical file size of F3 using ProTaper universal, rotary instruments depending on apical gauging. The canals were irrigated thoroughly with 2 mL of 3% sodium hypochlorite between successive files. Teeth were randomly divided in 4 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: 18% HEDP was prepared from commercially available HEDP in 3% NaOCl solution. Two capsules were mixed in 10mL 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), 200µm diameter fiber tips) for 20 seconds 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, 970nm+/-10, peak power 2 watts (CW), 200µm diameter fiber tips) for 20 seconds cycle.

ULTRASONICS ACTIVATION

Ultrasonic activation of 17% EDTA - Canals were irrigated with 1 mL of 17% EDTA with Passive Ultrasonic activation using no.25 ultrasonic tip for 30 seconds cycle.

Ultrasonic activation of 1:1 mixture of 3% NaOCl+18% HEDP - Irrigation of the canals was initially done with 1mL of 1:1 mixture of 3% NaOCl+18% HEDP with Passive Ultrasonic activation using no.25 ultrasonic tip for 30 seconds cycle.

Irrigation of canals was done with 3mL 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: 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 CLSM. The manufactures 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 remaining was condensed with a plugger. Caviton was used to seal the teeth and then they were incubated at 37 ^{0}C under humidified conditions for 7 days.

PREPARATION OF SAMPLES: Sectioning at apical third (3 mm from the apex) of each root to obtain 1mm 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 and 561 nm was used to acquire epifluorescence for Rhodamine dye.

CALCULATION OF 'DENTINAL TUBULE PENETRATION': Fiji Image J software was used to analyze images and longest penetration depth of sealer was measured. This depth was measured using the measuring tool in the "Image J software" and was calculated from the wall of the canal to point of deepest penetration of the sealer. Measurement analysis was done by a single operator and each measurement was repeated twice in order 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.

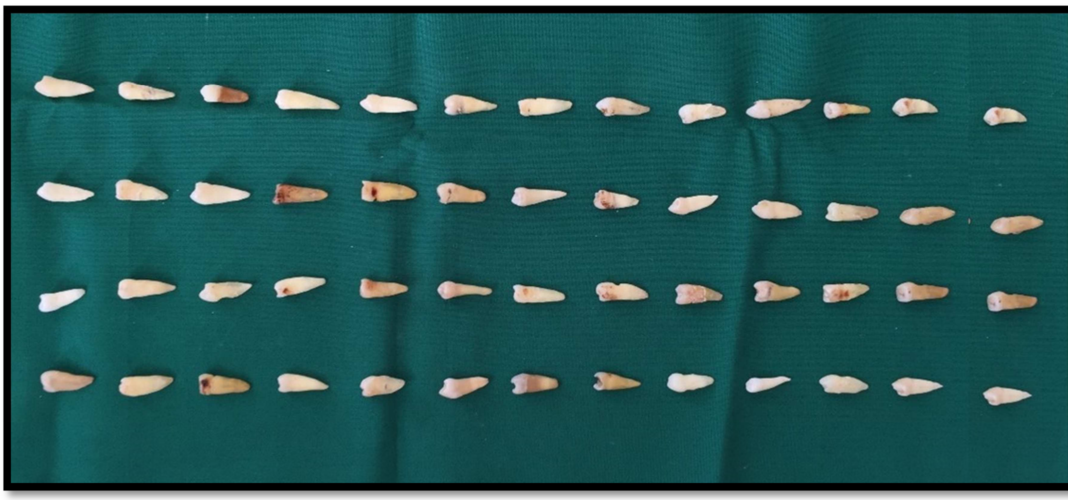


Fig 1: Human Mandibular Premolars

Fig 2: Armamentarium

Fig 3: Materials

Fig 4: Debris Removal

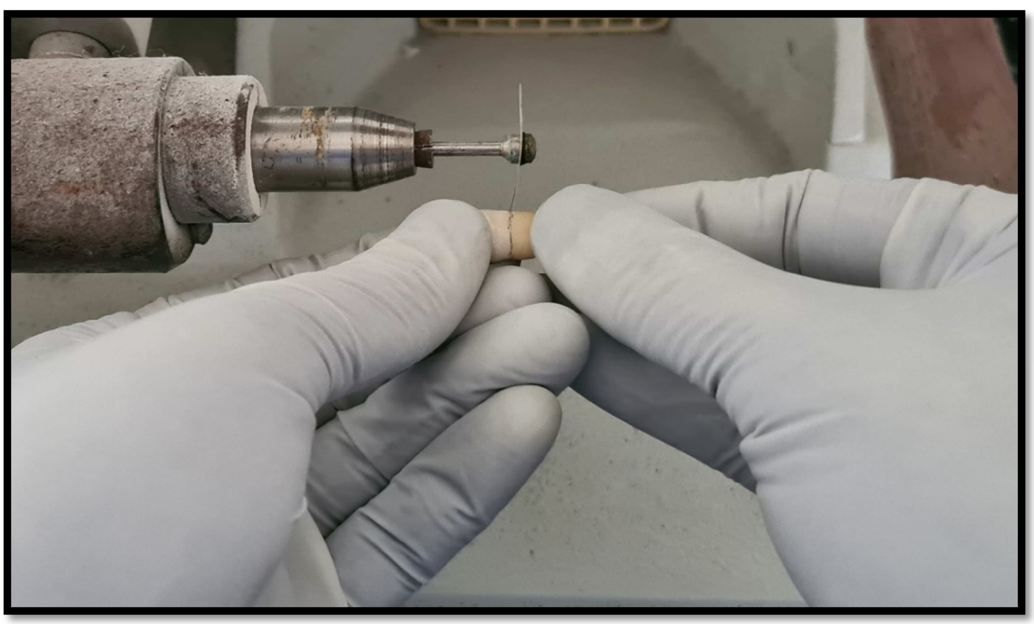


Fig 5: Decoronation

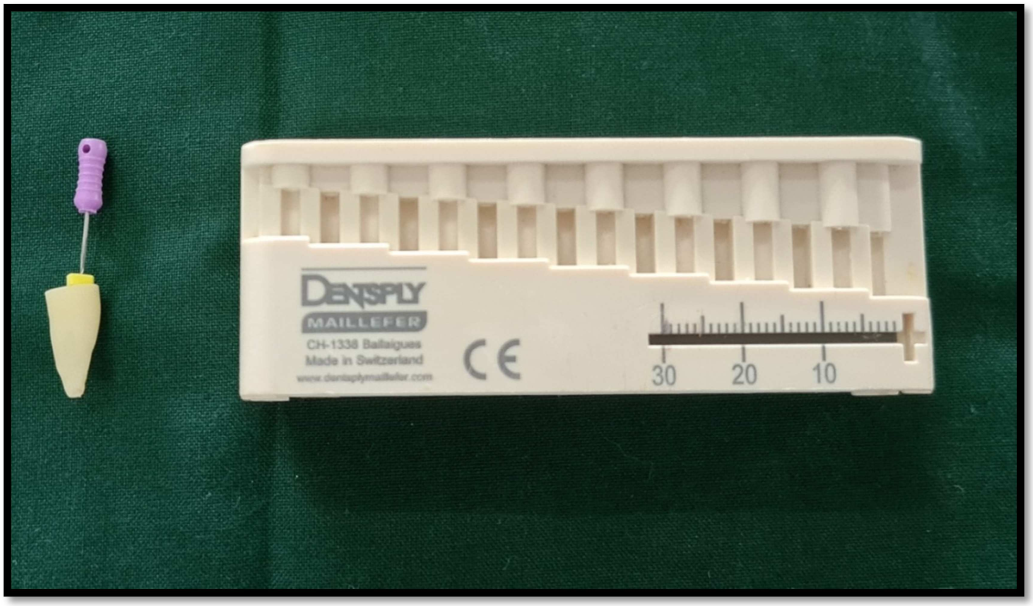


Fig 6: Working Length Determination

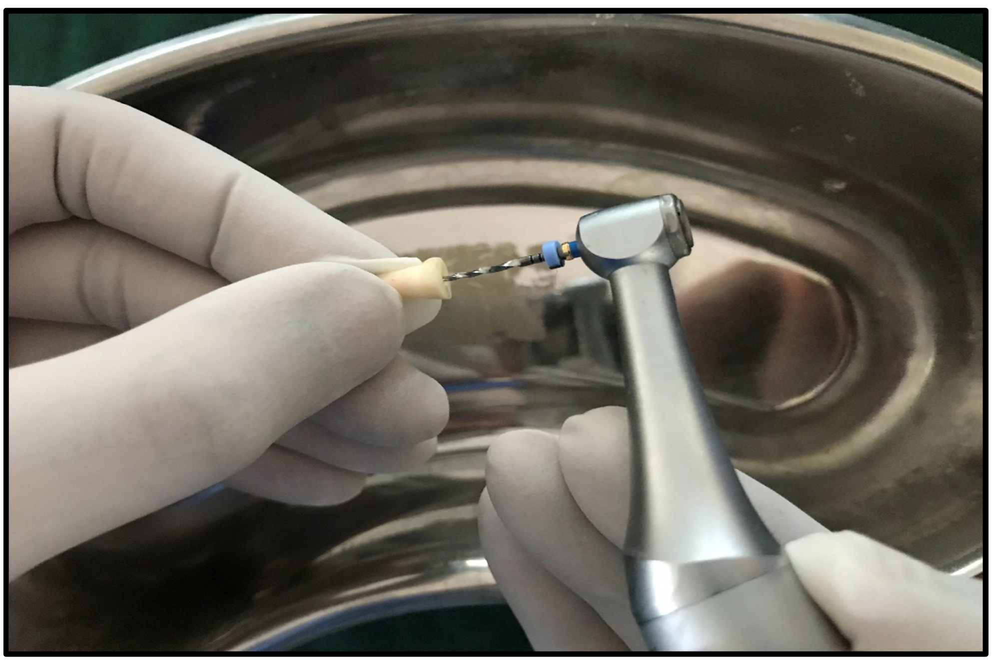


Fig 7: Bio-Mechanical Preparation

Fig 8: Irrigation

Fig 9: Irrigation activation with Diode Laser

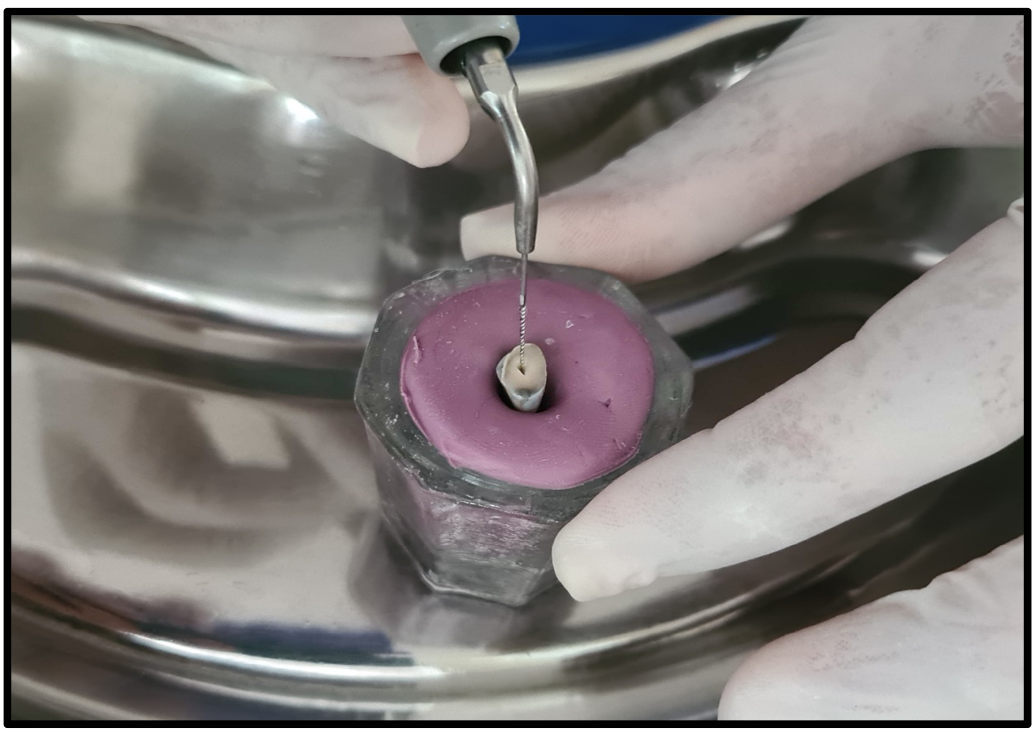


Fig 10: Irrigation activation with PUI

Fig 11: Drying canals with paper points

Fig 12: Dye incorporation in sealer

Fig 13: Obturation

Fig 14: Incubation

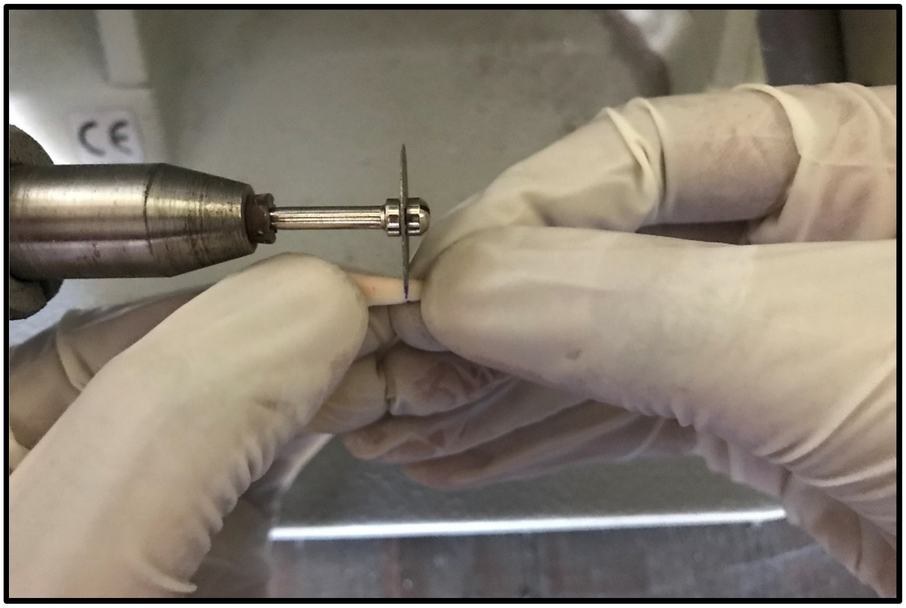


Fig 15: Sectioning

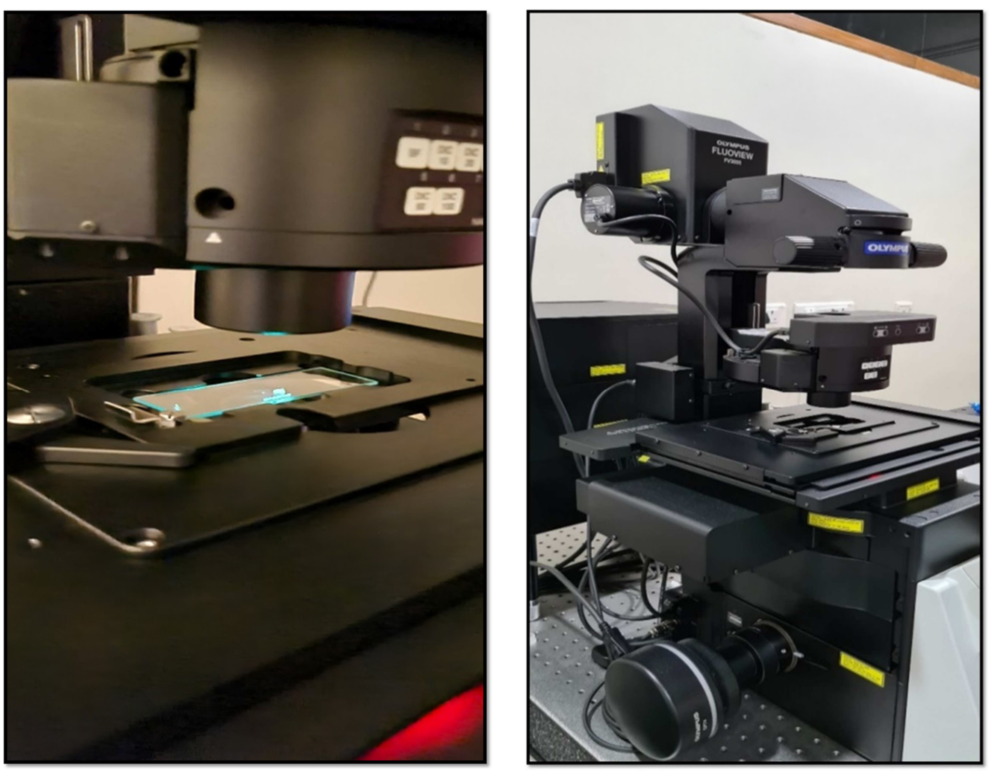


Fig 16: Confocal Laser Scanning Microscopy

RESULTS

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

Table 1 depicts, the mean depth of penetration, standard deviation and standard error of the four categories, consisting of 13 specimens per group by one-way ANOVA. 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. [Table 1]

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). 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. [Table 2 and Graph 1]

Tukey’s multiple post-hoc test was done for the comparison of the two sub groups in the apical section and highly significant difference was seen between “DIODE” (Sub group A) and “PUI” (Sub group 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. [Table 3 and Graph 2]

Table 4 represents the comparison of interactions of two main categories (Group 1 and Group 2) and two sub groups (Sub group A and Sub group B) with depth of penetration by Tukeys multiple posthoc 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, 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, highest sealer penetration was seen with HEDP with PUI activation.

Table 1: Summary of depth of penetration in two main groups (Group 1 and Group 2) and two sub groups (Sub group A and Sub group B)

Effects with level of Factor	N	Mean	Std.Dev.	Std.Err	95% CI for Mean	
					Lower	Upper
Total	52	626.57	308.24	42.74	540.76	712.39
Group 1	26	543.88	196.98	38.63	464.32	623.44
Group 2	26	709.27	375.23	73.59	557.71	860.83
Sub group A	26	453.53	145.69	28.57	394.68	512.37
Sub group B	26	799.62	332.12	65.14	665.47	933.77
Group 1 with Sub group A	13	486.78	150.32	41.69	395.94	577.61
Group 1 with Sub group B	13	600.98	226.23	62.74	464.28	737.69
Group 2 with Sub group A	13	420.28	138.68	38.46	336.47	504.08
Group 2 with Sub group B	13	998.26	305.18	84.64	813.84	1182.68

Table 2: Comparison of two main groups (Group 1 and Group 2) with depth of penetration by Tukey's multiple posthoc procedures

Groups	Group 1	Group 2
Mean	543.88	709.27
Std.Dev.	196.98	375.23
Group 1	-	
Group 2	P=0.0082,S	-

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

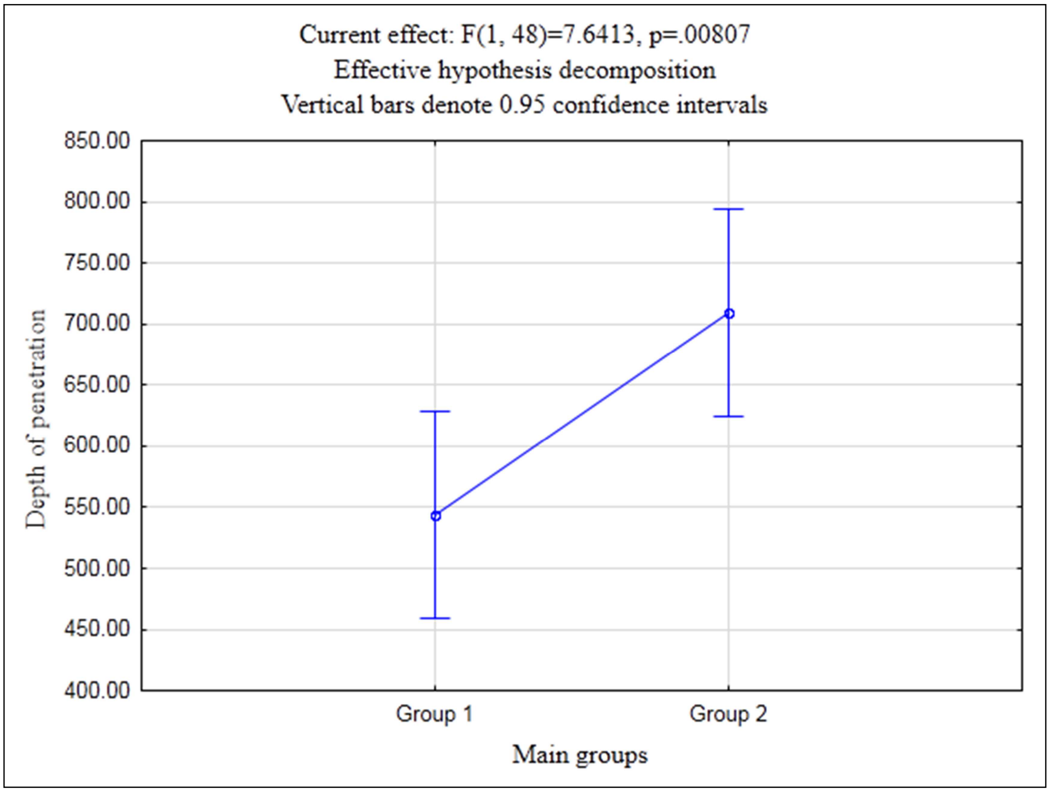


Table 3: Comparison of two subgroups (Group 1 and Group 2) with depth of penetration by Tukey's multiple posthoc procedures

Groups	Sub group A	Sub group B
Mean	453.53	799.62
Std.Dev.	145.69	332.12
Sub group A	-	
Sub group B	P=0.0001,S	-

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

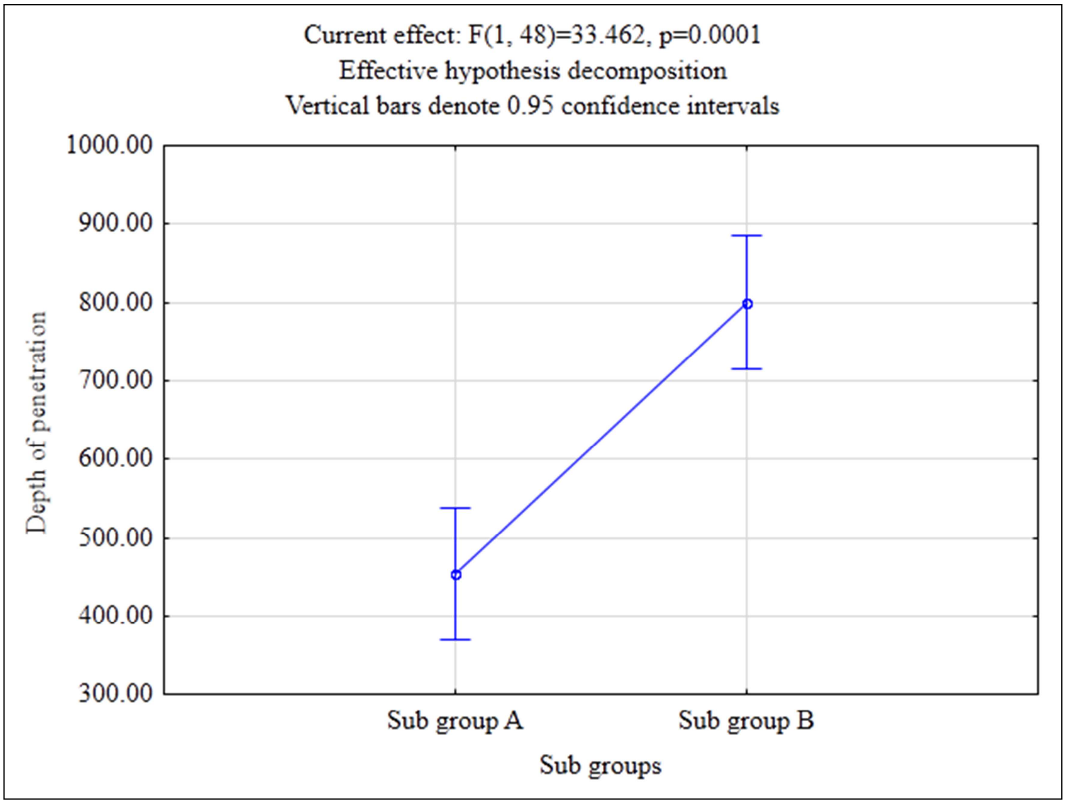
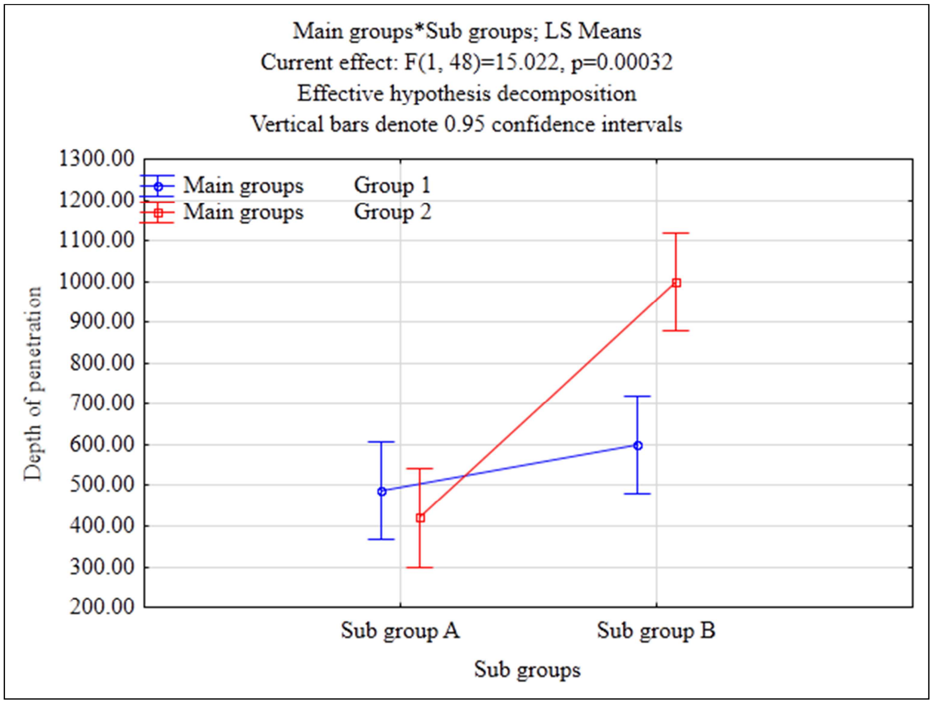
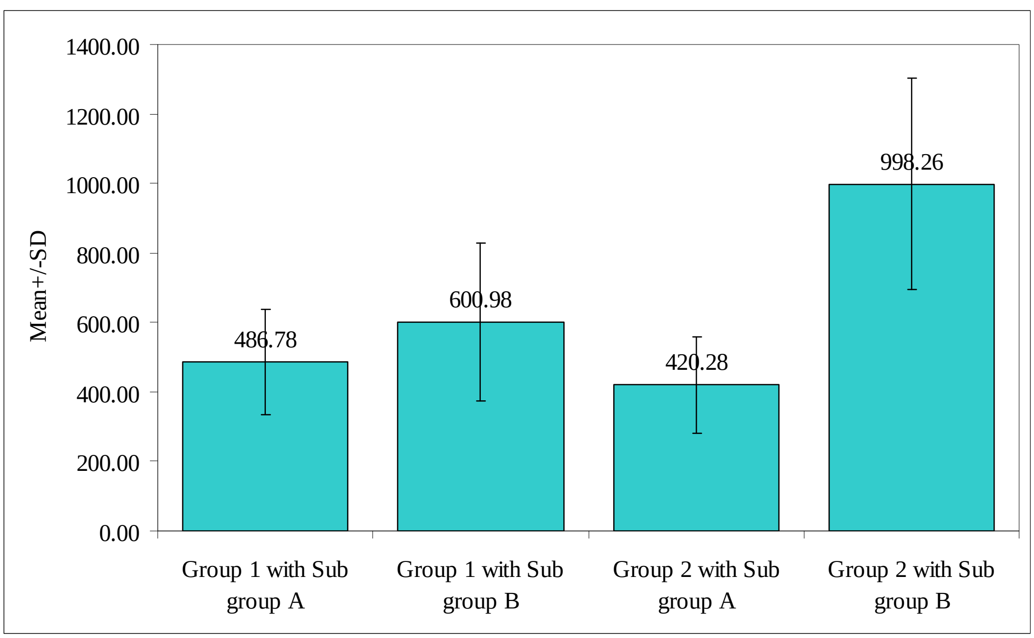


Table 4: Comparison of interactions of two main groups (Group 1 and Group 2) and two sub groups (Sub group A and Sub group B) with depth of penetration by Tukey's multiple posthoc procedures

Interactions	Group 1 with Sub group A	Group 1 with Sub group B	Group 2 with Sub group A	Group 2 with Sub group B
Mean	486.78	600.98	420.28	998.26
Std.Dev.	150.32	226.23	138.68	305.18
Group 1 with Sub group A	-			
Group 1 with Sub group B	P=0.5366,NS	-		
Group 2 with Sub group A	P=0.8605,NS	P=0.1567,NS	-	
Group 2 with Sub group B	P=0.0002,S	P=0.0003,S	P=0.0002,S	-

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





CLSM Images depicting penetration of AH Plus

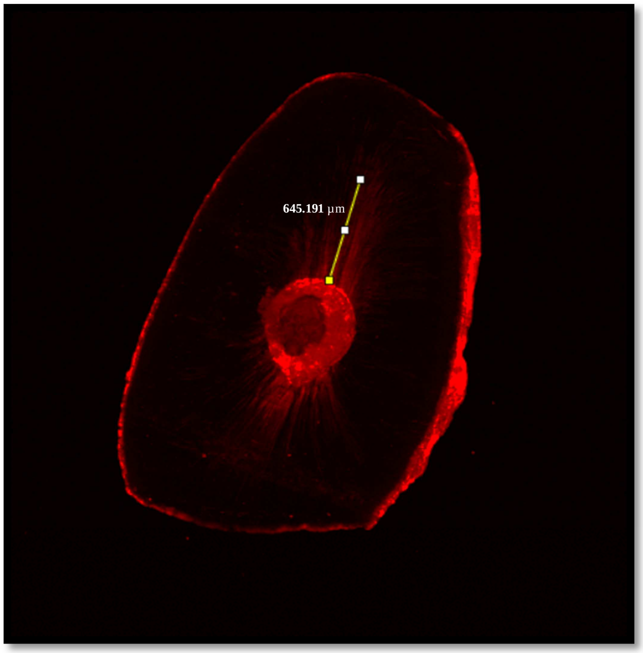


Fig: 17 CLSM image representing the depth of penetration of AH Plus sealer after EDTA and DIODE LASER activation

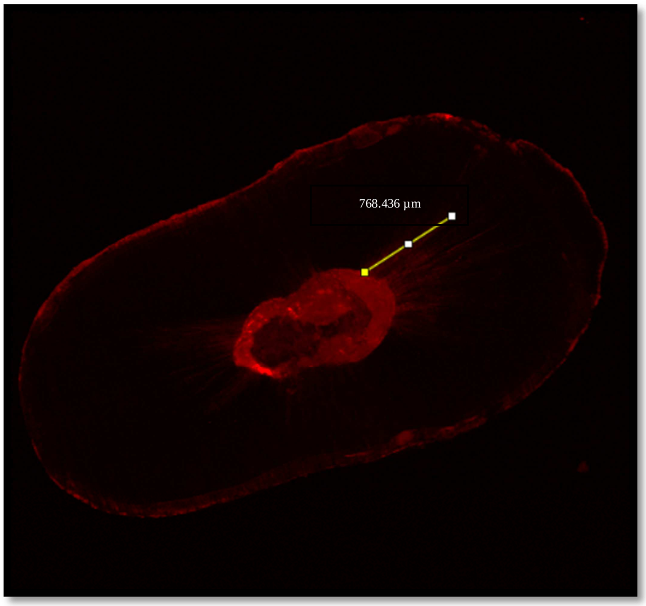


Fig: 18 CLSM image representing the depth of penetration of AH Plus sealer after EDTA and PUI activation

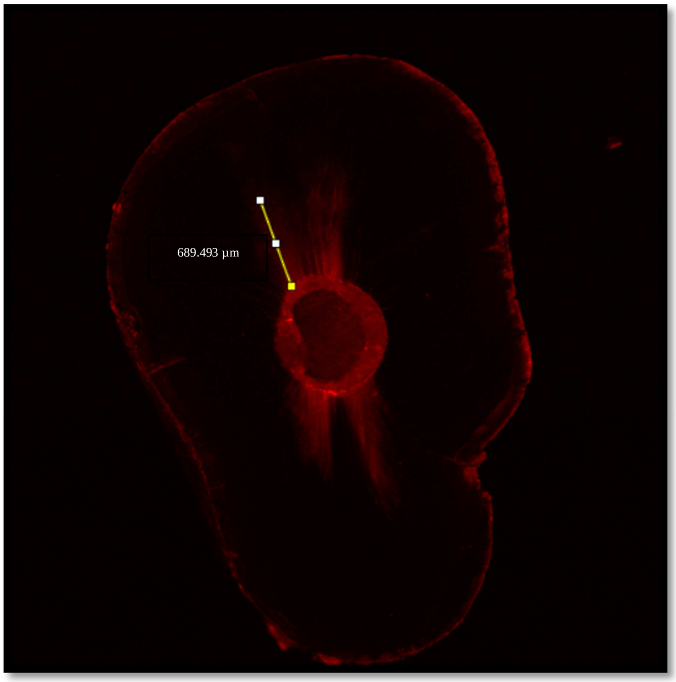


Fig: 19 CLSM image representing the depth of penetration of AH Plus sealer after HEDP and DIODE LASER activation

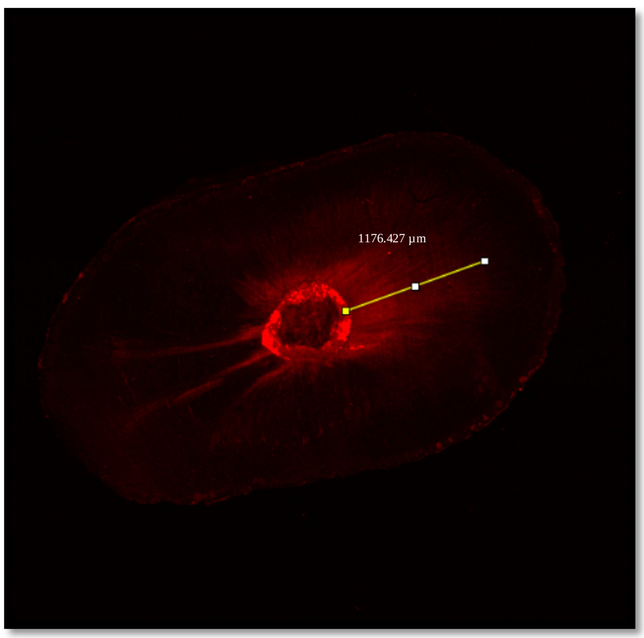


Fig: 20 CLSM image representing the depth of penetration of AH Plus sealer after HEDP and PUI activation

DISCUSSION

An important objective of root canal therapy is sterilization of the internal anatomy of the root canal. During instrumentation, a layer of debris comprising of dentin, traces of pulp tissue and 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, 14]

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. Sodium hypochlorite, (NaOCl) and ethylene diamine tetra-acetic acid (EDTA) solution are the most popularly used irrigants. Sodium hypochlorite has the ability to dissolve organic tissues and is bactericidal but not effective in removing the formed smear layer. The effective penetrability of sodium hypochlorite into the dentinal tubules is time, concentration, temperature and activation time dependent. [14, 15]

Other irrigants introduced like 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]

Multiple studies have stated that the effective removal of organic and inorganic debris is done with the help of the combination of sodium hypochlorite (2.5-5%) and EDTA (10-17%). EDTA is capable of removing smear layer because it is a Ca^{2+} chelating agent. The number of lateral canals to be filled increases as dentinal tubules open up after a final flush of EDTA. [16]

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 dentine. Changes in the diffusibility and soluble properties of human dentine is caused by any changes in Ca : P ratio which further affects the quality of root canal sealer adhesion. This results in sequalae of significant apical microleakage due to compromised sealer penetration. Moreover, EDTA and Citric Acid (component of MTAD) highly react with sodium hypochlorite, thereby making the agent incapacitated. Hence, it is essential to search a updated, biocompatible and efficient irrigating protocol during root canal procedures. [1]

Chelating agent such as HEDP, can be mixed with NaOCl. The ability to use both agents together clinically is time saving while sterilizing the root surfaces and clearing the smear layer, during the chemo-mechanical 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 attributes the to 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 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 root canal, which prevents penetration of the solution apically.[31]

In order 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. [10]

Due to their challenging anatomic variations and frequent extraction for orthodontic treatment, human mandibular premolars were chosen for the study. In order 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 bio-mechanical preparation as they possessed improved cutting efficiency and reduced torsional loading. [25, 26]

Irrigation protocol followed was- 3% sodium hypochlorite 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, 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, 27] To prevent microleakage Caviton was used over the gutta percha as a temporary restorative material.[28] 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 a period of one week.

To evaluate the tubular penetration depth in the study, Confocal Laser Scanning Microscope (CLSM) was the method of choice over SEM, due to its ability to create a 3D image, visualize sections at different levels and make depth measurement more precise. 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 like 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 microns depth. Minute amounts (0.1%) of the Rhodamine dye was needed to obtain the fluorescence and hence the sealer's properties remained unchanged. [29, 30]

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. Intra group comparison was done between the two sub groups in the apical section and highly significant difference was seen between DIODE (Sub group A) and PUI (Sub group B). In the present study, statistically 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. [15] [Table 3 and Graph 2] Literature supports that apical penetrability of irrigating solution can increase on ultrasonic agitation. [14] As oppose to the 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.[10]

The results of the past studies show that Diode Laser gave a 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.[14] Laser induces formation of cavitation-creating vapor inside the fluid. A force created on the collapse of these bubbles creates implosions that impact the root surfaces and cause surface deformation and surface material removal.[12] 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.[31]

In this present 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, 17] [Table 2 and Graph 1]. The results of this study confirm the improved efficiency of HEDP over EDTA.

In this present study 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 least efficiency seen when HEDP was activated by Diode Laser.

CONCLUSION

The irrigating solution activation techniques prominently influence the penetrability of the sealer in the dentinal tubules of the root. When penetrability of the sealer with various irrigation techniques and irrigants were evaluated, significant by greater levels of sealer penetration were attained with PUI activation of HEDP. To conclude, HEDP can be used as substitute to EDTA for smear layer removal. Taking in 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.

SUMMARY

Successful endodontic therapy is reliant on proper cleaning and debridement of the root canal system and a fluid tight impervious seal. Various newer equipments and materials have been introduced in order to achieve a highly sterile environment within the canal, resulting in successful outcomes. The concept of irrigant activation proved to enhance the efficiency of the root canal irrigating solution within the root canal as well as in complex anatomies present within the root canal system and dentinal tubules.[11]

Activation of irrigating solutions can be achieved by manual irrigation activation and also by using alternative methods such as sonic, ultrasonic, apical negative pressure irrigant system, plastic rotary files and lasers.

In this study, dentinal penetration after using EDTA and HEDP medicament has been evaluated as it has been observed that smear layer interferes with sealer penetrability. However, an association between these residues and the epoxy resin-based sealers was found which led to the aim of this study.

Decoronation of these teeth was done to achieve a standardized root length of 14±1mm with the help of a diamond disk. WL was calculated using a 10 K-file and the samples were divide into 2 groups, EDTA and HEDP group. Biomechanical preparation was completed with ProTaper Universal files up to size F3 followed by irrigation with 2 ml of 3% sodium hypochlorite between successive files. Teeth were categorized at random in 4 subgroups (n = 13) as per 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. The final rinse with 17% EDTA was done after the administration of NaOCl for the smear layer elimination. In another group, final flush was done with 1:1 mixture of 3% NaOCl+18% HEDP. Activation of irrigants was performed with Passive Ultrasonic Irrigation and diode laser. Final irrigation was performed with saline to banish the effect of the remanent oxygen from NaOCl on the polymerization of the sealers. [2, 27]

Obturation was done using a sealer labeled with 0.1% Rhodamine dye and the samples were incubated for a week. Sectioning was done for CLSM evaluation and images obtained were analyzed using Image J software.

The ‘null hypothesis’ that EDTA and HEDP on activation with diode laser and PUI did not influence the sealer penetration was rejected.

Highly significant results were obtained with HEDP after PUI activation, showing the highest penetration, followed by Group 1 subgroup B and Group 1 subgroup A. Least dentinal tubule penetration was seen with Group 2 subgroup A.

BIBLIOGRAPHY

1) Dineshkumar MK, Vinothkumar TS, Arathi G, Shanthisree P, Kandaswamy D. Effect of ethylene diamine tetra-acetic acid, MTAD™, and HEBP as a final rinse on the microhardness of root dentin. J Conserv Dent. 2012 Apr;15(2):170-3. doi: 10.4103/0972-0707.94587. PMID: 22557818; PMCID: PMC3339014.

2) Kuçi A, Alaçam T, Yavaş Ö, Ergul-Ulger Z, Kayaoglu G. Sealer penetration into dentinal tubules in the presence or absence of smear layer: a confocal laser scanning microscopic study. Journal of endodontics. 2014 Oct 1;40(10): 1627-31.

3) Sen BH, Wesselink PR, Türkün M. The smear layer: a phenomenon in root canal therapy. Int Endod J. 1995 May;28(3):141-8. doi: 10.1111/j.1365-2591.1995.tb00289.x. PMID: 8626198.

4) Emre Erik C, Onur Orhan E, Maden M. Qualitative analysis of smear layer treated with different etidronate concentrations: A scanning electron microscopy study. Microsc Res Tech. 2019 Sep;82(9):1535-1541. doi: 10.1002/jemt.23318. Epub 2019 Jun 12. PMID: 31187914.

5) Kfir A, Goldenberg C, Metzger Z, Hülsmann M, Baxter S. Cleanliness and erosion of root canal walls after irrigation with a new HEDP-based solution vs. traditional sodium hypochlorite followed by EDTA. A scanning electron microscope study. Clin Oral Investig. 2020 Oct;24(10):3699-3706. doi: 10.1007/s00784-020-03249-w. Epub 2020 May 16. PMID: 32418013.

6) Mathias zhender dual rinse for root canal clinical use

7) JOUR Kaki, Gülter Başer Kolcu, Mukadder- 2018/08/10- Effect of Dual Rinse® HEDP Root Canal Irrigation Solution On Coronal Dentin Adhesion DOI - 10.17343/sdutfd.424119

8) Zollinger A, Mohn D, Zeltner M, Zehnder M. Short-term storage stability of NaOCl solutions when combined with Dual Rinse HEDP. Int Endod J. 2018 Jun;51(6):691-696. doi: 10.1111/iej.12875. Epub 2017 Dec 5. PMID: 29121393

9) Kumar VR, Bahuguna N, Manan R. Comparison of efficacy of various root canal irrigation systems in removal of smear layer generated at apical third: An SEM study. J Conserv Dent. 2015 May-Jun;18(3):252-6. doi: 10.4103/0972-0707.157267. PMID: 26069415; PMCID: PMC4450535.

10) Mozo S, Llena C, Forner L. Review of ultrasonic irrigation in endodontics: increasing action of irrigating solutions. *Med Oral Patol Oral Cir Bucal*. 2012;17(3):e512-e516. Published 2012 May 1. doi:10.4317/medoral.17621

11) Abraham S, Vaswani SD, Najan HB, Mehta DL, Kamble AB, Chaudhari SD. Scanning electron microscopic evaluation of smear layer removal at the apical third of root canals using diode laser, endoActivator, and ultrasonics with chitosan: An *in vitro* study. J Conserv Dent. 2019 Mar-Apr;22(2):149-154. doi: 10.4103/JCD.JCD_337_18. PMID: 31142984; PMCID: PMC6519194.

12) Hmud R, Kahler WA, George R, Walsh LJ. Cavitational effects in aqueous endodontic irrigants generated by near-infrared lasers. J Endod. 2010 Feb;36(2):275-8. doi: 10.1016/j.joen.2009.08.012. Epub 2009 Dec 4. PMID: 20113789.

13) S, Llena C, Forner L. Review of ultrasonic irrigation in endodontics: increasing action of irrigating solutions. Med Oral Patol Oral Cir Bucal. 2012;17(3):e512-e516. Published 2012 May 1. doi:10.4317/medoral.17621

14) Rajakumaran A, Ganesh A. Comparative Evaluation of Depth of Penetration of Root Canal Irrigant After Using Manual, Passive Ultrasonic, and Diode Laser-

Assisted Irrigant Activation Technique. J Pharm Bioallied Sci. 2019;11(Suppl 2):S216-S220. doi:10.4103/JPBS.JPBS_300_18

15) Amin K, Masoodi A, Nabi S, Ahmad P, Farooq R, Purra AR, Ahangar FA. Effect of diode laser and ultrasonics with and without ethylenediaminetetraacetic acid on smear layer removal from the root canals: A scanning electron microscope study. J Conserv Dent. 2016 Sep-Oct;19(5):424-7. doi: 10.4103/0972-0707.190005. PMID: 27656060; PMCID: PMC5026101

16) Kuruvilla A, Jaganath BM, Krishnegowda SC, Ramachandra PK, Johns DA, Abraham A. A comparative evaluation of smear layer removal by using edta, etidronic acid, and maleic acid as root canal irrigants: An in vitro scanning electron microscopic study. J Conserv Dent. 2015;18(3):247-251. doi:10.4103/0972-0707.157266

17) Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. Int Endod J. 2009 Apr;42(4):335-43. doi: 10.1111/j.1365-2591.2008.01514.x. Epub 2009 Feb 7. PMID: 19220516.

18) Lagemann M, George R, Chai L, Walsh LJ. Activation of ethylenediaminetetraacetic acid by a 940 nm diode laser for enhanced removal of smear layer. Aust Endod J. 2014 Aug;40(2):72-5. doi: 10.1111/aej.12039. Epub 2013 Aug 6. PMID: 25244221

19) Caron G, Nham K, Bronnec F, Machtou P. Effectiveness of different final irrigant activation protocols on smear layer removal in curved canals. J Endod. 2010 Aug;36(8):1361-6. doi: 10.1016/j.joen.2010.03.037. Epub 2010 May 13. PMID: 20647097.

20) Kuah HG, Lui JN, Tseng PS, Chen NN. The effect of EDTA with and without ultrasonics on removal of the smear layer. Journal of endodontics. 2009 Mar 1;35(3):393-6.

21) Kamble AB, Abraham S, Kakde DD, Shashidhar C, Mehta DL. Scanning electron microscopic evaluation of efficacy of 17% ethylenediaminetetraacetic acid and chitosan for smear layer removal with ultrasonics: an in vitro study. Contemporary clinical dentistry. 2017 Oct;8(4):621.

22) Wang X, Sun Y, Kimura Y, Kinoshita JI, Ishizaki NT, Matsumoto K. Effects of diode laser irradiation on smear layer removal from root canal walls and apical leakage after obturation. Photomedicine and laser surgery. 2005 Dec 1;23(6):575-81.

23) Ciucchi B, Khettabi M, Holz J. The effectiveness of different endodontic irrigation procedures on the removal of the smear layer: a scanning electron microscopic study. International Endodontic Journal. 1989 Jan;22(1):21-8.

24) Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. International endodontic journal. 2003 Dec;36(12):810-30.

25) Uygun AD, Kol E, Topcu MK, Seckin F, Ersoy I, Tanriver M. Variations in cyclic fatigue resistance among ProTaper Gold, ProTaper Next and ProTaper Universal instruments at different levels. International endodontic journal. 2016 May;49(5):494-9.

26) Nishad SV, Shivamurthy GB. Comparative analysis of apical root crack propagation after root canal preparation at different instrumentation lengths using ProTaper universal, ProTaper next and ProTaper gold rotary files: an in vitro study. contemporary clinical dentistry. 2018 Jun;9(Suppl 1):S34.

27) Balguerie E, van der Sluis L, Vallaeys K, Gurgel-Georgelin M, Diemer F. Sealer penetration and adaptation in the dentinal tubules: a scanning electron microscopic study. Journal of endodontics. 2011 Nov 1;37(11):1576-9.

28) Sivakumar JS, Kumar BN, Shyamala PV. Role of provisional restorations in endodontic therapy. Journal of pharmacy & bioallied sciences. 2013 Jun;5(Suppl 1):S120.

29) Perdigao JO, Lambrechts P, Vanherle G. Microscopy investigations: techniques, results, limitations. Am. J. Dent. 2000;13:3D18D.

30) Tedesco M, Chain MC, Bortoluzzi EA, Garcia LD, Alves AM, Teixeira CS. Comparison of two observational methods, scanning electron and confocal laser scanning microscopies, in the adhesive interface analysis of endodontic sealers to root dentine. Clinical oral investigations. 2018 Jul;22(6):2353-61.

31) Jambagi N, Kore P, Dhaded NS, Patil SA, Shankar M. Comparison of Antimicrobial Efficacy of Diode Laser, Ultrasonic Activated and Conventional Irrigation with 2.5% NaOCl during RCT: An Interventional Study. J Contemp Dent Pract. 2021 Jun 1;22(6):669-673. PMID: 34393125.

ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC   Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362   Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640   E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1473

CERTIFICATE

This is to Certify that the synopsis titled "Comparitive evaluation of depth of penetration of an epoxy resin based sealer following a final rinse of 17% EDTA and 18% HEDP with diode laser and PUI: an in-vitro CLSM - study" *Submitted by Dr. _____ _____ P. G. Student / Staff, Guided by _____ from Department of* Department of Conservative Dentistry & Endodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE II

BIOSTATISTICS CLEARANCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)
0831-2470362 **Web**: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 **E-mail**: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Dr….** ………., **Post Graduate Student**, under the guidance of **Dr.** ………..M.D.S, **Professor, Department of** CONSERVATIVE DENTISTRY AND ENDODONTICS………., entitled "COMPARATIVE EVALUATION OF THE DEPTH OF PENETRATION OF AN EPOXY RESIN BASED SEALER FOLLOWING A FINAL RINSE OF 17% EDTA AND 18% HEDP, WITH DIODE LASER & PUI ACTIVATION" AN IN-VITRO CONFOCAL LASER SCANING MICROSCOPY STUDY. has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 27/09/2022

Name & Signature of Biostatistician
(Dr. S. B. Javali)

ANNEXURE III

PLAGIARISM CHECK CERTIFICATE

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State

Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Date : 26|12|2022 Serial No. : 138

PLAGIARISM CHECK REPORT

Name of the Applicant :
UG / PG / Ph.D / Staff : Post graduate student
Batch & Year : 2020 - 2023
Department : conservative Dentistry

The soft copy of Research Work / Manuscript by ..... . entitled "comparative evaluation of depth of penetration of an epoxy resin based sealer following a final rinse of 17% EDTA and 18% HEDP with diode laser and PUI an in vitro CLSM - study" under the guidance of ……... ……….has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of ....9..........%, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi