KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

BELAGAVI, KARNATAKA

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT AND PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"COMPARATIVE EVALUATION OF THE EFFECT OF DIFFERENT FINAL IRRIGATION REGIMENS 1:1 MIXTURE OF 3% NAOCL+18% HEDP AND 17% EDTA ON THE DENTINAL TUBULE PENETRATION OF TWO DIFFERENT ROOT CANAL SEALERS: A CONFOCAL LASER SCANNING MICROSCOPIC STUDY"** is a bonafide research work done by **REG. NO. IE0221002**

Head of Department

Dr. SONAL B. JOSHI M.D.S
Dean & Head of Department,
Department of Conservative Dentistry and Endodontics,
KAHER's V K Institute of Dental Sciences,
Belagavi - 590010
Date: 23rd APRIL 2024
Place: Belagavi

Principal

Dr. ALKA KALE M.D.S.
Principal,
KAHER's V K Institute of Dental Sciences, Nehru Nagar,
Belagavi-590010.
Date: 23/4/24
Place: Belagavi

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

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	**mL**	Milliliter
10	**° C**	Degrees Celsius
11	**mm**	Millimeter
12	**hrs**	Hours
13	**min**	Minutes
14	**n**	Number of specimens
15	**p-value**	Probability value
16	**i.e.**	That is

17	**nm**	Nanometer
18	**<**	Less than
19	**>**	Greater than
20	**SE**	Standard error
21	**PM**	Premolar
22	**PUI**	Passive ultrasonic irrigation
23	**WL**	Working Length
24	**SC**	Single Cone
25	**NS**	Not significant
26	**DW**	Distilled Water
27	**PP**	Paper Point
28	**GP**	Gutta Percha

ABSTRACT

Aim- To evaluate the effect of 17% EDTA and 1:1 mixture of 3% NaOCl+18% HEDP as final irrigating solution on the maximum depth of penetration of an epoxy resin-based sealer (AH Plus) and BIOACTIVE GLASS sealer into the dentinal tubules at the apical areas of root canals using Confocal Laser Microscopic Study.

Methodology: Eighty 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 F2/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= 20 according to the intervention. Passive ultrasonic irrigation was used to activate the irrigants. Final irrigation was performed with distilled water. The teeth were obturated using AH plus sealer and Bioactive glass sealer coated with rhodamine dye. Depth of sealer penetration into dentinal tubules was assessed using Confocal Laser Scanning Microscope

Results – Highly significant difference was seen between the groups with EDTA and HEDP, with EDTA + AH plus group demonstrating the highest penetration. The least penetration was seen with HEDP + BIOACTIVE sealer group

Conclusion- Type of irrigating solution and flowability of sealer significantly influence the penetration of sealer into root dentinal tubules. When penetration of sealer with different irrigants were evaluated significant greater level of sealer penetration were attained with EDTA and HEDP respectively.

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

INTRODUCTION

The long-term success of root canal therapy relies remarkably upon termination of microorganism by-products[1]. This is accomplished through chemo-mechanical disinfection and the appropriate use of antimicrobial sealers in the root canal ecosystem which involves the use of mechanical instruments and antimicrobial reagents to sculpt the canal's ecosystem[2,3].

Smear layer removal plays a crucial role for optimal root canal filling retention. Sealer penetration into dentin tubules post-removal of said layer indicates successful smear layer elimination[4]. This enhances antibacterial efficacy of reagents entering the canal[5]. Auxiliary chelating solutions aid in smear layer removal during final root canal irrigation with NaOCl as a dominant irrigant.

Various chelating solutions for aid of smear layer removal including organic acids like citric acid and inorganic acids such as ethylenediamine tetraacetic acid (EDTA) are incorporated[6]

However, studies indicate that EDTA has shortcomings in the apical third of the root canal in terms of dentinal surface loss due to its strong demineralizing effect[7]. Prolonged EDTA contact leads to dentinal tubule enlargement, dentin softening, and collagen fibre denaturation, prompting researchers to seek alternatives to EDTA due to its erosive and toxic effects on dentinal and periapical tissues[8,9].

To overcome this hurdle, a novel endodontic chelating irrigant called 1-hydroxyethylidene 1,1-diphosphonate (HEDP) is devised. HEDP has comparable potential in devouring smear layer without contradicting NaOCl[10].

The novel transient HEDP competence with sodium hypochlorite (NaOCl) allows for the combined use of NaOCl/HEDP irrigation root canal therapy. Importantly, HEDP doesn't contradict NaOCl's capability of proteolysis. Continuous chelation, secured notable traction in endodontics in recent years[11,12].

Ultrasonic energy has been utilized in endodontics for a significant period to clean the root canal and aid in disinfection. (PUI) involves activating the irrigant solution through acoustic micro streaming generated by an oscillating file or smooth wire[13]. (PUI) in synergism with acoustic micro streaming operates by to and fro vigorous motion files or smooth wires at 30kHz frequency[14]. The resulting rapid vortex-like motion of the liquid and cavitation, lead to the formation of spontaneous cavities throughout the liquid, enhancing the penetration of irrigants in canal wall ecosystem.

The triumph of root canal treatment compiles three stages: shaping and cleaning, disinfection, and 3D hermetic seal. To achieve proper obturation, it is crucial to eliminate any space amongst canal wall and the core material. Root canal sealers play a critical role in this process[15].

Resin sealers showcased better results compared to zinc oxide-based sealers[16]. Epoxy resin based sealers in conjunction with AH Plus, are known for their desirable properties such as greater dimensional stability, low solubility rates, high radio-opacity, and optimal adhesiveness to the root dentin[17,18,19].

There are three basic types of bioceramics available: bioinert, bioactive, and bioresorbable ceramics. Bioactive glass, a type of bioceramic-based sealer, novel biomaterial for root canal core in endodontics. It is a second generation bioceramic sealer and offers various bioactivities, including biocompatibility and the ability to expedite the regeneration of periapical tissues. This sealer contains a specific composition of Na_2O-CaO-SiO_2-P_2O_5 glass. The bioceramic sealer's performance is elucidated by small particle size, hydrophilicity, and low contact angle with spread across the root canal's dentin. This creates a gap-free chemical contact between the sealer and the dentinal walls, making it constructive, sealer wise[20].

The depth of sealer penetration relies on both physical and chemical properties of the sealers, making it essential to compare their penetrability for informed material selection[17]. Currently, no study has compared the tubular ecosystem penetration of bioactive sealer in

conjunction epoxy resin-based sealer, AH Plus, using (17% EDTA and equal proportion of 18 percentage HEDP with Hypochlorite) as final irrigating solutions.

Thus, the study aims to assess the impact of 17% EDTA and equal proportion of 18 percentage HEDP as final irrigating solutions on optimum sealer discernment of AH Plus and bioactive glass sealer in tubule at the culminating (apical) regions using CLSM.

AIMS AND OBJECTIVES

AIM OF STUDY

To evaluate and compare the effect of 17% EDTA and 1:1 mixture of 3% NaOCL+18% HEDP as a final irrigating solution on sealer discernment of two root canal sealers: Confocal Laser Scanning Microscopic Study

OBJECTIVES

- To evaluate the dentinal tubule discernment of an epoxy resin-based sealer after using different final irrigating solutions (17% EDTA and 1:1 mixture of 3% NaOCl+18% HEDP)
- To evaluate the dentinal tubule penetration of a bioactive glass sealer after using different final irrigating solutions (17% EDTA and equal proportion of 18 percentage HEDP with three percentage Hypochlorite)
- To compare the dentinal discernment of an epoxy resin-based sealer with bioactive glass sealer after using different final irrigating solutions (17% EDTA and 1:1 mixture of 3% NaOCl+18% HDP)

HYPOTHESIS

NULL HYPOTHESIS:-

There will be no disparity in the effect of 17% EDTA and equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite as final irrigating solution on depth of sealer penetration of epoxy resin based sealer and bioactive glass sealer.

ALTERNATE HYPOTHESIS:-

There will be difference in the effect of 17% EDTA and equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite as final irrigating solution on depth of sealer discernment of epoxy resin based sealer and bioactive glass sealer.

REVIEW OF LITERATURE

1. A study by Ayat H. Adham comparing continuous chelation (CC) with sequential chelation (SC) protocols using different irrigants found comparable effectiveness in smear layer eradication across overall thirds of the canal wall. However, CC bespeak horrendous (POBS) of Bio-C sealer compared to SC. SEM analysis showed clean dentin in coronal and middle thirds but smeared dentin in the apical third. HEDP showcased superior smear layer termination in the apical third.POBS was highest with NaOCl\HEDP irrigation, while NaOCl alone showed the least effectiveness. EDTA had lower POBS than HEDP, possibly due to its effect on sealer setting reaction. Modes of failure were predominantly mixed. However, the study's in vitro nature limits generalization to clinical settings, and dynamic load simulations are needed for a more realistic assessment[21]
2. In accordance to Germain Sfeir et al targeted the impact of irrigation protocols on the anchoring of Total Fill Sealer® (CSBS) when measured with AH Plus Jet®. EDTA/Total Fill Sealer® and NaOCl/Total Fill Sealer® demonstrated superior POBS compared to HEDP/Total Fill Sealer®. POBS being superior in the apex than in the upper third, with superior SD The study emphasized the importance of POBS in assessing endodontic sealer. Different irrigation protocols influenced POBS, with EDTA activation resulting in comparable POBS for both sealers, while HEDP activation showed the best results for AH Plus Jet®[22].
3. A study by Aishwarya Sanjay Awati et al stated that NaOCl and EDTA are commonly used irrigants, but newer methods like MTAD offer enhanced smear layer removal. Chelating agents like HEDP, mixed with NaOCl, provide effective smear layer removal without compromising NaOCl's properties. Activation techniques such as passive ultrasonic irrigation (PUI) enhance sealer penetration, with HEDP activated by PUI showing the deepest penetration. In contrast, diode laser activation showed the least penetration, possibly due to low power. HEDP outperformed EDTA in terms of sealer

penetration, suggesting its potential as an alternative. Further in vivo studies are needed to validate these findings and explore different concentrations and activation techniques for HEDP[23]

4. A study by Ayat H. Adham concluded that Continuous chelation involves using a mix of HEDP and NaOCl throughout root canal preparation, maintaining NaOCl's antimicrobial activity. HEDP, a biocompatible chelator, forms an all-in-one solution with NaOCl, simplifying irrigation and enhancing debris removal. Electronic searches found relevant papers from 2005 to 2022, favoring English-language articles. This approach offers a single solution for cleansing and decontamination, saving time and enhancing root canal wall conditioning. Continuous chelation facilitates the clinical procedure and improves debris removal, benefiting endodontic treatments. The combination of HEDP with NaOCl offers a promising alternative to sequential irrigation with NaOCl followed by EDTA. Sodium hypochlorite/Dual Rinse HEDP may serve as a viable substitute for NaOCl + EDTA due to its superior antibacterial effect[24].
5. A study by Shalan Kaul et al stated that fate of canal sealing is to cease microorganism ingress, with TotalFill BC sealer showing the least leakage due to its small particle size and hydrophilicity. GuttaFlow synergize sealer and gutta-percha, containing nanosilver for antimicrobial properties. AH Plus, an epoxy-based sealer, exhibited higher leakage, possibly due to inadequate bonding with gutta-percha. Dye penetration studies revealed BC sealer had the best sealing ability, followed by GuttaFlow and AH Plus. However, all sealers showed some level of leakage, indicating incomplete apical sealing. Further in vivo studies are needed to confirm these findings and assess their clinical efficacy in preventing bacterial ingress into root canals [25].
6. In accordance to Nina Novozhilova et al the impact of EDTA plus HEDP on sodium hypochlorite (SH) activity against C. albicans and E. faecalis. HEDP formulations, except those with 15% and 18% ID, showed no significant decrease in SH activity against C. albicans. The effect on E. faecalis was minimal, with SH + ID15% and SH + ID18% showing no effect. Different studies have varied results on HEDP's effect, with some

suggesting superior activity against E. faecalis compared to EDTA. pH changes influenced antibacterial activity, with solutions of higher pH showing greater effectiveness. HEDP formulations, particularly SH + DR9%, demonstrated significant effectiveness against both microorganisms, with outcomes varying by concentration and manufacturer. However, these findings are limited to in vitro conditions and further research is needed to assess clinical applicability [26].

7. Takashi Komabayashi et al stated that sealers for root canal treatment (NSRCT) have many combinations and effects. The article categorises types of endodontic sealers used today, by their setting reaction type, contents, and effects on canal: ZOE, methacrylate resin sealers based, GIC based, silicone based, epoxy resin based, tricalcium silicate based. Time taken to set, solubility, ability to seal, antimicrobial, and cell toxicity are aspects that define a good sealer. The microleakage of all the sealers in the article was measured by way of a meta-analysis. AH Plus kept as a control, tricalcium silicate show least microleakage. Tricalcium silicate sealers also showed most favorable antimicrobial effect and best biocompatibility. Future sealers must add a hermetic seal with other effects [27].

8. A study by Talita Tartari investigated the superiority of contrasting irrigation protocols on the long-term bond strength (BS) of AH Plus sealer. Various irrigation solutions were tested, affecting dentin surface characteristics and subsequently BS. Groups using NaOCl + EDTA and NaOCl + HEDP showed the highest initial BS values, while NaOCl alone yielded the lowest.. These results were attributed to differences in dentin surface preparation and interaction with the sealer. Overall, protocols promoting clean surfaces with high roughness and wettability achieved the best long-term BS results, emphasizing the importance of understanding adhesive interfaces in endodontic treatment [28].

9. The study by Lamiaa A. Ibrahim explored irrigation protocols' impact on root canal sealers' adaptation. A 3% NaOCl concentration was used to balance antimicrobial efficacy with tissue toxicity. Dual Rinse HEDP with NaOCl maintained NaOCl's activities while preventing adverse reactions. Sealer adaptation and adhesion were

affected by smear layer removal and tubule penetration. EndoSeal MTA sealers exhibited uniform adaptation regardless of irrigation, owing to self-adhesiveness and hydroxyapatite formation. Adseal sealer showed fewer marginal gaps with NaOCl \ Dual Rinse HEDP irrigation. The study contradicted findings on epoxy resin vs. bioceramic sealers' similarity, attributing it to different study parameters. Conclusion: Endoseal MTA provided superior adaptation, while Adseal benefited from NaOCl \ Dual Rinse HEDP irrigation for optimal adaptation [29].

10. The study by Raffaella Castagnola evaluated Triton, a new endodontic irrigating solution, compared to Dual Rinse HEDP in synergism with NaOCl/EDTA. Triton showed superior efficacy in debris removal across root canal thirds and equivalent efficacy to NaOCl/EDTA in smear layer removal. It also exhibited the highest antimicrobial efficacy averse to C. albicans, followed by Dual Rinse HEDP and NaOCl/EDTA. All solutions effectively reduced E. faecalis. While Triton's continuous chelation approach proved promising for antimicrobial activity and debris removal, further studies are warranted to explore its performance under different conditions. Limitations include in vitro design and mono-species biofilm testing. Nonetheless, Triton offers a potential alternative to current irrigation protocols for enhanced antimicrobial and debris removal effectiveness [30].

11. The study by Poornika Gandhi et al gauged resin sealer wettability and BioRoot RCS sealers with different irrigating solutions. Maleic acid (MA) demonstrated superior wettability compared to other solutions, attributed to its strong chelating action and acidic nature. SmearOFF, a combination of EDTA, chlorhexidine, and surfactants, showed good wettability due to its surfactant content. EDTA exhibited poorer wettability than MA but better than Dual Rinse® HEDP and distilled water, attributed to its high pH and limited chelating effectiveness. Dual Rinse® HEDP showed the lowest wettability results due to its weak chelating properties, although it improved wettability compared to distilled water for AH Plus sealer. Controlled volume and standardized environmental conditions were maintained for accurate measurements. Overall, MA as a final irrigant

demonstrated the best wettability for both AH Plus and BioRoot RCS sealers in this study.

12. The study by Aastha Dureja et al evaluated the wettability of CeraSeal and AH Plus sealers with different chelating agents. Contact angle measurements were used to assess wettability, with smaller angles indicating better adhesion. Controlled sealer volumes and standard environmental conditions were maintained for accurate measurements. Surface roughness was standardized using silicon carbide paper. CeraSeal exhibited optimal wettability with 9% HEDP due to its minimal dentin damage and increased surface energy. AH Plus showed best wettability with 7% MA, attributed to its strong demineralization effect and increased surface roughness. AH Plus demonstrated superior wettability compared to CeraSeal, possibly due to its longer setting time and higher creep capacity. Clinical variations may affect contact angle values, necessitating further clinical studies for validation. Overall, 9% HEDP and 7% MA were identified as the most favorable final irrigants for CeraSeal and AH Plus sealers, respectively [32].

13. In accordance to study Twin Kleen and NaOCl + EDTA irrigation protocols, finding greater sealer discernment depth with Twin Kleen, AH Plus along with Perma Evolution sealers showed deep discernment in dentin channels, attributed to favorable properties, physically. Being highest at highest 1/3rd of canals, enhanced smear layer removal and greater tubular density. Perma Evolution displayed superior sealer discernmrnt in medial and distal end, possibly due to its unique microcapsule technology and hydrophilic composition. Group 2 exhibited superior discernment depth compared to Group 1 throughout the canal. Overall, Twin Kleen demonstrated superior sealer discernment compared to NaOCl + EDTA, while AH Plus and Perma Evolution were at par in the upper third but 2nd sealer had deeper extension in the lower two thirds [33]

14. The study by Dr. Talita Tartari et al investigated the effects of various agents attacking inorganic content and mixtures of reagents on dentine content, rejecting the null hypotheses. Different agents altered dentine composition at varying rates, with HEDP and EDTANa4 causing minor demineralization, while EDTAH Na3 and PAA resulted

in significant demineralization, depending on concentration and immersion time. NaOCl degraded collagen more rapidly when exposed, and different reagent mixtures produced dentin with various exposed chemical groups matching various adhesion types with dental sealers. Treatment with Hypo and agents acting on inorganic content can manipulate dentine surface for adhesion with sealers, influencing sealing ability and microbial adhesion. Further research should analyze the impact of dentine [34]

15. A study by S. Deari et al, investigated the influence of pH and sodium content upon fruitfulness of EDTA and HEDP, removing smear layer from dentine. A combination of atomic absorption spectroscopy (AAS) and optical measurements was used for robust analysis. While EDTA proved to be a stronger chelator than HEDP, higher pH solutions, caused by tetrasodium salts, reduced their decalcifying effects. Despite efforts to standardize dentine samples, variance remained high due to dentine heterogeneity. 3D laser scanning microscopy was employed for sample analysis, providing advantages over SEM. The study highlights the importance of pH and salt composition in chelating effectiveness. Future investigations might prioritize AAS analysis for comparative assessments of such agents. Overall, EDTA exhibited superior calcium chelation and smear layer removal compared to HEDP [35].

16. A study by Nidambur Vasudev Ballal et al stated that tricalcium silicate-based sealers, widely used in radicular ecosystem treatment, pose challenges for complete removal due to their bonding and interaction with dental tissues. Activated irrigation methods, like Er:YAG laser and ultrasonics, were employed to enhance removal efficacy, but none achieved complete removal. Factors affecting removal included activation method, irrigation solution, and sealer type. Er:YAG laser activation resulted in higher amounts of residual sealer compared to ultrasonics, possibly due to differing mechanisms. Maleic acid and Dual Rinse HEDP, galavanised utilising ultrasonics, showed better sealer removal, consistent with their reported higher smear layer removal capacities. However, direct comparison is limited due to lack of knowledge on their effect specifically on

tricalcium silicate-based sealers. Further research, possibly utilizing advanced imaging techniques like micro-CT, is warranted to gauge the effectiveness while eradicating these sealers from root canals [36].

17. A study by Betul Aycan Alim Uysal et al stated that various microscopy techniques, including SEM, TEM, stereomicroscopy, and CLSM, have been utilized to study the sealer-dentin interface. The Cytation 5 reader, combined with Gen5 software, offers advantages in rapid imaging and data analysis, though it's not been previously used in dentistry for sealer penetration studies. EDTA, commonly used as a chelation solution, has limitations in removing the entire smear layer, leading to exploration of alternative irrigation solutions. MA, less abrasive to dentine, and HEBP, compatible with NaOCl, have been investigated. While previous studies found differences in sealer penetration depths, our study revealed no significant disparities among sealer types. However, EndoSequence BC Sealer exhibited greater penetration depth overall. Penetration depths were higher in coronal thirds due to thinner and less sclerosed dentin compared to apical thirds. Future research should consider anatomical complexities and alternative obturation techniques for comprehensive evaluation. EndoSequence BC Sealer displayed superior penetration, and MA positively influenced sealer penetration compared to EDTA and HEBP groups in the apical third [37].

18. The study by Merve Akcay et al aimed to evaluate dentinal tubule penetration of four sealers after three final irrigation techniques: CUI, PIPS, and PUI, using a confocal laser scanning microscope. iRoot SP showed significantly greater penetration than AH Plus, MTA Fillapex, and Gutta Flow Bioseal, attributed to its small particle size and high viscosity. PIPS and PUI techniques achieved higher penetration than CUI, likely due to acoustic energy and fluid motion. Er:YAG laser activation with PIPS or PUI showed significant improvement in penetration compared to the control group.i Root SP, in conjunction with PIPS or PUI, demonstrated advantageous dentinal tubule penetration. The study recommends further research to validate these results. The ImageJ program

was utilized for measurement, overcoming limitations of single/multiple measurements.[38]

19. A study by Pattath Kunjan Anju et al stated that root canal irrigation with chelating agents can alter dentin properties, influencing the bond strength of root canal fillings. In accordance, 7% MA and 17% EDTA showed higher Push-Out Bond Strength (POBS) values, rejecting the null hypothesis. The superior POBS of 7% MA is attributed to its acidic nature, enhancing demineralization and surface roughness for better adhesion. EDTA's alkaline pH led to increased surface roughness and improved wettability, resulting in higher POBS compared to HEDP. HEDP exhibited weaker chelating action and lower POBS. NeoMTA Plus showed increased POBS when used with chelating agents due to closer contact between cement and dentin. POBS values decreased from coronal to apical thirds, with cohesive failure patterns indicating strong material bonding to dentin. Future studies are needed to compare NeoMTA Plus with other sealers, considering AH Plus as a gold standard. Overall, EDTA, MA, and HEDP improved NeoMTA Plus POBS, with MA and EDTA showing the highest values [39]

20. The in vitro study by Reham Hassan et al assessed anchorage of Total Fill Hi Flo sealer with Dual Rins chelation and warm obturation under CLSM. Sealer penetration showed significantly higher in upper two thirds when compared to the lowest third due to dentinal tubule density differences. Continuous chelation with Hypo/Dual Rins HEDP had most sealer discernment in upper two thirds. EDTA inhibited tricalcium silicate cement hydration, leading to higher sealer extension in the apical third with NaOCl and EDTA combined irrigation. The study highlights the importance of irrigation strategy in promoting sealer penetration, potentially impacting treatment success. Limitations include the laboratory setting and variation in samples. More studies on various activation techniques is warranted for greater cleansing and sealer extension [40].

21. A study by Pooja Gandhe said that hypo and HEDP in combination effectively decreased smear layer accumulation during canal instrumentation, comparable to hypo and EDTA. HEDP does not hinder hypo’s dissolution ability and. Group A (HEDP&NaOCl) showed

much greater anchorage than control and Hypo + CHX groups, due to HEDP's effect on CSCs hydration properties. Less HEDP conc. (9%) are as effective as greater conc. (18%) in removing smear layer. Group C (Chlorhexidine + Hypo) shows poor anchorage when measured with hypo and HEDP, in accordance to findings on CHX's impact on CSCs. Biodentine as control showed reduced anchorage due to presence of water during setting. Cohesive bond failure dominated, showing Biodentine strong anchorage to root canal. Exposure to hypo increases Biodentine sealing potential by increasing CH particle size and releasing calcium. Clinical decisions should take patient factors to maximize long prognosis. Overall, combining 2.5% hypo/9% HEDP enhances Biodentine anchorage to root canal dentin compared to other irrigation protocols [41].

22. A study by Shaimaa et. Al. on mandibular premolars prepared with ProTaper Next were irrigated with hypo, hypo/Dual Rinse, or hypo/EDTA, then filled with BC sealer using a single-cone obturation. CLSM showed great differences in anchorage area among groups ($P < 0.05$), with Hypo/Dual Rinse having greatest amount of sealer covering the canal and highest penetration. Hypo/Dual Rinse demonstrated better sealer extension into tubules compared to Hypo/EDTA, while hypo alone showed the least extension [42]

23. A report by Kothandaraman Sathyanarayanan stated that good management of external root resorption must cover the trauma, the type of infection, and the body's inflammatory pathology. RCTs using HEDP with Hypo and etidronate, succeeded by CH dressing and MTA filling till CEJ, is usually advocated. XP endo shaper reduces forces on dentin walls during BMP. MTA's alkaline pH helps Ca2+ ion release and tissue generation. Combined treatment advances complete stoppage of resorption and ensures good prognosis with regular follow-up [43].

24. A study by Inderpal S. Sappal et. al. compared the efficacy of acetic acid (AA), chloroform, and H_3PO_4 in removing BC Sealer during RCT. AA allowed quicker and more reproducible calculation of working length (WL) and patency when measured to chloroform or H_3PO_4, without affecting dentin. Although chloroform permitted filing to original WL in all cases, AA surpassed its efficiency. H_3PO_4 did not show better result

over AA or chloroform. Limitations of the study were assessment of only accessible areas. Research should study AA's efficacy in total BC Sealer removal and its effect on root dentin. Overall, 5 percent AA shows promise for good Re RCT but needs further investigation [44].

25. A study by Özgür İlke Ulusoy et. al. studied the result of chelating agents on CSCs used in RCTs. Control had minimal effect while other reagents compromised cement anchorage. Dual R HEDP showed weak chelating and maintained anchorage without altering dentin. Ortho MTA or Pro-Root MTA with Dual R HEDP for smear layer removal is advocated. But mixing HEDP and hypo had a mild effect on anchorage when levelled with EDTA. Clinical replications are required to confirm these findings. Using weak chelators like Dual R HEDP during procedures may avoid failures in cement disnaturing from canal dentin. More research in vivo is needed to study long-term cement stability [45].

MATERIALS AND METHODOLOGY

STUDY DESIGN:-

In Vitro Study

DATA SOURCE/LABORATORY DETAILS:-

Study was performed in the Department of Conservative Dentistry and Endodontics, VKIDS Belagavi with laboratory apparatus performed in Dr. Prabhakar Kore's Basic Science Research Laboratory, KLE University, Belagavi.

Specimens were assessed by Confocal Laser Scanning Microscope at BITS PILANI, GOA Campus

Extracted human mandibular premolar teeth, indicated for orthodontic extraction were collected from Department of Oral and Maxillofacial Surgery, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi

INCLUSION CRITERIA

- Extracted human mandibular bicuspid with singular obvious canals.
- Apical width corresponding 20 K-file/less

EXCLUSION CRITERIA

- Apical width more than #20 K-file size
- Calcified canals
- Root caries
- Fracture/crack or a restoration
- Internal and external root resorption
- Presence of anatomic variations
- Curvature, more than 5^0 curvature

PERMISSIONS TO BE TAKEN

- Ethical committee approval
- Dr. Prabhakar Kore's Basic Science Research Centre (BSRC), KLE University, Belagavi.
- Specimens were evaluated under the Confocal Laser Scanning Microscope at Birla Institute of Technology and Science, Pilani, K.K Birla Goa Campus

SAMPLE SIZE ESTIMATION

Sample size at 95 % confidence interval and 95% power

$$n = \frac{\left(z_{1-a/2} + z_{1-\beta}\right)^2 \left(SD_1^2 + SD_2^2\right)}{\left(\underline{x}_1 - \underline{x}_2\right)^2}$$

$\underline{x}_1 = 490.48$

$\underline{x}_2 = 379.0$

$SD_1 = 158.22$

$SD_2 = 114.02$

$z_{1-a/2} = 1.96$

$z_{1-\beta} = 1.64$

n = 40 per group

MATERIALS:

- Human mandibular premolar teeth
- 0.1% thymol (S D FINE-CHEMICALS LIMITED, MUMBAI)
- 3% Sodium Hypochlorite (VISHAL DENTOCARE, AHEMDABAD)
- 17% Ethylenediamine tetraacetic Acid (EDTA) (CANALARGE)
- 3% NaOCl+18% 1-hydroxyethylidene-1,1-biphosphonate (SIGMA-ALDRICH)
- Paper points (Diadent Group International)
- Distilled water (NICE LIFE CARE, NEW DELHI)
- GP points (Diadent Group International)
- Rhodamine B dye
- AH Plus sealer (Dentsply, Germany)
- Bioactive glass sealer (Cerkamed, Poland)

ARMAMENTARIUM

- K Files (#10-40)
- ProTaper Universal nickel-titanium files
- Airotor
- Endomotor
- Lentulospiral
- Micromotor
- 5 ml, 30-gauge syringe
- Diamond disk
- Confocal Laser Scanning Microscope

Human lower jaw bicuspid engaging singular roots and single canals were hand picked following its preservation in 0.1% thymol suspension. After RVG assessment and meeting inclusion criteria, teeth were sliced up to 14 mm for evenness. Working length determination,

and root canal instrumentation was proceeded captivating ProTaper universal rotary upto F3 master culminating file size. Chemomechanical procedure with three percent sodium hypochlorite was mastered followed by interim filing. Subject teeth were assigned to one of four intervention subgroups.

- SUBGROUP 1A: AH plus Sealer sealer + final flush with 17% EDTA
- SUBGROUP 1B: BIOACTIVE GLASS sealer + final flush with 17% EDTA
- SUBGROUP 2A: AH plus Sealer + final flush with equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite
- SUBGROUP 2B: BIOACTIVE GLASS sealer + final flush with equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite

Devising flushing chemical: 18% HEDP being devised from mercantile available company and manipulated in deionized water. Solution was manipulated 24hrs prior the experimental day and stored at 5^0 C. NaOCl and HEDP combinations were freshly mixed 1:1 (v/v) before the experiments.

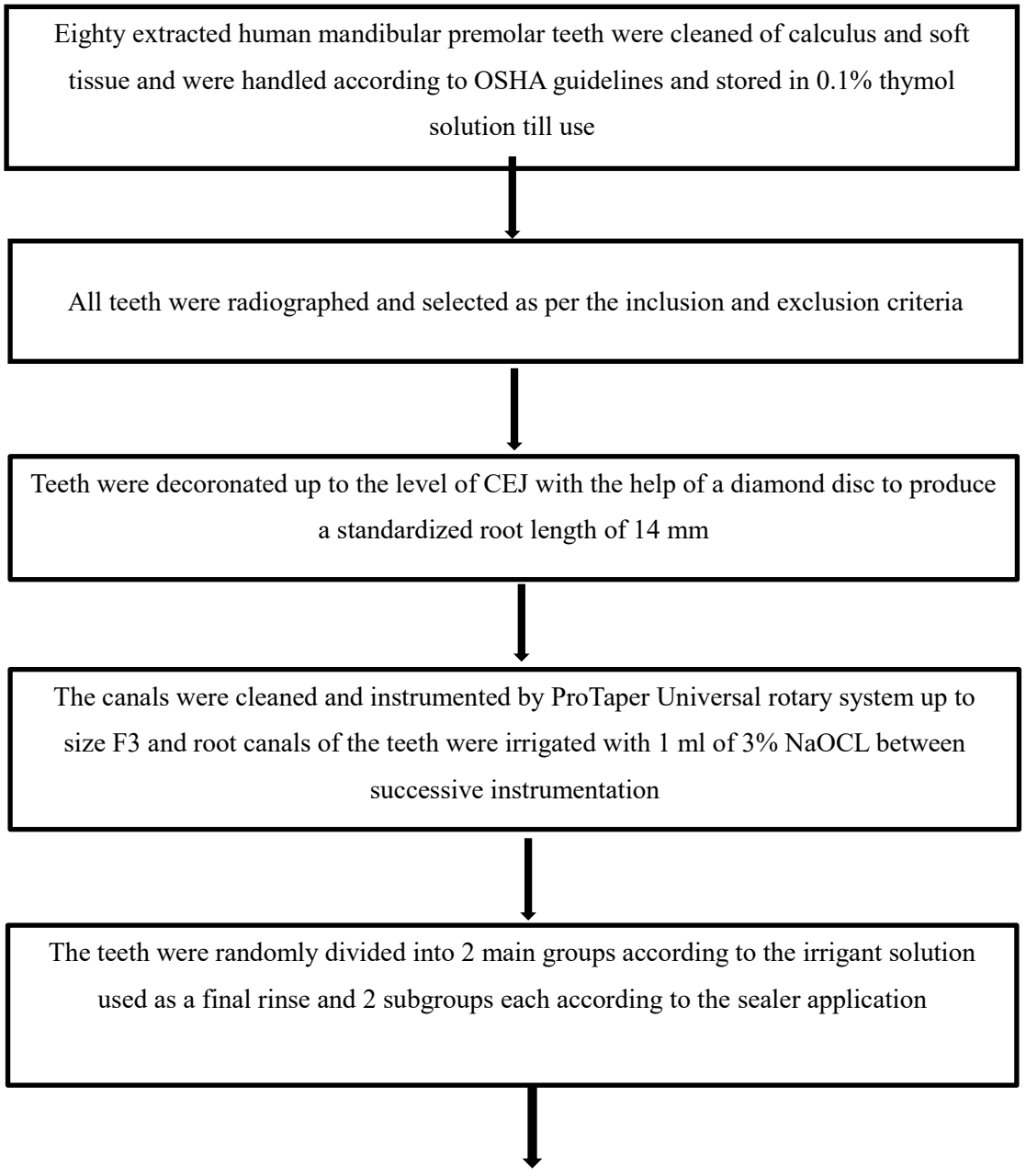
All irrigation solutions were administered utilizing five millimeter, 30-gauge syringe inserted one millimeter meniscal than the working length. Each specimen received a 5 ml flush of the experimental irrigating solution for 1 minute. Following this, radicular wall were thoroughly irrigated with ten millimeter distilled water for 1 minute plus dried using PP.

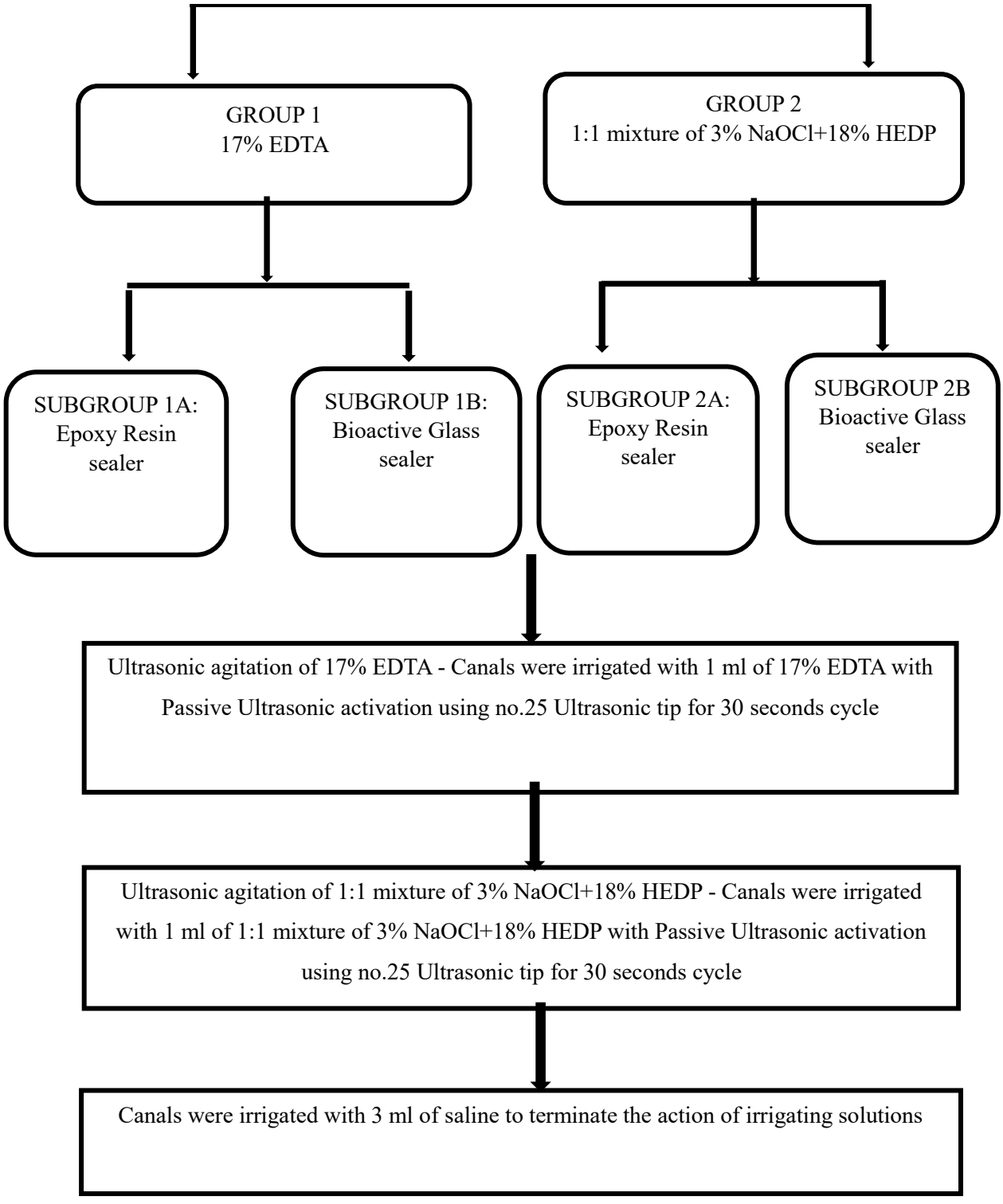
Agitation of the irrigating solution: Ultrasonic agitation of 17% EDTA and a with equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite with PUI utilizing no.25 ultrasonic tip for a 30-second cycle enhances root canal cleaning. This method involves irrigating canals with 1mL of the respective solution, effectively removing debris and smear layer. Research suggests that ultrasonic activation combined with an irrigant yields superior cleaning compared to syringe irrigation alone.

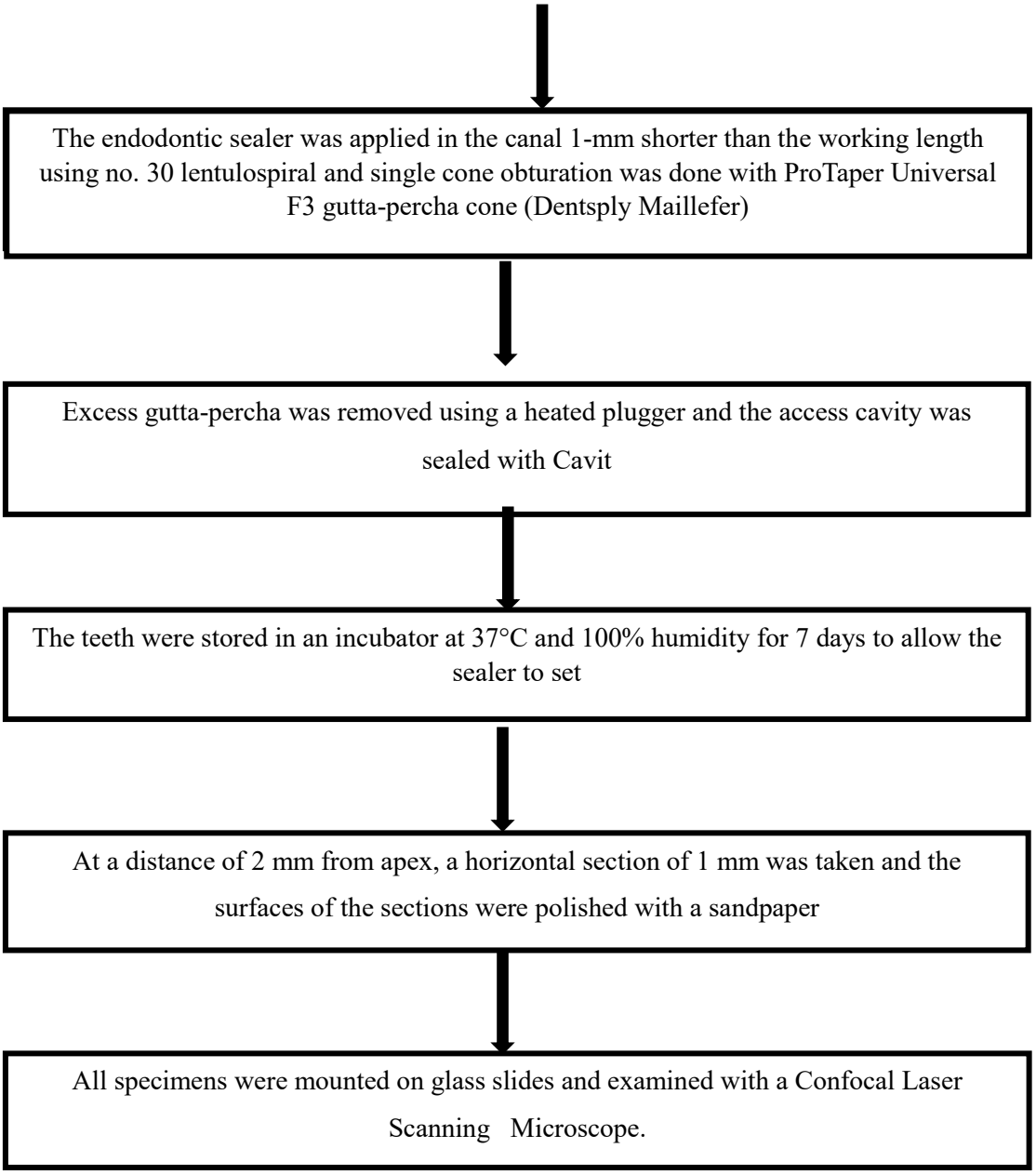
To enhance fluorescence for visualization under confocal laser scanning microscopy, Rhodamine B isothiocyanate dye was added to AH Plus and Bioactive glass sealers at a

concentration of approximately 0.1% by weight during manipulation. Labeled sealers were applied into canal wall using a no. 30 lentulospiral, with GP cones coated in the labeled epoxy resin-based sealer placed to the working length. Surplus GP was sheared with cavit seal. Teeth were preserved at temperature 37°C with hundred percentage humidity for 7 days for sealer polymerisation. After complete setting, teeth will be horizontally sectioned at the apical third with a diamond disc, followed by polishing. Specimens were ascended on glass slides and assessed using CLSM

METHODOLOGY WITH A FLOW CHART




GROUP 1
17% EDTA
GROUP 2
1:1 mixture of 3% NaOCl+18% HEDP
SUBGROUP 1A:
Epoxy Resin
sealer
SUBGROUP 1B:
Bioactive Glass
sealer
SUBGROUP 2A:
Epoxy Resin
sealer
SUBGROUP 2B
Bioactive Glass
sealer
Ultrasonic agitation 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 agitation of 1:1 mixture of 3% NaOCl+18% HEDP - Canals were irrigated with 1 ml of 1:1 mixture of 3% NaOCl+18% HEDP with Passive Ultrasonic activation using no.25 Ultrasonic tip for 30 seconds cycle
Canals were irrigated with 3 ml of saline to terminate the action of irrigating solutions


The endodontic sealer was applied in the canal 1-mm shorter than the working length using no. 30 lentulospiral and single cone obturation was done with ProTaper Universal F3 gutta-percha cone (Dentsply Maillefer)
Excess gutta-percha was removed using a heated plugger and the access cavity was sealed with Cavit
The teeth were stored in an incubator at 37°C and 100% humidity for 7 days to allow the sealer to set
At a distance of 2 mm from apex, a horizontal section of 1 mm was taken and the surfaces of the sections were polished with a sandpaper
All specimens were mounted on glass slides and examined with a Confocal Laser Scanning Microscope.

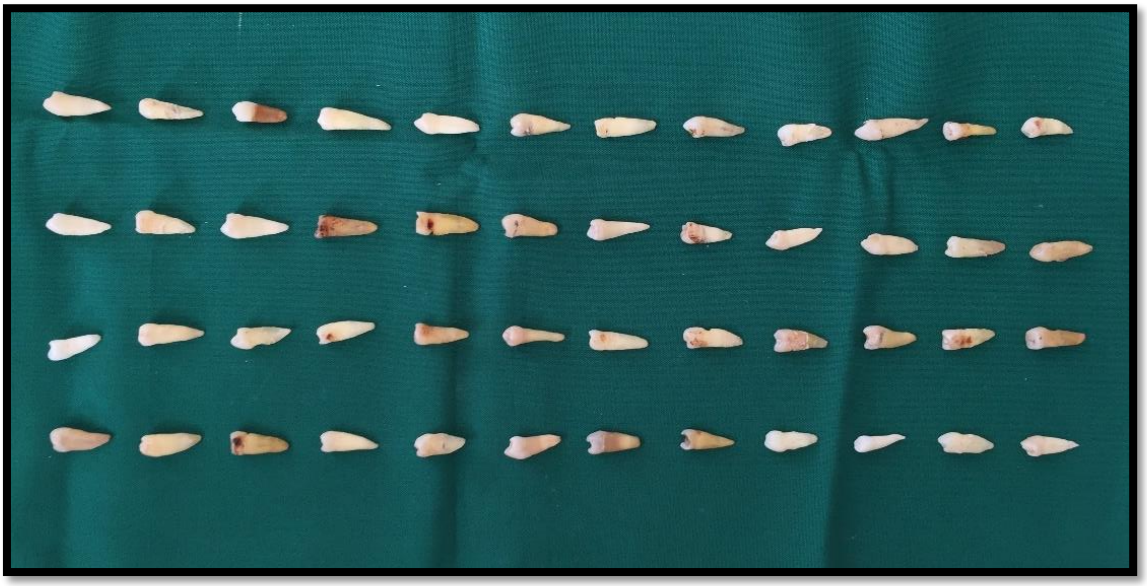


Fig 1: Human Mandibular Premolars

Fig 2: Armamentarium

Figure 3: Materials

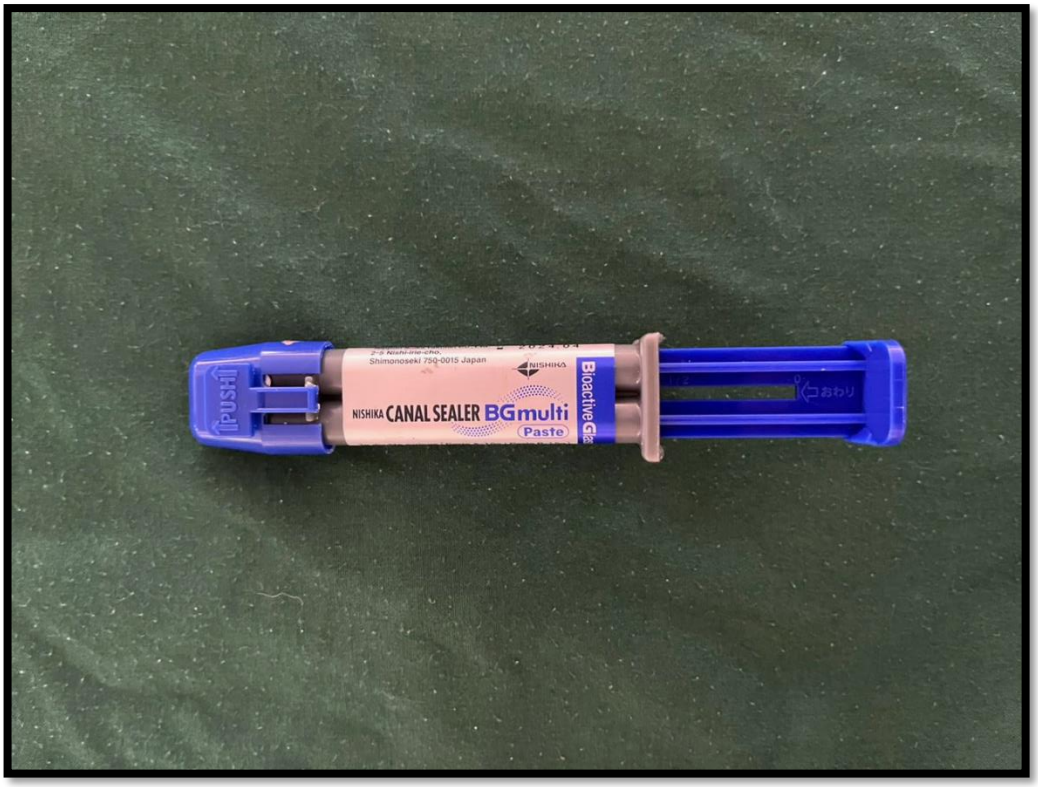


Fig 4: Debris Removal

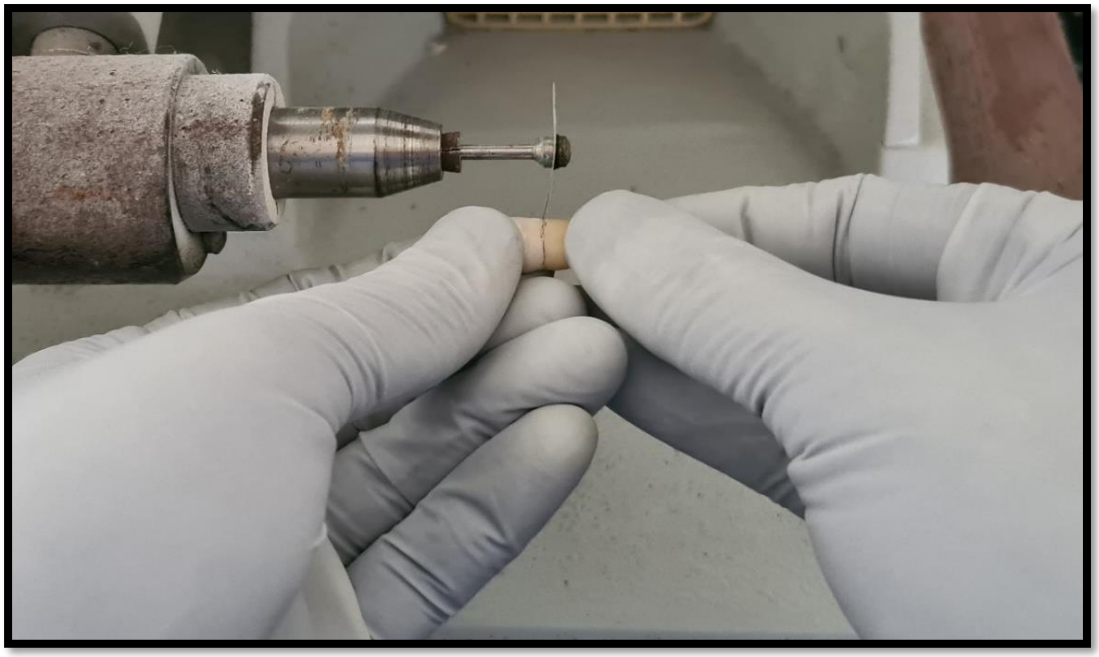


Fig 5: Decoronation

Fig 6: Working Length Determination

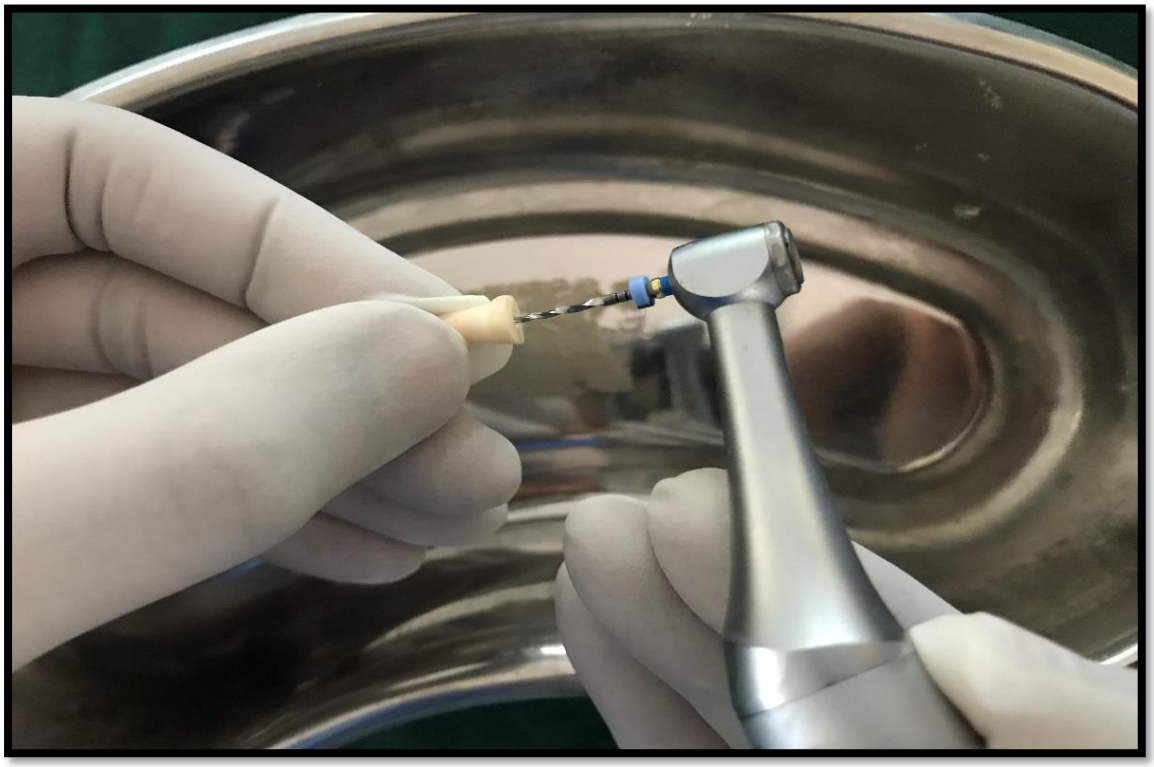


Fig 7: Bio-Mechanical Preparation

Fig 8: Irrigation with EDTA and HEDP

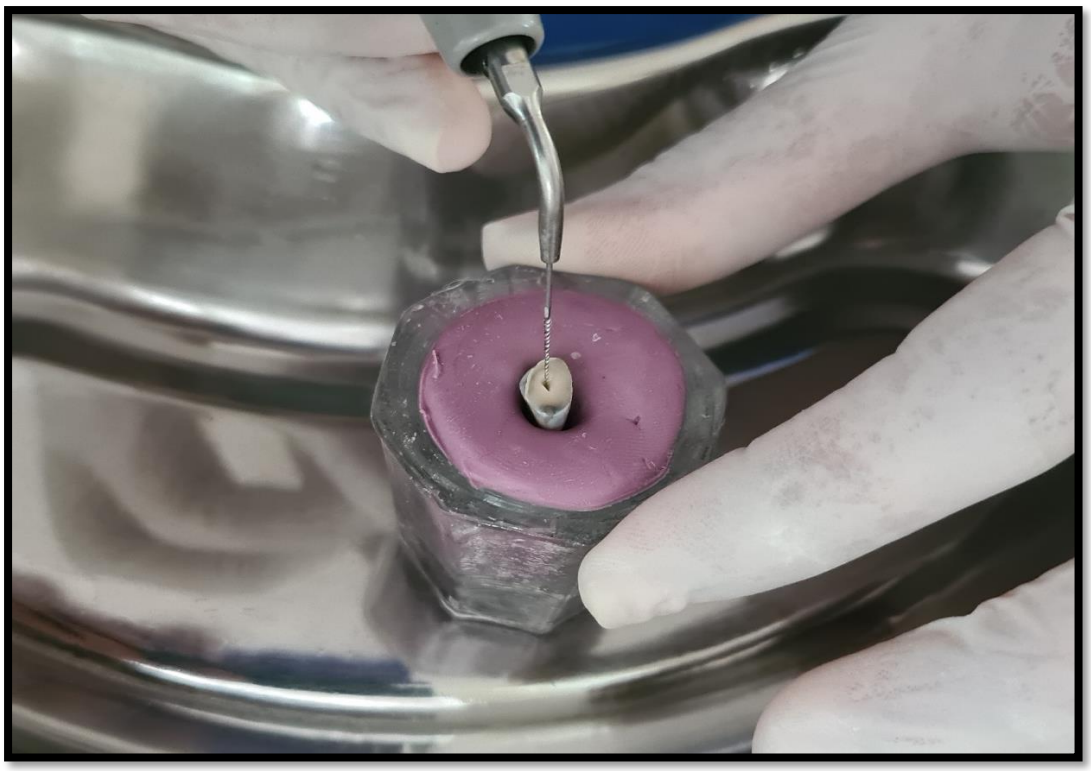


Fig 9: Irrigation activation with PUI

Fig 10: Drying canals with paper points

Fig 11: Drying Can

Fig 12: Obturation

Fig 13: Incubation

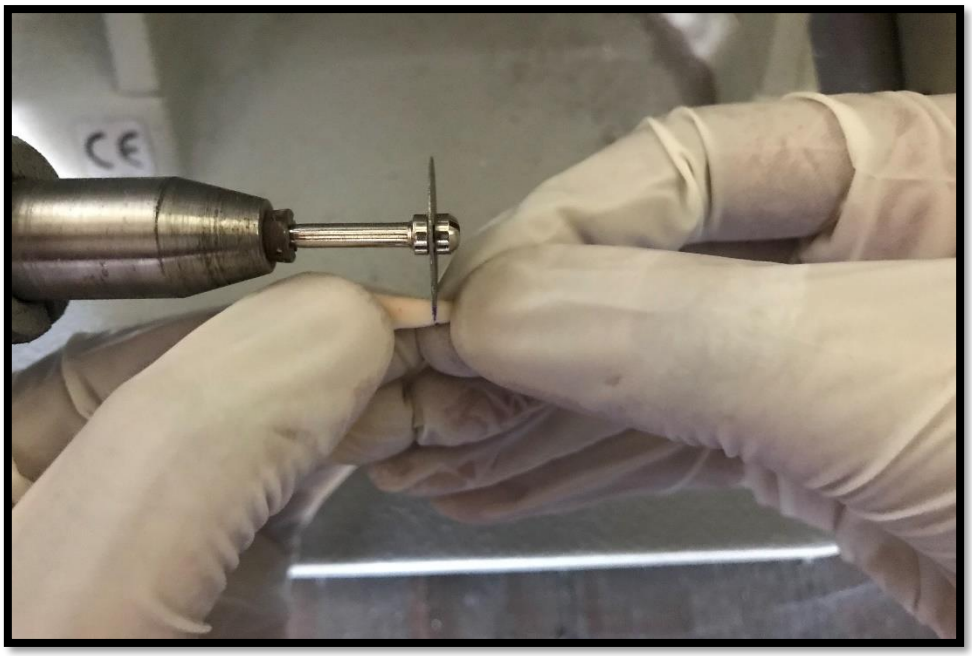


Fig 14: Sectioning

Fig 15: Confocal Laser Scanning Microscope

RESULTS

In total, 20 samples were examined in each subgroup to assess apical third for sealer discernment. Table 1 summarizes the average penetration depth, SD, and SE for secondary groups, comprising 20 test samples per group, analysed using ANOVA. Spectacular mean penetration assessed apical 3rd with EDTA/AH Plus after PUI, reaching 1053.89 µm, with statistically significant results (P=0.0001). Conversely, specimens cored with bioactive sealant succeeding HEDP irrigation exhibited the least penetration, at 869.234 micrometre.

Tukey's multiple post-hoc test revealed a superior transition in penetration amongst EDTA and HEDP in the apical section. There are statistically significant disparities between the two primary groups. Additionally, superior transition was notable statistically amongst two subgroups when comparing AH Plus and bioactive. The analysis indicated a dynamic disparity of (p=0.0001) between these subgroups, highlighting positive impact of AH Plus on sealer penetration.

Further comparison showed that the EDTA+AH Plus sealer penetration group exhibited superior penetration compared to the EDTA + Bioactive, HEDP + AH Plus, and HEDP + Bioactive sealer groups.

HEDP + AH Plus	EDTA + Bioactive	HEDP + Bioactive
p=0.0178*	p=0.0001*	p=0.0009*

Table: Levene Test of Homogeneity of Variances

Variable	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F-value	p-value
Dentinal tubule penetration	43641.30	3.00	14547.1	916409.44	76	12058.02	1.2064	0.3132

Variances are homogeneous. Therefore, parametric one way ANOVA was applied

Table:1 Summary of dentinal tubule discernment in secondary groups

Group	Mean	Std.Dev.	Std.Err.	95% CI for mean	
				Lower	Upper
EDTA + AH Plus	1053.89	145.16	32.46	985.96	1121.83
HEDP + AH Plus	914.62	214.77	48.02	814.10	1015.13
EDTA + Bioactive Glass	890.34	89.07	19.92	598.65	682.03
HEDP + Bioactive Glass	869.23	99.88	22.33	822.48	915.97

Table: 2 Four groups with mean dentinal tubule discernment by one way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	3	1771001.48	590333.82	27.7450	0.0001*
Within groups	76	1617062.74	21277.14		
Total	79	3388064.22			

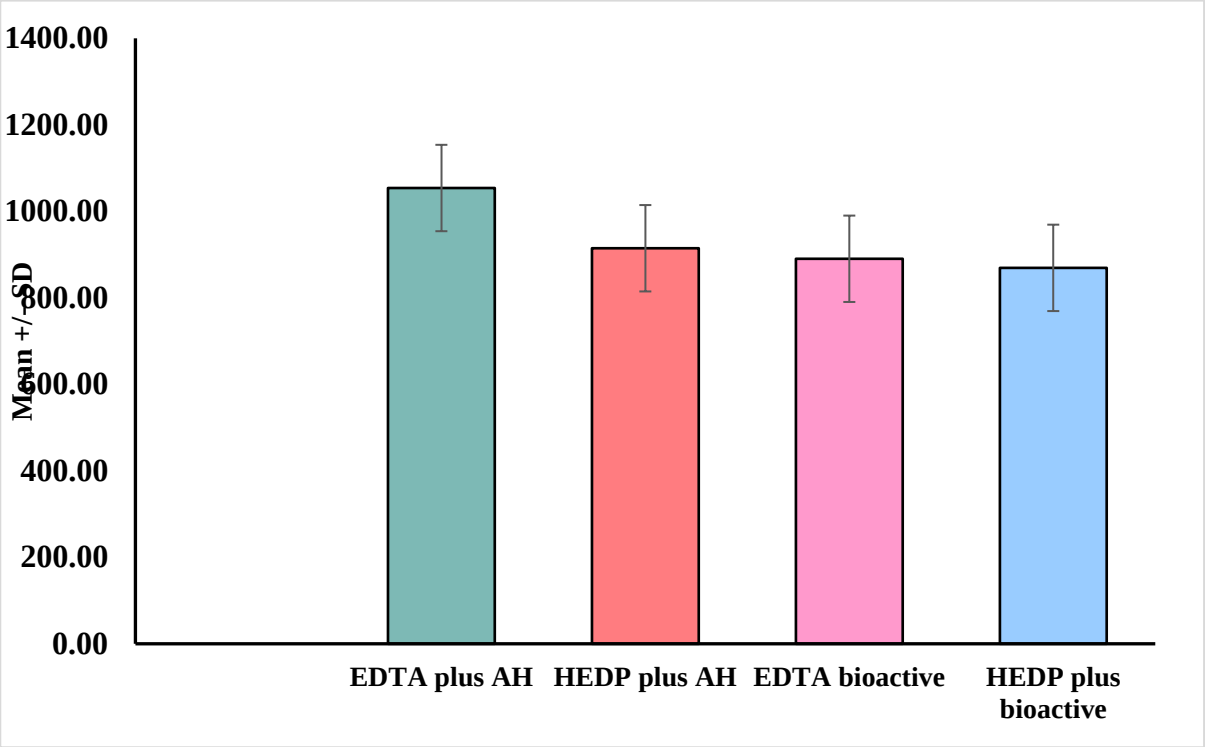
*p<0.05

Table:3 Pair wise comparison of four groups with mean dentinal tubule discernment by Tukeys multiple posthoc procedures

Group	EDTA + AH Plus	HEDP + AH Plus	EDTA + bioactive	HEDP+ bioactive
Means	1053.89	914.62	890.34	869.23
Std.Dev.	145.16	214.77	89.07	99.88
EDTA + AH Plus	-			
HEDP + AH Plus	p=0.0178*	-		
EDTA+Bioactive Glass	p=0.0001*	p=0.0001*	-	
HEDP + Bioactive Glass	p=0.0009*	p=0.7590	p=0.0002*	-

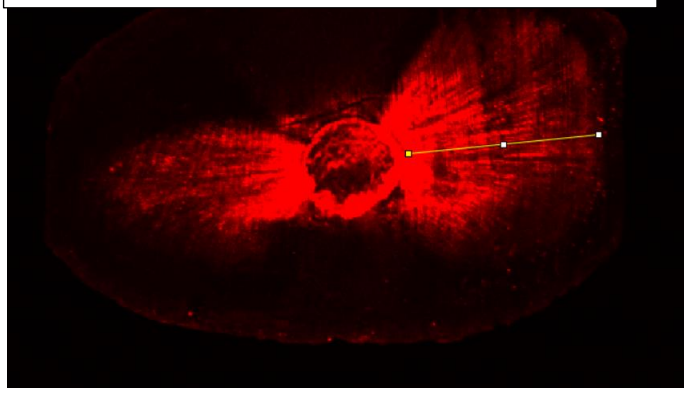
*p<0.05

Figure: 4 Comparison of four groups with mean dentinal tubule discernment



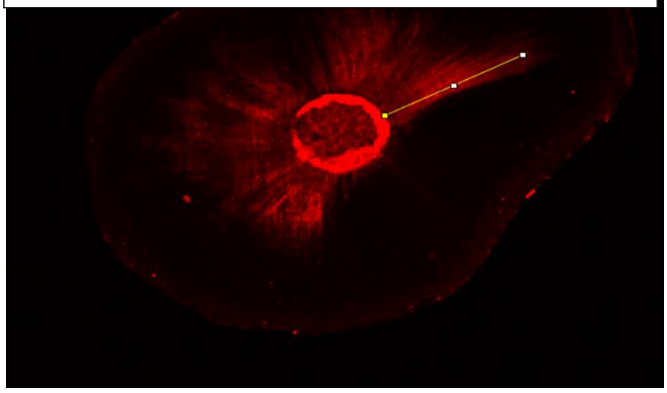
1196.69 µm

CLSM image representing the depth of penetration of AH Plus sealer after EDTA activation



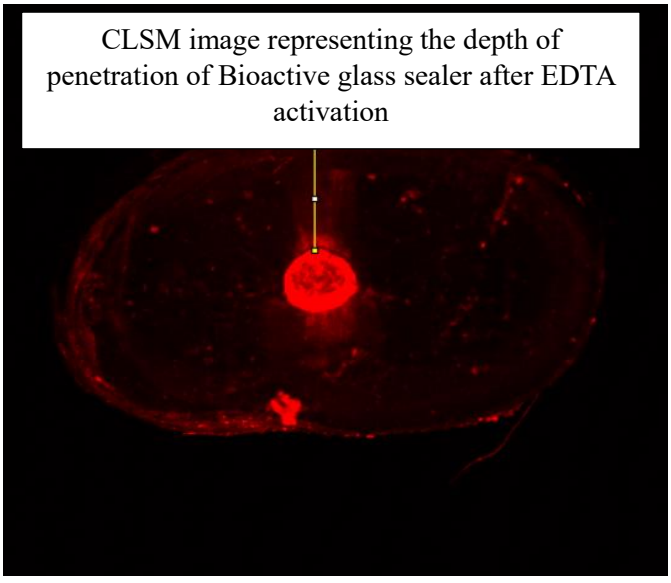
954.59 µm

CLSM image representing the depth of penetration of AH Plus sealer after HEDP activation



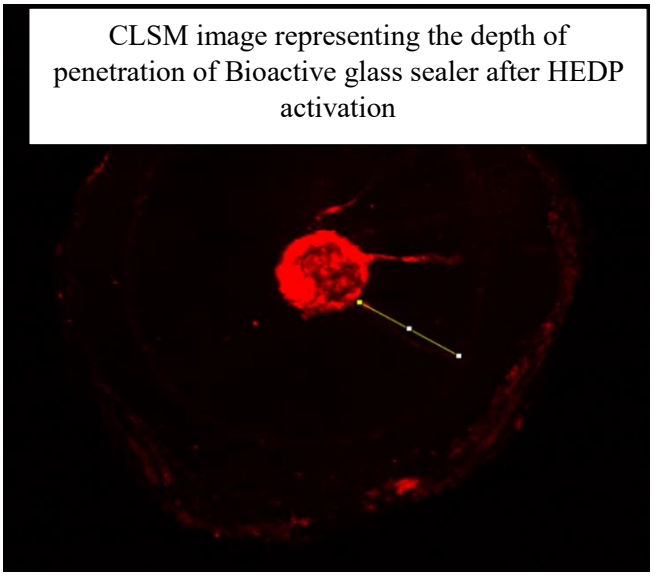
943.394 µm

CLSM image representing the depth of penetration of Bioactive glass sealer after EDTA activation



895.709 µm

CLSM image representing the depth of penetration of Bioactive glass sealer after HEDP activation



DISCUSSION

The bullseye of therapeutic root canal is to thoroughly disinfect internal structure of the root canal [46]. While cleaning process, dentin mixture, pulp tissues makes residual layer, known as the smear layer, and is spread across the internal walls, thus creating a hurdle impeding Toto bonding penetration of core material[47,48,49].

Currently, amalgamation of smear removal techniques were crafted to get rid of smear layer[50].

Irrigating solutions are employed to eliminate the smear layer that has been created. Sodium hypochlorite is adept at dissolving organic tissues and possesses bactericidal properties, although it is not particularly efficient at eliminating remains [51].

Amalgamation of sodium hypochlorite (2.5-5%) with EDTA (10-17%) were unique at removing both organic and inorganic contaminants with EDTA being a Ca2+ chelating agent when applied as a final flush [52,53].

In accordance to literature these chelators alter Ca and P [48]. Furthermore, sodium hypochlorite and EDTA strongly react, rendering the agent ineffective [54,55]. Therefore, during root canal therapies, it is imperative to look for an updated, biocompatible, and effective alternative irrigation technique [56].

A chelating agent, HEDP, compatible with NaOCl, aids in root canal preparation by expediting sterilization and removing the smear layer. HEDP is safe to root dentin due to its basic pH, preserving Ca++ ions [57]. Despite this, it maintains smear eradication potential comparable to EDTA without compromising NaOCl's tissue dissolution. HEDP's optimal concentration for smear layer removal is recommended to be at least 18%. Manual and mechanised techniques, including sonic, ultrasonic, and apical negative pressure systems, enhance effectiveness of endodontic irrigants [58].

When compared to traditional syringe and needle irrigation, these approaches provide a significant improvement in canal cleaning [40].

Human mandibular premolars, with complex anatomical contrast with removal for orthodontic purposes, were utilized in the research. To prevent fungal growth, specimens were stored in a 0.1% thymol solution . Decoronation was performed to achieve a standardized radicular length of 14 ± 1 mm associated flat coronal area. ProTaper Universal file were employed for BMP due to their cutting potential with diminished torsional stress [49].

Irrigation procedure involved using three percentage sodium hypochlorite between successive instrumentations for its tissue-dissolving competence with final rinse 17% EDTA to remove the smear layer after NaOCl usage. In another experimental group, a final rinse was performed using equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite. PUI was used to activate the irrigants. Ultrasonic energy has been utilized in endodontics for a significant period to pulverize debris and aid disinfection [50]. When combined with an irrigant, ultrasonics provide constructive root wall ecosystem debridement compared to orthodox methods. PUI involves activating the irrigant, generating ultrasonic frequency of 30 kHz. The resulting rapid vortex-like motion of the liquid and cavitation lead to the formation of spontaneous cavities throughout the liquid, enhancing full blown pulverization of canal system [35].

Effective root canal treatment comprises three key phases: shaping and cleansing, disinfection, and hermetic seal. Ensuring tight contact between the radicular wall and the core filling material is essential for proper obturation, with root canal sealers playing a pivotal role in achieving this outcome [48].

Caviton sealed the cavity. Maintaining 37 degree Celsius with humid conditions for 7days test subjects were preserved to ensure complete setting and polymerization of the sealer, mimicking oral conditions

To gauge the tubular penetration depth, Confocal Laser Scanning Microscope (CLSM) was elected over SEM – pertaining to its capability to sanction three dimensional image, documenting phases of radicular dentine at contrasting levels and evaluating precise depth measurement. Adding another feather to cap of CLSM is , it vanquishes artifacts [23], because of analysis under 20-30microns.

The study discovered that AH Plus sealer demonstrated greater penetration at the deepest third when washed with EDTA and HEDP.

Specimens filled with bioactive sealer following EDTA and HEDP activation showed the least penetration. Significant differences were noted between secondary groups in observed sections, with a drastic unique disparity overall (p=0.0001) among the four subgroups. Specifically, the EDTA+ AH Plus sealer group demonstrated superior penetration compared to the EDTA + Bioactive, HEDP + AH Plus, and HEDP + Bioactive sealer groups. Factors contributing to this discrepancy include the irrigation activation method and sealer flow ability.

PUI has affirming ascendancy upon sealer penetration conferred by Amin et al. Ultrasonic agitation can boost the apical penetration of an irrigating solution.

The study found AH Plus sealer exhibited superior tubular penetration when EDTA and HEDP accompanied it, likely due to its pseudo plastic nature it experiences decreased viscosity and increased flow under compaction, aided by higher shear rates. The chemical composition of sealers is crucial; hydrophilic sealers penetrate deeper than hydrophobic ones. AH Plus, a hydrophobic sealer, is affected by compaction pressure and elevated temperatures during obturation, leading to enhanced flowability and deeper penetration.

CONCLUSION

The type of irrigating solution and flowability of sealer influences depth of penetration. Evaluation of sealer penetration reported , significant greater level with EDTA and HEDP respectively. To conclude, HEDP can implicitly replace EDTA. In accordance to study associated with EDTA shortcoming, in-vivo assessment of HEDP with bioceramic sealers should executed to bolster up the current thesis results.

SUMMARY

Endodontic triumph is reliant upon cleaning, debridement of the canal ecosystem and a fluid tight impervious seal. Various newer 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 irrigant within the canal ecosystem as well in complex anatomies present.

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

Standardized root length of 14±1mm with the help of a diamond disk was achieved. 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 till F3. Teeth were categorized at random 4 secondary groups (n = 20).

SUBGROUP 1A: AH Plus sealer + final flush with 17% EDTA

SUBGROUP 1B: Bioactive glass sealer + final flush with 17% EDTA

SUBGROUP 2A: AH Plus sealer + final flush with equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite

SUBGROUP2B: Bioactive glass sealer + final flush with equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite

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

Null hypothesis of no disparity in advent of 17% EDTA and equal composition of equal proportion of 18 percentage HEDP with 3 percentage Hypochlorite as final flush on sealer discernment of epoxy resin based sealer and bioactive glass sealer was rejected.

Superior results were attained with EDTA + HEDP, with EDTA + AH Plus group demonstrating the tremendous penetration with inferior HEDP + Bioactive sealer group.

LIMITATIONS

In the present study, HEDP + Bioactive sealer group showed least penetration. Bioactive glass sealer is a novel second generation bioceramic sealer with limited number of evidence based studies. The flow rate of Bioactive glass sealer is 18.25mm which is less than ISO requirement of 20mm which may explain its less penetration. In future modification in its composition such as particle size can be done to enhance its penetration. Within the limitation of this study, HEDP though being a weak chelating agent along with Bioactive sealer showed comparable sealer penetration to EDTA + AH Plus group.

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.
2. Sen BH, Wesselink PR, Türkün M. The smear layer: a phenomenon in root canal therapy. Int Endod J. 1995 May;28(3):141-8.
3. 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.
4. 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.
5. 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.
6. 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):512-516.
7. 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.
8. 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.

9. 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.
10. 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.
11. Kaki GD, Recen D, Baser Mİ, Guvenc P. Effect of Dual Rinse® HEDP Root Canal Irrigation Solution on Coronal Dentin Adhesion. Medical Journal of Suleyman Demirel University. 2018;25(4):54-63..
12. 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.
13. 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.
14. 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.
15. 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;22(1):21-8.
16. 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.
17. 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):512-516.
18. 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.

19. 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;9(1):34-38.
20. 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;37(11):1576-9.
21. Adham A, Ahmed AL. The effectiveness of continuous versus sequential chelation in the removal of smear layer and their influence on push-out bond strength of Bio-C sealer (An in vitro study). Cumhuriyet Dental Journal. 2023 Jun 23;26(2):112-20.
22. Sfeir G, Bukiet F, Hage W, El Hachem R, Zogheib C. Impact of final irrigation protocol on the push-out bond strength of two types of endodontic sealers. Materials. 2023 Feb 21;16(5):1761.
23. Awati AS, Dhaded NS, Mokal S, Doddwad PK. 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. Journal of Conservative Dentistry and Endodontics. 2024 Jan 1;27(1):87-94.
24. Adham AH, Ali AH, Mannocci F. Continuous Chelation Concept in Endodontics. Journal of Baghdad College of Dentistry. 2022 Dec 15;34(4):59-69.
25. Kaul S, Kumar A, Badiyani BK, Sukhtankar L, Madhumitha M, Kumar A. Comparison of sealing ability of bioceramic sealer, AH Plus, and guttaflow in conservatively prepared curved root canals obturated with single-cone technique: an in vitro study. Journal of Pharmacy and Bioallied Sciences. 2021 Jun 1;13(Suppl 1):S857-60.
26. Novozhilova N, Babina K, Polyakova M, Sokhova I, Sherstneva V, Zaytsev A, Makeeva I, Mikheikina A. The Effect of Different Compositions and Concentrations of Etidronate-Containing Irrigants on the Antibacterial Activity of Sodium Hypochlorite against Enterococcus faecalis and Candida albicans. Dentistry Journal. 2024 Feb 21;12(3):46.

27. Komabayashi T, Colmenar D, Cvach N, Bhat A, Primus C, Imai Y. Comprehensive review of current endodontic sealers. Dental materials journal. 2020 Sep 28;39(5):703-20.
28. Tartari T, Wichnieski C, Silva RM, Letra A, Duarte MA, Bramante CM. Final irrigation protocols can be used to promote stable long-term bond strength of AH Plus to dentin. Journal of Applied Oral Science. 2023 May 26;31:e20230005.
29. Ibrahim L. Assessment of marginal adaptation of two different root canal sealers using an innovative irrigant: An in vitro study. Egyptian Dental Journal. 2021 Oct 1;67(4):3731-9.
30. Castagnola R, Martini C, Colangeli M, Pellicciotta I, Marigo L, Grande NM, Bugli F, Plotino G. In Vitro Evaluation of Smear Layer and Debris Removal and Antimicrobial Activity of Different Irrigating Solutions. European Endodontic Journal. 2024 Jan 1;9(1):81-8.
31. Gandhi P, Ballal NV, George JE, George SD, Narkedamalli RK. Effect of chelating agents on the wettability of BioRoot RCS and AH Plus sealers. Saudi Endodontic Journal. 2020 May 1;10(2):100-5.
32. Dureja A, Purayil TP, Ballal NV, George SD. Effect of different chelating agents on the wettability of CeraSeal and AH Plus sealers to intraradicular dentin: An in vitro study. Saudi Endodontic Journal. 2023 Sep 1;13(3):248-53.
33. Kamin R, Vikram R, Meena N, Kumari RA, Adarsha MS, Murthy CS. Effect of final irrigating solutions on penetration depth of resin-based sealers into dentinal tubules. Journal of Conservative Dentistry and Endodontics. 2021 Jul 1;24(4):374-8.
34. Tartari T, Bachmann L, Zancan RF, Vivan RR, Duarte MA, Bramante CM. Analysis of the effects of several decalcifying agents alone and in combination with sodium hypochlorite on the chemical composition of dentine. International endodontic journal. 2018 Jan;51:e42-54.
35. Deari S, Mohn D, Zehnder M. Dentine decalcification and smear layer removal by different ethylenediaminetetraacetic acid and 1-hydroxyethane-1, 1-diphosphonic acid species. International endodontic journal. 2019 Feb;52(2): 237-43.

36. Ballal NV, Ulusoy Öİ, Rao S, Gandhi P. The efficacy of different irrigation protocols in removing tricalcium silicate-based sealers from simulated root canal irregularities. Microscopy research and technique. 2019 Nov;82(11):1862-8.
37. Alim Uysal BA, Kotan G, Guneser MB, Dincer AN, Senturk H, Rafiqi AM. Investigation of the effect of different chelation solutions on penetration of resin-based and bioceramic sealers with a novel method. Microscopy research and technique. 2021 Jul;84(7):1571-6.
38. Akcay M, Arslan H, Durmus N, Mese M, Capar ID. Dentinal tubule penetration of AH Plus, iRoot SP, MTA fillapex, and guttaflow bioseal root canal sealers after different final irrigation procedures: A confocal microscopic study. Lasers in surgery and medicine. 2016 Jan;48(1):70-6.
39. Anju PK, Purayil TP, Ginjupalli K, Ballal NV. Effect of chelating agents on push-out bond strength of NeoMTA Plus to root canal dentin. Pesquisa Brasileira em Odontopediatria e Clínica Integrada. 2022 Mar 7;22:e210058.
40. Hassan R, Roshdy NN. Effect of continuous chelation on the dentinal tubule penetration of a calcium silicate–based root canal sealer: a confocal laser microscopy study. BMC Oral Health. 2023 Jun 9;23(1):377.
41. Gandhe P, Gaikwad A, Pandit V, Sengupta A, Deshmukh P. Comparative Evaluation of Pushout Bond Strength of Biodentine after Root Dentin Conditioning with Different Irrigating Solutions-An In Vitro Study.
42. Shekhar S, Mallya PL, Ballal V, Shenoy R. To evaluate and compare the effect of 17% EDTA, 10% citric acid, 7% maleic acid on the dentinal tubule penetration depth of bio ceramic root canal sealer using confocal laser scanning microscopy: an in vitro study. F1000Research. 2022;11.
43. Sathyanarayanan K, Poornima L, Narayan KV. Non-Surgical Endodontic Management of External Root Resorption in a Permanent Maxillary Central Incisor Tooth-A 3 Year Follow-Up of a Rare Case Scenario. Journal of Evolution of Medical and Dental Sciences. 2021 Jul 26;10(30):2350-4.

44. Sappal IS. In Vitro Evaluation of the Solubility of EndoSequence BC Sealer in Acidic Solutions.
45. Ozdemir HO, Buzoglu HD, Calt S, Stabholz A, Steinberg D. Effect of ethylenediaminetetraacetic acid and sodium hypochlorite irrigation on Enterococcus faecalis biofilm colonization in young and old human root canal dentin: in vitro study. Journal of endodontics. 2010 May 1;36(5):842-6.
46. Sivakumar JS, Kumar BN, Shyamala PV. Role of provisional restorations in endodontic therapy. Journal of pharmacy & bioallied sciences. 2013;5(1):120-135.
47. Perdigao JO, Lambrechts P, Vanherle G. Microscopy investigations: techniques, results, limitations. Am. J. Dent. 2000;13(4):14-26.
48. 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.
49. Chadha R, Taneja S, Kumar M, Gupta S. An in vitro comparative evaluation of depth of tubular penetration of three resin-based root canal sealers. J Conserv Dent 2012;15:18-21.
50. Iqbal A. Antimicrobial irrigants in the endodontic therapy. Int J Health Sci (Qassim) 2012;6:186-92.
51. Violich DR, Chandler NP. The smear layer in endodontics – A review. Int Endod J 2010;43:2-15.
52. Crumpton BJ, Goodell GG, McClanahan SB. Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. J Endod 2005;31:536-8.
53. Clarkson RM, Podlich HM, Moule AJ. Influence of ethylenediaminetetraacetic acid on the active chlorine content of sodium hypochlorite solutions when mixed in various proportions. J Endod 2011;37:538-43.

54. Arias-Moliz MT, Ordinola-Zapata R, Baca P, Ruiz-Linares M, Ferrer-Luque CM. Antimicrobial activity of a sodium hypochlorite/ etidronic acid irrigant solution. J Endod 2014;40:1999-2002.
55. Girard S, Paqué F, Badertscher M, Sener B, Zehnder M. Assessment of a gel-type chelating preparation containing 1-hydroxyethylidene-1, 1-bisphosphonate. Int Endod J 2005;38:810-6.
56. Paqué F, Rechenberg DK, Zehnder M. Reduction of hard-tissue debris accumulation during rotary root canal instrumentation by etidronic acid in a sodium hypochlorite irrigant. J Endod 2012;38:692-5.
57. 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;42:335-43.
58. Moogi P, Sayyad A, Rathore V, Ghosh S, Ambalia S, Amin A, et al. Comparative evaluation of marginal adaptation of two resin-based sealers: A scanning electron microscopic study. Endodontology. 2020;32:137.

ANNEXURE – I – ETHICAL CLEARANCE LETTER

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

Sl. No. : 1598

CERTIFICATE

EC/NEW/INST/2021/2435
Research & Ethics Committee

This is to Certify that the synopsis titled

Effect of final irrigation regimens on the dentinal tubule penetration of two different root canal sealers. A confocal Laser scanning Microscope In-Vitro study

Submitted by

Dr. REG.NO. IE0221002 *P. G. Student /*

Staff, Guided by _____ *from Department of*

Conservative Dentistry & Endodontic *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date :

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

MEMBER SECRETARY
Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences

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

Accredited 'A+' grade by NAAC (3rd 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

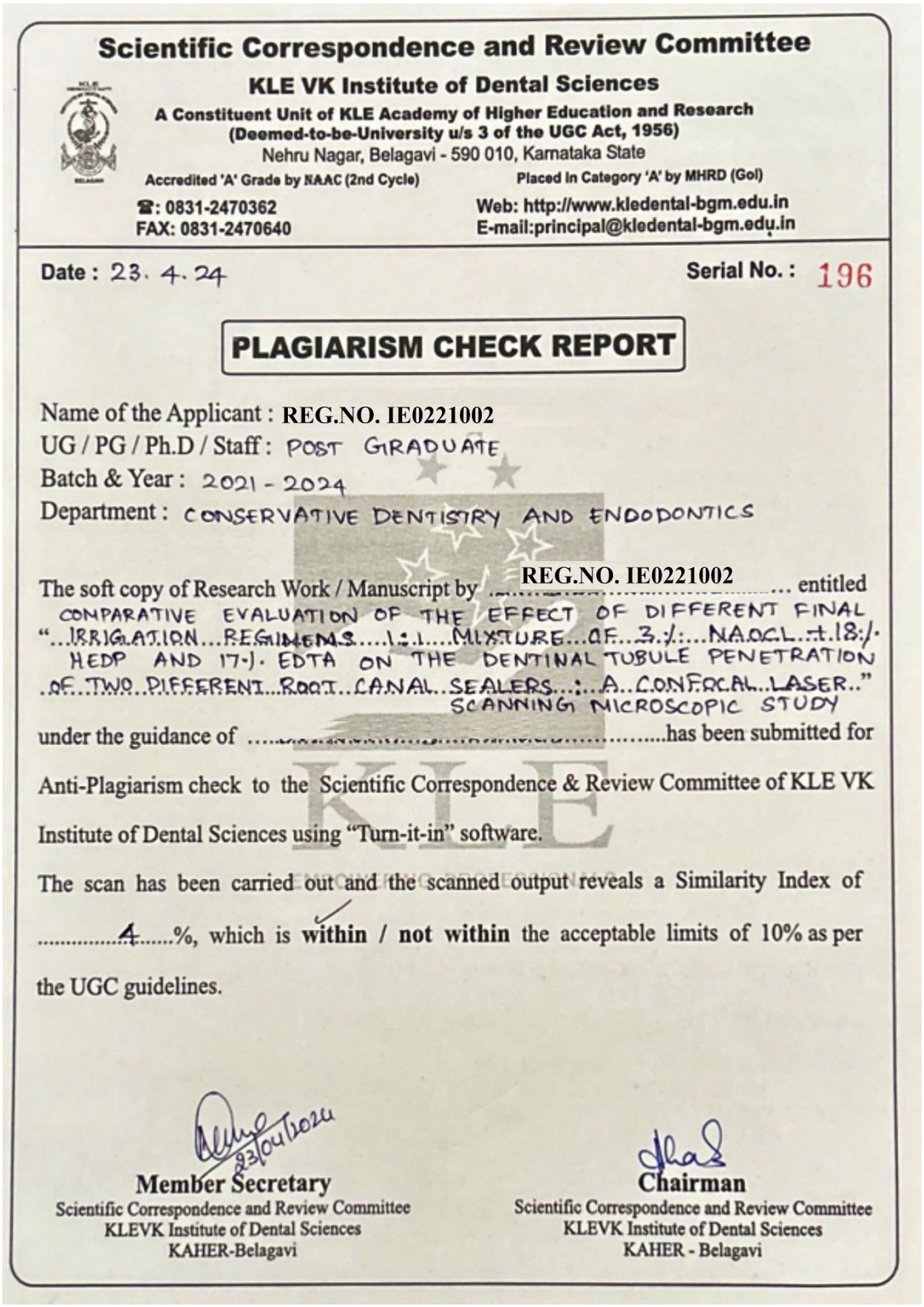
Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of this dissertation/ Thesis work of **REG.NO. IE0221002** post-graduate student, under the guidance of **Department of Conservative Dentistry and Endodontics, entitled "COMPARATIVE EVALUATION OF THE EFFECT OF FINAL IRRIGATION REGIMENS ON THE DENTINAL TUBULE PENETRATION OF TWO DIFFERENT ROOT CANAL SEALERS: A CONFOCAL LASER SCANNING MICROSCOPIC IN-VITRO STUDY."** has been done under my guidance and completed satisfactorily.

Place: Belagavi
Date:

Name & Signature of Biostatistician
Dr. S. B. JAVALI Ph.D.
Sr. Associate Professor in Statistics
Department of Community Medicine
USM KLE International Medical Programmes
BELAGAVI-590010.

ANNEXURE – III – PLAGIARISM CHECK REPORT



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 : 23.4.24 Serial No. : 196

PLAGIARISM CHECK REPORT

Name of the Applicant : **REG.NO. IE0221002**
UG / PG / Ph.D / Staff : POST GRADUATE
Batch & Year : 2021 - 2024
Department : CONSERVATIVE DENTISTRY AND ENDODONTICS

The soft copy of Research Work / Manuscript by **REG.NO. IE0221002** ... entitled "COMPARATIVE EVALUATION OF THE EFFECT OF DIFFERENT FINAL IRRIGATION REGIMENS : MIXTURE OF 3% NAOCL + 18% HEDP AND 17% EDTA ON THE DENTINAL TUBULE PENETRATION OF TWO DIFFERENT ROOT CANAL SEALERS : A CONFOCAL LASER SCANNING MICROSCOPIC 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 .....4.....%, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

23/04/2024

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