"COMPARATIVE EVALUATION OF THE EFFECT OF 0.2% CHITOSAN WITH OR WITHOUT PASSIVE ULTRASONIC ACTIVATION USED AS A FINAL RINSE ON THE TUBULE PENETRATION OF EPOXY RESIN BASED AND BIOCERAMIC SEALERS ON RADICULAR DENTIN: AN IN-VITRO CONFOCAL LASER SCANNING MICROSCOPY STUDY."

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LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	<	Less than
2	>	Greater than
3	° C	Degree Celsius
4	μm	Micrometers
5	3D	Three Dimensional
6	AASF	Atomic Absorption Spectrophotometry with Flame
7	ANOVA	Analysis of Variance
8	BC	Bioceramic sealer
9	CA	Citric Acid
10	CHX	Chlorhexidine
11	CI	Conventional irrigation
12	CLSM	Confocal Laser Scanning Mircroscope
13	EDTA	Ethylene Diamine Tetra-acetic Acid
14	GF-B	Guttaflow-Bioseal
15	GP	Gutta percha

16	i.e.	That is
17	kHz	Kilohertz
18	LM	Light microscope
19	MA	Malic Acid
20	MDA	Manual Dynamic Agitation
21	ml	Milliliter
22	mm	Millimeter
23	MTA	Mineral Trioxide Aggregate
24	n	Number of specimens
25	NaOCl	Sodium Hypochlorite
26	OSHA	Occupational Safety and Health Administration
27	PUI	Passive Ultrasonic Irrigation
28	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
29	RCS	Root canal system
30	RCT	Root canal treatment
31	SD	Standard Deviation

32	SE	Standard error
33	SEM	Scanning Electron Microscope
34	SI	Sonic irrigation
35	SP	Sealer Penetration
36	WL	Working Length
37	ZOE	Zinc-oxide Eugenol

ABSTRACT

Aim and Objectives: To evaluate and compare the tubular penetration of Epoxy resin and Bioceramic sealers following a final rinse of 0.2% Chitosan, with or without Ultrasonic activation using Confocal laser scanning microscopy (CLSM).

Study design: Hundred extracted human mandibular premolar teeth were selected, disinfected and decoronated to obtain samples with standardized root length of 14 mm. The samples were prepared with ProTaper Universal rotary files upto F3 (size 30, 0.09 taper) and were randomly divided into two groups of 50 samples each based on irrigating technique used.

Group I: 3% NaOCl and 0.2% Chitosan + Conventional Irrigation for 2 minutes.

Group II: 3% NaOCl and 0.2% Chitosan + Passive Ultrasonic Irrigation for 1 minute.

In both the groups, 5 ml of 3% NaOCl was used as an initial rinse for 1 min followed by 5ml of 0.2% Chitosan as a final rinse for 2 min. Each group was further divided into two subgroups of 25 samples each depending on the type of root canal sealer used.

Group IA: Epoxy resin based sealer (AH Plus)

Group IB: Bioceramic sealer (Ceraseal)

Group IIA: Epoxy resin based sealer (AH Plus)

Group IIB: Bioceramic sealer (Ceraseal)

Fluorescent dye (Rhodamine B isothiocynate) was added to the sealer during manipulation at an approximate ratio of 0.1% (weight). The root canals were obturated with Gutta-percha points and sealer. Teeth were then sealed with Cavit (3M, ESPE) and incubated at 37°C and 100% humidity for a week to simulate clinical conditions. Specimens were sectioned horizontally with a diamond disc at coronal (8mm from apex), middle (5mm from apex) and apical third (2mm from apex) of each root and these sections were categorized as a, b and c respectively. All sections were polished with silicon carbide abrasive paper (25 micro-meter) and specimens were mounted onto glass slides and examined and evaluated for depth of penetration of sealer by using Confocal Laser Scanning Microscopy (CLSM).

Results: The mean depth of penetration of Ceraseal was highest when irrigated with Chitosan and PUI (Group IIB=1476.86±37.18) than when using CI (Group IB=1230.60±39.86), with AH Plus however, the mean depth of dentinal tubule penetration was lower as compared to Ceraseal irrespective of the irrigation technique used, Groups IA (1021±34.11) and IIA (1230.18±38.36). In addition, PUI produced greater penetration of AH Plus compared to CI.

When the sections of the root were considered, the highest depth of sealer penetration was observed at coronal third (1506.19±34.59) followed by middle third (1223.34±31.40) and least was at the apical third (989.50±19.93) among all the groups. The higher tubule penetration values were observed with the Ceraseal groups in all the sections observed as compared to AH Plus, irrespective of the irrigation technique used.

However, when the interactions between the various sealers, activation technique and sections of the tooth was analysed, the mean depth of sealer penetration

was observed to be the highest for Bioceramic at the coronal third with PUI (1711.88±210.42) and the least was for AH plus at the apical third with CI (810.19±145.19).

Conclusion: Within the limitations of the present study, we can conclude that Bioceramic sealer (Ceraseal) showed higher depth of penetration into the dentinal tubules when compared with Epoxy resin (AH Plus). PUI further improved the effectiveness of Chitosan irrigant. Cleaning the apical third of root canal still remains an area of concern due to the reasons clearly discussed.

Key words: Chitosan, Passive Ultrasonic irrigation, Conventional Irrigation, Rhodamine dye, Ceraseal, Confocal Laser Scanning Microscope

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

ANNEXURES

INTRODUCTION

Key to successful root canal therapy relies on meticulous chemicomechanical preparation of infected pulpal tissue, dentin debris, and microorganisms.[1]

When root canals are instrumented during cleaning and shaping, material containing organic and inorganic content is deposited on the canal walls. This layer is known as SMEAR layer.[2] It is an amorphous structure which is about 1-2 μm thick. This layer functions as a tangible obstruction, hindering the infiltration of irrigants and medicaments, as well as impeding the attachment of sealers.[3]

Research indicates that the elimination of the smear layer is necessary for the long-term viability of root canal therapy.[2,4] Irrigants can aid preparation by eliminating debris, disintegrating organic and inorganic content, and cleaning the root canal system.[5] In teeth with intricate structures, like fins or other irregularities, chemical debridement may play a significantly role, especially in areas that might be missed during instrumentation.[1,5]

Sodium hypochlorite (NaOCl) is the primary choice of irrigant in endodontic procedures, esteemed as the gold standard for its remarkable antimicrobial efficacy and its ability to eradicate organic tissue.[6] However, it does not affect the inorganic content or the smear layer.[7] Hence, the integration of auxiliary chelating solutions during chemo-mechanical preparation aims to assist in the removal of this smear layer.[8] Moreover, elimination of this smear layer improves the sealer's penetration and retention. This penetration serves as a reliable indicator that, smear layer has indeed been effectively eliminated from the specific area.[9]

To eliminate the smear layer, a variety of chelating solutions have been used, including organic acids like Maleic acid (MA) and Citric acid (CA) and inorganic acids like phosphoric acid and ethylenediaminetetraacetic acid (EDTA).[10]

EDTA remains the preferred choice in clinical practice owing to its chelating properties, which efficiently dissolve the inorganic components of dentin.[11] This process significantly widens dentinal tubules, facilitating deeper penetration of irrigants.[12] As a result, it not only improves root canal disinfection but also enhances the penetration of sealers.[13] However, extended exposure to EDTA can pose limitations and drawbacks as a root canal irrigant, potentially leading to erosion of dentin and decrease in its microhardness of.[11,14] Additionally, EDTA is considered as a pollutant due to its synthetic nature and limited antimicrobial properties.[15]

Owing to all these concerns, a new chelating agent Chitosan, has attracted attention of dental researchers. Chitin, the second-most prevalent natural polysaccharide, is made up of repeating units of N-acetyl glucosamine monomer. Chitosan is produced as a result of partial breakdown of chitin.[16] Previous studies have proven Chitosan to be highly biocompatible, biodegradable, bioadhesive, and least toxic.[17] Chitosan has demonstrated numerous applications in dentistry and has been employed in medicine as an antibacterial, anticancer, wound healing accelerator and as an excipient.[16,18] Ballal et al., demonstrated that the incorporation of Chitosan into calcium hydroxide paste leads to prolonged release of calcium hydroxide ions within root canals.[19] Additionally, Silva et al., suggested that a 0.2% Chitosan exhibited comparable efficacy to higher concentrations of EDTA (15%) and CA (10%) in eliminating the smear layer.[20]

Endodontics has a deep-rooted history of utilizing ultrasonic energy to clean root canals and facilitate disinfection.[21] PUI activates irrigating solution through acoustic-microstreaming at 30 kHz, creating cavitation and hydrodynamic movement which enhances irrigant penetration into dentinal tubules.[22]

Following shaping and cleaning, it is essential to achieve 3D obturation of the RCS for the long term success.[23] Obturation typically consist of a low-viscosity sealing substance (sealer) and a core material (gutta-percha), which work together to seal the dentinal tubules, ramifications, and lateral canals while also making up for any defects in the prepared root canal.[24]

Based on their chemical makeup, range of sealers are employed in clinical practice such as ZOE, Epoxy resin, Calcium hydroxide, Silicone and recently Bioceramic based sealers.[25]

AH Plus, resin sealer, effectively eliminates microorganisms beyond depth of penetration, with pseudoplastic behavior enhancing flow during filling procedures and currently serve as gold standards for comparison.[26] Its desirable properties include greater stability, low solubility, radiopacity, and optimal adhesiveness. Despite advancements, it continues to have limitations, including the possibility of mutagenicity, cytotoxicity, inflammatory responses, and hydrophobicity.[25,26] To overcome these challenges, new calcium silicate based bioceramic sealers have been established.[27]

Bioceramic root canal sealers were first introduced by Krell and Wefel, its major advantages being their biocompatibility.[28] These sealers yield a molecular composition and crystal lattice similar to that of bone and tooth, thereby enhancing the bond between the dentin and sealer.[28,29]

Ceraseal is a new calcium silicate-based root canal sealer which is available in premixed form.[30] It is composed of calcium silicates, thickening agent, and zirconium oxide. It has numerous advantages such as short setting time, high flow rate, alkalinization ability, radio-opacity and volumetric expansion on setting.[30,31]

Literature has shown that Chitosan is a safe compound to use and it has many positive properties in restorative dentistry with no side effects.[20,32] There is a scarcity of literature exploring the impact of Chitosan on radicular dentin and tubular penetration of various sealers.

Keeping these concepts in mind, the current study is crafted to analyse the effect of Chitosan, with or without passive ultrasonic agitation on tubule penetration of different sealers.

OBJECTIVES

AIM OF THE STUDY:

To evaluate and compare the dentinal tubular penetration of Epoxy resin and Bioceramic sealers following a final rinse using 0.2% Chitosan with or without the use of passive ultrasonic activation.

OBJECTIVES:

1. To evaluate the tubular penetration of an Epoxy resin sealer following a final rinse using 0.2% Chitosan with or without passive ultrasonic activation using Confocal laser scanning microscopy.

2. To evaluate the tubular penetration of a Bioceramic sealer following a final rinse using 0.2% Chitosan with or without passive ultrasonic activation using Confocal laser scanning microscopy.

3. To compare the tubular penetration of Epoxy resin and Bioceramic sealers following a final rinse using 0.2% Chitosan with or without passive ultrasonic activation using Confocal laser scanning microscopy.

HYPOTHESIS

NULL HYPOTHESIS:

There is no difference in the tubular penetration of Epoxy resin and Bioceramic sealers following a final rinse using 0.2% Chitosan, with or without passive ultrasonic activation.

ALTERNATE HYPOTHESIS:

There is a difference in the tubular penetration of Epoxy resin and Bioceramic sealers following a final rinse using 0.2% Chitosan, with or without passive ultrasonic activation.

REVIEW OF LITERATURE

1. Wael and associates examined Ceraseal and Bio-C Sealer's interfacial adaptability in relation to AH Plus. AH Plus, Bio-C Sealer, and Ceraseal were the three groups into which the 66 human premolars with single roots were split. Next, SEM was used to assess each group, and CLSM was employed to ascertain the extent of infiltration. According to the study's findings, Bio-C sealer had the best sealer penetration into the dentinal tubules, whereas AH Plus demonstrated good sealer adaption to the root canal wall.[32]
2. Shaker M and coworkers investigated the effect of PUI on the depth of penetration of BS (CeraSeal) and Resin sealer (Adseal) into the dentinal tubules with cold lateral compaction obturation technique. The study concluded that irrigation activation is an essential step in canal preparation for better smear layer removal that results in deeper sealer penetration and higher sealing ability of obturation which was examined using SEM and also concluded that Bioceramic sealers have higher sealing ability than Epoxy resin sealers.[33]
3. Hussein ER and coworkers investigated efficacy of EDTA, Chitosan nanoparticles and Citric acid (CA) in dissolving smear layer using different needles (IrriFlex, ProRinse) and viewed under SEM. Four experimental groups and one control group was studied. The study concluded that Chitosan nanoparticles showed same efficiency as other irrigants but was more efficient in the apical region. IrriFlex tips were more effective compared to ProRinse tips when used with EDTA and CA.[34]
4. Rekha R et al., investigated the effect of sonic activation on dentinal penetration of bio-ceramic sealers in root canals of lower molars filled with EndoSequence BC sealer, which were divided into two groups i.e, with or without sonic

activation and evaluated using CLSM. The study concluded that the agitation of bio-ceramic cement using sonics showed higher penetration.[35]

5. Chew STH et al., examined the differences between three distinct bio-ceramic-based sealers and an epoxy resin-based sealer for their interfacial adaptability and depth of penetration. Forty lower premolars were split into five groups: CeraSeal, Nishika bioactive glass sealer and EndoSeal MTA and AH Plus. The penetration depth was assessed using a CLSM. Nishika bioactive glass sealer had higher adaptability to radicular dentin than EndoSeal MTA in apical and middle region of canal. When compared to AH Plus and EndoSeal MTA, Nishika sealer has a substantially deeper penetration depth at the coronal third of radicular dentin.[36]

6. Abidin T et al., investigated the impact of the Chitosan oligo-saccharide irrigating solution on penetration of two endodontic sealer. 3 groups of mandibular premolars were made -2% Chitosan oligosaccharides, 2.5% NaOCl and 0.2% Chitosan nanoparticles combination, and 2.5% NaOCl and 17% EDTA combination. Obturation was performed with CeraSeal Bioceramic and AH Plus sealer and evaluated with a confocal laser scanning microscope. The study concluded that the irrigation group employing Chitosan oligosaccharide had the highest mean penetration depth, and furthermore, bio-ceramic based CeraSeal sealer performed better than resin based AH Plus sealer.[37]

7. Veeramachaneni C and coworkers investigated the bond strength of bio-ceramic and epoxy sealers after irrigation using 5% glycolic acid, 17% glycolic acid, 0.2% Chitosan, 17% EDTA and 0.9% saline. Samples were obturated and subjected to push-out test. According to the study's findings, Bio C sealer performed best followed by glycolic acid with no significant difference among 5% and 17% glycolic acid.[38]

8. Ratih DN et al., examined the impact of Chitosan nanoparticles as last irrigant on the root dentin's surface roughness, microhardness, and elimination of smear layers. The study found that, when compared to 17% EDTA, the final treatment with 0.2% Chitosan nano-particles had same effect on inorganic smear layer removal however lower surface roughness and higher micro-hardness was seen.[39]
9. Bogari DF examined the mechanical and biological effects of various irrigation techniques on the bioceramic sealer's ability to adhere to root canal dentin walls. 3 irrigation activation techniques- the syringe needle irrigation method, ultrasonic activation and Nd:YAG laser activation were evaluated. According to study, no difference between syringe needle irrigation and PUI group, and also concluded that RC system irrigation protocol had effect on the BC sealers adaptation and bond strength to radicular dentin.[40]
10. Matos FS et al., compared CI and PUI with 17% EDTA and QMiX on sealer penetration into dentinal tubules. 80 lower premolars were divided into 4 groups: QMiX + CI, EDTA + CI, QMiX + PUI and EDTA + PUI and were examined by SEM and CLSM, following obturation with a gutta-percha cone and AH Plus sealer. PUI protocols showed higher rates of debris/smear layer removal with both QMiX and EDTA. PUI was superior in the middle third interms of sealer penetration.[41]
11. Caceres C and coworkers evaluated penetration of dentinal tubules and adaptability of AH Plus and Bio-C sealers. In this investigation, thirty wide, single, straight canals were prepared endodontically, split into two groups at random based on the sealer, and were observed under SEM. Study found that Bio-C sealer had greater penetration and adaptability in all the sections section in contrast to AH Plus sealer.[42]

12. Antunovic M et al., assessed the bacterial leakage of four different bioceramic RCS (MTA+, MTA Fillapex, Totalfill BC, BioRoot) when compared to the epoxy resin sealer for evaluating Enterococcus faecalis leakage, scanning electron microscopy was utilised to examine adaptation between sealer and dentine wall. According to this study, TotalFill BCS sealer outperformed MTA Plus and AH Plus sealers in terms of sealing ability.[43]
13. Antunes PVS et al., evaluated dentin microhardness, push-out strength, and sealer dentin tubule after final flush with Chitosan and EDTA. The study found that 0.2% Chitosan and 15% EDTA act similarly, and that both solutions effects are enhanced when Endovac is used.[44]
14. Arathi G et al., used a light microscope to assess the depth of canal irrigants penetration into dentinal tubules with and without ultrasonics. 40 non-carious mandibular premolars were divided into four groups of ten each and was irrigated with 2% Chlorhexidine (CHX) and agitated ultrasonically, 2% Chlorhexidine, 2% Chitosan and ultrasonically agitated, 2 % Chitosan. In comparison to Chitosan, the study indicated that 2% chlorhexidine, when used as an irrigant with ultrasonic agitation, had the largest depth of penetration into the dentinal tubules.[45]
15. Abraham S et al., examined efficacy of Endoactivator, diode laser, and passive ultrasonics for removing smear layers from root canals containing 0.2% Chitosan at the apical third. Forty mandibular premolars were prepared and irrigated using several methods of 0.2% Chitosan activation: diode laser, endoActivator, passive ultrasonics, and Chitosan activated with 0.2% Chitosan. When compared to passive ultrasonic irrigation, the study found that Diode laser and Endoactivator with 0.2% Chitosan performed more effective in removal of inorganic debris.[46]

16. Souza CC et al., studied three activation methods used in the final irrigation of endodontic treatments. The 80 uniradicular teeth were separated into four groups: control, passive ultrasonic irrigation, continuous ultrasonic irrigation, and simple clean. The irrigant's penetration into the samples was assessed by Image J application. The study concluded that employing a positive syringe and needle pressure did not properly transport the irrigant to the fabricated lateral channels, although PUI, CUI, and easy clean were all equally effective.[47]
17. Vineetha CS et al., studied ability of inorganic debris removal by ultrasonic agitation of EDTA and Chitosan which was examined by SEM. The study demonstrated that ultrasonic activation of EDTA had a superior ability to remove smear layer, while Chitosan performed better when compared to saline, however, EDTA outperformed Chitosan.[48]
18. Zand V et al., investigated the impact of various NaOCl agitation methods on the penetration of Sure Seal Root bioceramic sealer into dentinal tubules. It divided single-rooted human teeth into four groups (10 each): XP-Finisher file, Er: YAG laser, PUI and conventional needle irrigation. The PUI technique showed a deeper penetration of the sealer into dentinal tubules.[49]
19. Bertan Kesim and associates examined the efficacy of utilizing Chitosan, EDTA, and citric acid during the final irrigation process. 70 mandibular premolars were obturated using a resin-based sealant and were observed under CLSM. The investigators concluded that the percentage of SP for coronal thirds was greatly increased by Chitosan, EDTA, and CA.[50]
20. Thota MM and coworkers examined the impact of irrigating solutions on tubular penetration of sealer in human mandibular premolar teeth. The teeth were divided into two groups and irrigated with EDTA and Chitosan. After obturation and

sealing with AH 26 sealer, it was observed that higher sealer penetration was seen in EDTA at coronal section, but in apical section higher penetration was observed in Chitosan.[51]

21. Singh CV et al., conducted a study on the penetration depth of resin-based sealers (AH plus, Resino Seal, and Zinc Oxide Eugenol) into dentinal tubules after eliminating smear layer using PUI and NaOCl (2.5%). After obturating 30 extracted maxillary central incisors with three sealers, sections of the roots were sputtered with gold and examined under a SEM. According to the study, AH Plus penetrated dentinal tubules to the greatest depth.[52]
22. Praveen M et al., conducted a study on the effectiveness of 10% CA, 4% Chitosan citrate and 2% Chitosan as final flush during irrigation regimen. The study involved 60 upper anterior teeth, and found that the groups using 2%, 10%, and 4% citric acid had the least erosion, debris, and smear layer in the root canal.[53]
23. Darrag AM et al., studied smear layer removal in 50 maxillary central incisors after irrigation with Chitosan (0.2%), Citric acid (10%), EDTA (17%) and Biopure MTAD solutions. Results showed that Chitosan at 0.2% had the lowest mean smear layer scores, followed by MTAD. Chitosan was highly effective in dissolving smear layer, suggesting it as a superior irrigant.[54]
24. Silva et al., conducted a study on the effectiveness of different chelators in removing smear layers from root canals. They created 25 canals and measured Ca^{+} in solutions using AASF. Results showed that 0.2% Chitosan, 10% citric acid, 15% EDTA, and 1% acetic acid effectively eliminated the inorganic layer in middle and apical regions of root.[55]

MATERIALS AND METHODOLOGY

STUDY DESIGN: In-vitro study.

SOURCE OF DATA

- The study was conducted in Department of Conservative Dentistry and Endodontics, KLE Academy of Higher Education & Research (KAHER), KLE V K Institute of Dental Sciences, Belagavi.
- Extracted human mandibular premolar teeth were collected from Department of Oral and Maxillofacial surgery, KLE Academy of Higher Education & Research (KAHER), KLE V K Institute of Dental Sciences Belagavi.
- Chitosan (0.2% Solution) was prepared at the KLE College of Pharmacy, KAHER, Belagavi, Karnataka.
- Confocal Laser Scanning Microscopy was carried out at the Birla Institute of Technology and Science (BITS), Pilani, Goa.

SAMPLE SIZE ESTIMATION:

Sample size was estimated at 95% Confidence Interval and 80% power using the formula,

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

Where ,

$\bar{x}_1$ = 1.96

$\bar{x}_2$ = 0.81

SD_1 = 1.73

SD_2 = 0.80

$Z_{1-\alpha/2}$ = 1.96

$Z_{1-\beta}$ = 0.85

n = 22 per group

(The sample size for each group was rounded off to 25 for convenience)

There are four main groups, so the total sample size n= 25 x 4 = 100

All the teeth were evaluated using radiographs and magnification and selected based on the following inclusion and exclusion criteria:

INCLUSION CRITERIA

- Extracted human Intact single rooted single straight canal mandibular premolar teeth.
- Teeth with apical width corresponding to #20 K-file or less.

EXCLUSION CRITERIA:

- Calcified canals.
- Fracture/crack or a restoration.
- Internal and external root resorption.
- Anatomic variations.

MATERIALS USED FOR STUDY

- Extracted Human mandibular premolar teeth
- 0.1% Thymol solution (S D FINE-CHEMICALS LIMITED, MUMBAI)
- 3% Sodium hypochlorite (VISHAL DENTOCARE, AHMEDABAD)
- Chitosan powder (SIGMA-ALDRICH, BANGALORE)
- Paper points (DIADENT GROUP INTERNATIONAL, KOREA)
- Rhodamine B dye (SIGMA ALDRICH, BANGALORE)
- Distilled water (NICE LIFE CARE, NEW DELHI)
- Normal saline solution (AISHWARYA, SOLAN, ODISHA)
- AH Plus sealer (DENTSPLY, GERMANY)
- Ceraseal sealer (METABIOMED CO., KOREA)
- Gutta-percha points (DIADENT GROUP INTERNATIONAL, KOREA)
- Cavit (3M, ESPE, USA)
- Lentulospiral (No-30) (MANI INC, JAPAN)

ARMAMENTARIUM:

- Micromotor (NSK, JAPAN)
- Gates Glidden Drills (MANI, JAPAN)
- #10 K- File (MANI INC, JAPAN)
- ProTaper Universal nickel-titanium files (DENTSPLY MAILLEFER, SWITZERLAND)
- Endomotor (X SMART, DENTSPLY, GERMANY)
- Surgical disposable needles and syringes (27 GAUGE, 5ML, DISPOVAN, INDIA)

- Diamond disc (KWALITY DIAMOND TOOLS, MUMBAI)
- Incubator (BIO TECHNICS, MUMBAI, INDIA)
- Ultrasonic scaler (WOODPECKER, GUANGZHOU, CHINA)
- PUI adapter (E1, WOODPECKER, GUANGZHOU, CHINA)
- Confocal laser scanning microscope (OLYMPUS FLUOVIEW FV3000)
- Dental operating microscope (CARL ZEISS, GERMANY)
- Image J software (FIJI)
- X-ray unit (AMS, BANGALORE, INDIA)
- X- ray sensor (SCHICK 33, DENTSPLY SIRONA, NEW YORK, USA)

<u>Preparation of Study materials:</u>

Preparation of 0.2%Chitosan irrigant-

0.2g of Chitosan mixed with 100 ml of 1% acetic acid, and stirred continuously for 2hrs using a magnetic stirrer.

METHODOLOGY: -

Hundred lower premolar teeth extracted from human subjects were used and handled according to OSHA guidelines. All the teeth were evaluated using radiographs and magnification. Root length was standardized to 14mm by decoronating them with diamond disc under water. WL was estimated by subtracting 1 millimeter from the point where K-file (#10) exits the foramen. BMP was carried out using Protaper Universal instruments, achieving an apical size of F3 (size 30, 0.09 taper). Samples were allocated into two groups (n=50) depending upon technique of irrigation.

Group I: 3% NaOCl and 0.2% Chitosan + CI for 2 minutes.

Group II: 3% NaOCl and 0.2% Chitosan + PUI for 1 minute.

In both the groups, 5 ml of 3% NaOCl was used as an initial rinse for 1 minute followed by final flush with 5ml of Chitosan for 2 minutes, and further divided depending on type of root canal sealer (n=25 per group)

Group IA: Epoxy resin-based sealer (AH Plus)

Group IB: Bioceramic sealer (Ceraseal)

Group IIA: Epoxy resin-based sealer (AH Plus)

Group IIB: Bioceramic sealer (Ceraseal)

A Fluorescent dye (Rhodamine B isothiocynate) was incoperated into the sealer during mixing at a ratio of 0.1% (weight), to promote fluorescence under CLSM. The endodontic sealer was applied using no. 30 lentulospiral and then a gutta percha coated with sealer was placed in canal. Cavit (3M, ESPE) was used for sealing teeth and incubated at 37°C and 100% humidity for a week to simulate clinical conditions.

<u>Sample Preparation:</u>

Specimens, each with a thickness of 1mm, were prepared by sectioning teeth horizontally with a diamond disc at coronal (8mm from apex), middle (5mm from apex) and apical thirds (2mm from apex) of each root. These sections were categorized as **a, b** and **c** respectively. All sections were polished with silicon carbide abrasive paper (25 micro-meter). All specimens were mounted onto glass slides and the specimens were examined using CLSM.

Calculation of 'dentinal tubule penetration' (in μm):

CLSM images were assessed using the Fiji Image J software and maximum penetration was measured. The depth of penetrability was gauged using the measuring tool in the Image J software.

STATISTICAL ANALYSIS:

The data was statistically analyzed using-

- Two-way ANOVA- for intra group comparison
- Tukey's Multiple Post hoc test- for inter group comparison

Study design

Hundred extracted human mandibular premolar teeth were selected and handled according to OSHA guidelines.

↓

All the teeth were radiographed, observed under magnification and selected according to the inclusion and exclusion criteria

↓

Calculus and soft tissue debris were cleaned using Ultrasonic scaler and immersed in 0.1% Thymol

↓

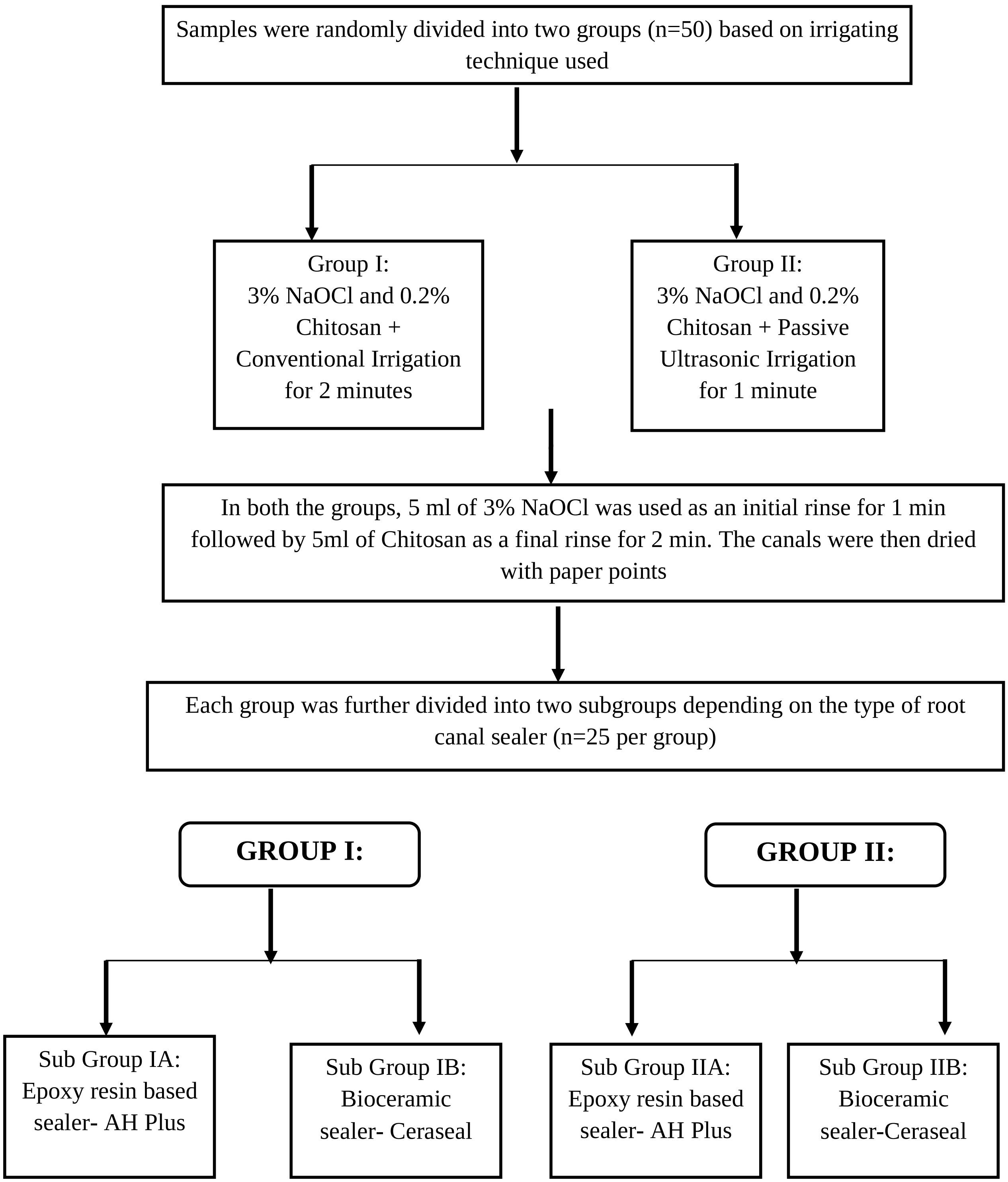
The teeth were decoronated using a diamond disc under copious water spray to acquire a standardized root length of 14 mm from the apex

↓

Working length was established 1mm short of the length where #10 K-file exits the apical foramen

↓

Cleaning and shaping was performed using Protaper Universal (DENTSPLY) NiTi rotary instrument till size F3


Samples were randomly divided into two groups (n=50) based on irrigating technique used
Group I: 3% NaOCl and 0.2% Chitosan + Conventional Irrigation for 2 minutes
Group II: 3% NaOCl and 0.2% Chitosan + Passive Ultrasonic Irrigation for 1 minute
In both the groups, 5 ml of 3% NaOCl was used as an initial rinse for 1 min followed by 5ml of Chitosan as a final rinse for 2 min. The canals were then dried with paper points
Each group was further divided into two subgroups depending on the type of root canal sealer (n=25 per group)
GROUP I:
GROUP II:
Sub Group IA: Epoxy resin based sealer- AH Plus
Sub Group IB: Bioceramic sealer- Ceraseal
Sub Group IIA: Epoxy resin based sealer- AH Plus
Sub Group IIB: Bioceramic sealer-Ceraseal

Fluorescent dye (Rhodamine B isothiocynate) was added to the sealer during manipulation at an approximate ratio of 0.1% (weight), to promote fluorescence under CLSM

↓

The endodontic sealer was applied in the canal 1mm short of the working length using no. 30 lentulospiral and then master cone coated with sealer was placed into the canal

↓

Excess Gutta-percha was cut off at orifice level using a heated plugger

↓

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

↓

Specimens were sectioned horizontally with a diamond disc at coronal (8mm from apex), middle (5mm from apex) and apical third (2mm from apex) of the root to obtain slices of 1mm thickness each and were categorized as a, b and c respectively.

↓

All sections were then polished with silicon carbide abrasive paper (25 micro-meter)

↓

All specimens were mounted onto glass slides and the specimens were examined by using Confocal Laser Scanning Microscope (CLSM)

↓

Image analysis was done using the Fiji Image J software

↓

The collected data was statistically analysed using Two-way ANOVA and Tukey's Multiple Post hoc test

MATERIALS USED FOR THE STUDY:

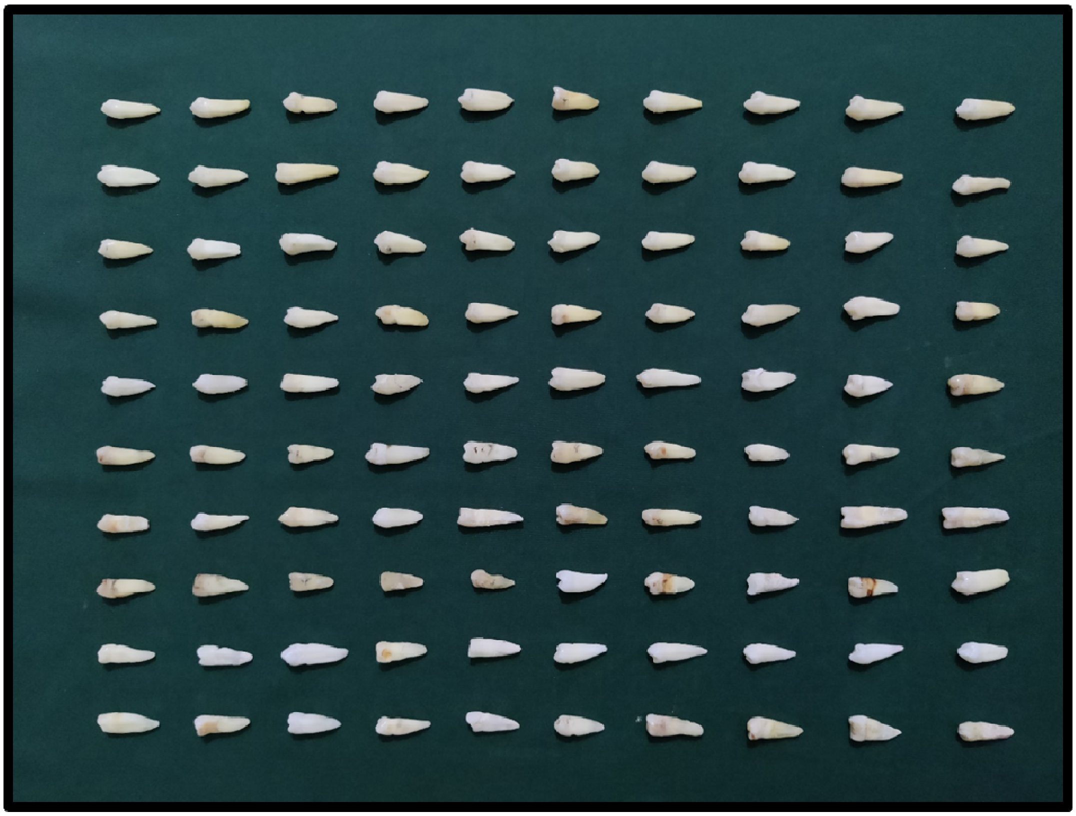


Fig 1: Extracted Human mandibular Premolars (n=100)

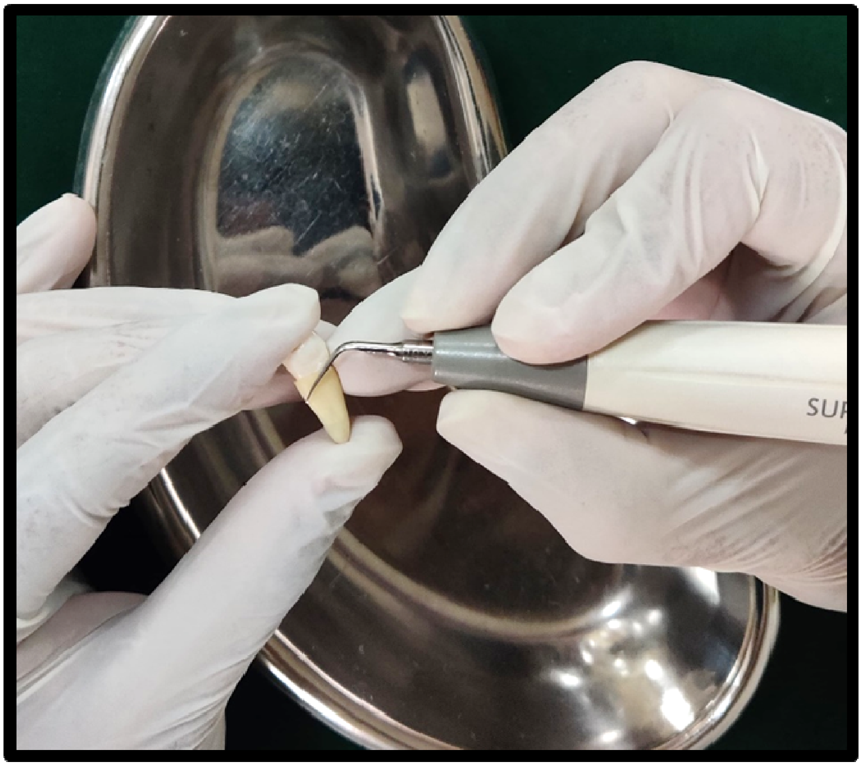


Fig 2: Debris Removal

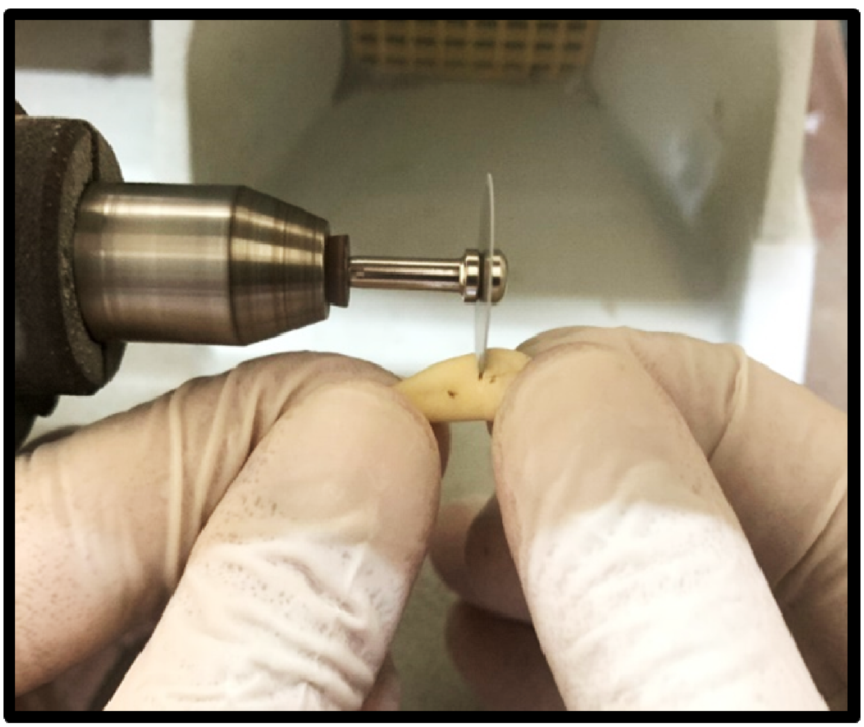


Fig 3: Premolar decoronated at the level of CEJ using Diamond disc

Fig 4: Materials and armamentarium used for chemomechanical preparation

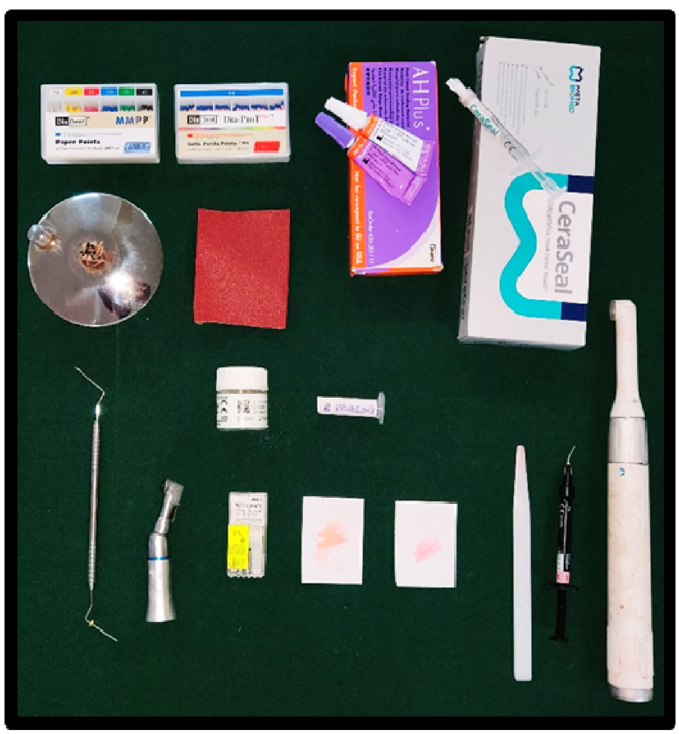


Fig 5: Materials and armamentarium used for obturation

Fig 6: Working Length Determination

Fig 7: Bio-Mechanical Preparation

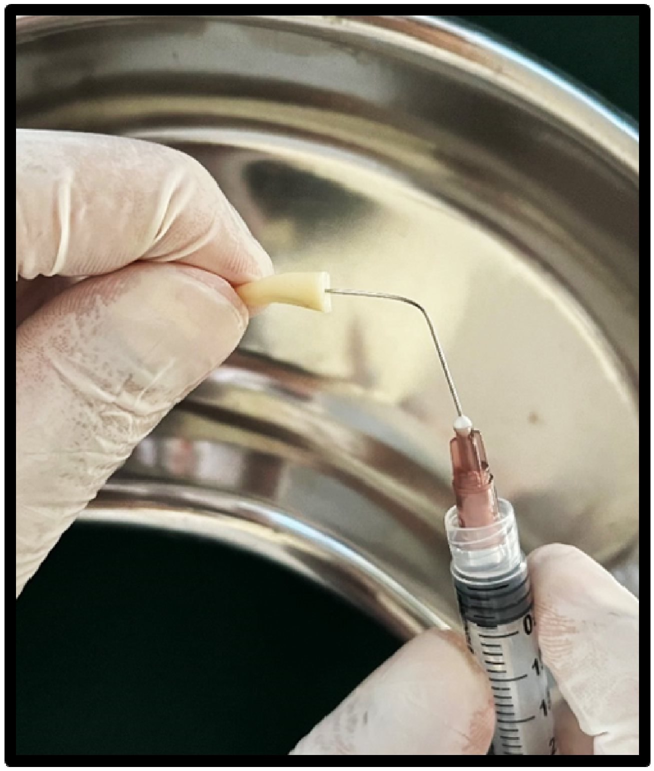


Fig 8: Sodium hypochlorite Irrigation in between instrumentation

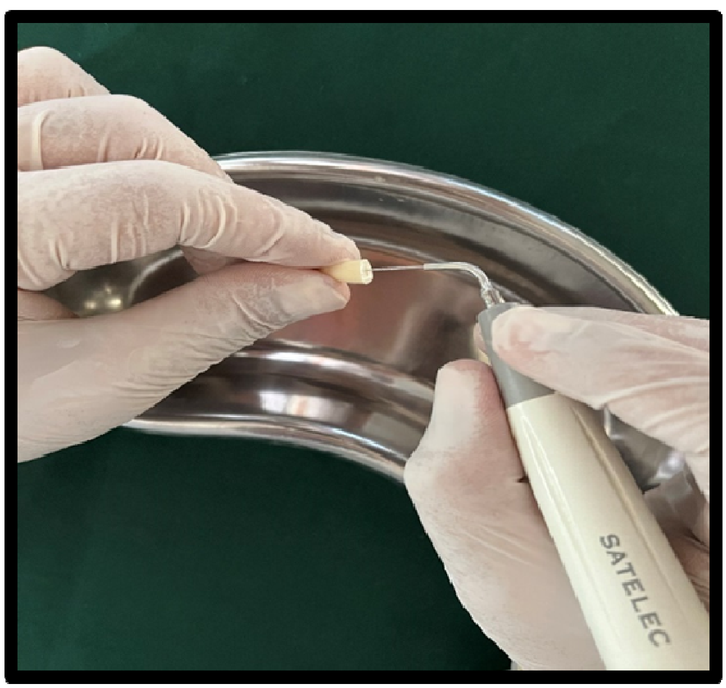


Fig 9: Passive Ultrasonic Irrigation

Fig 10: Drying canals with paper points

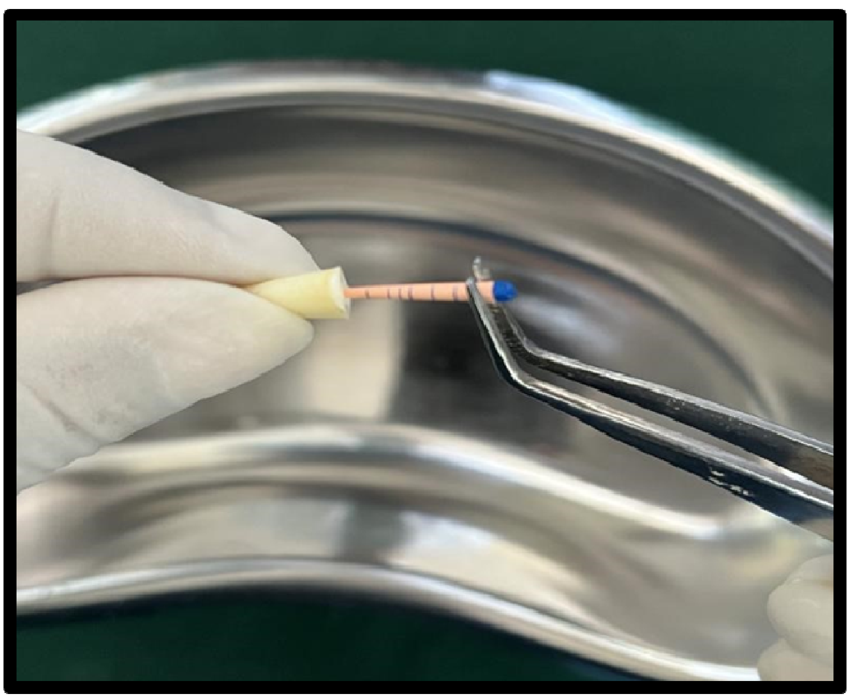


Fig 11: Obturation

Fig 12: Incubator

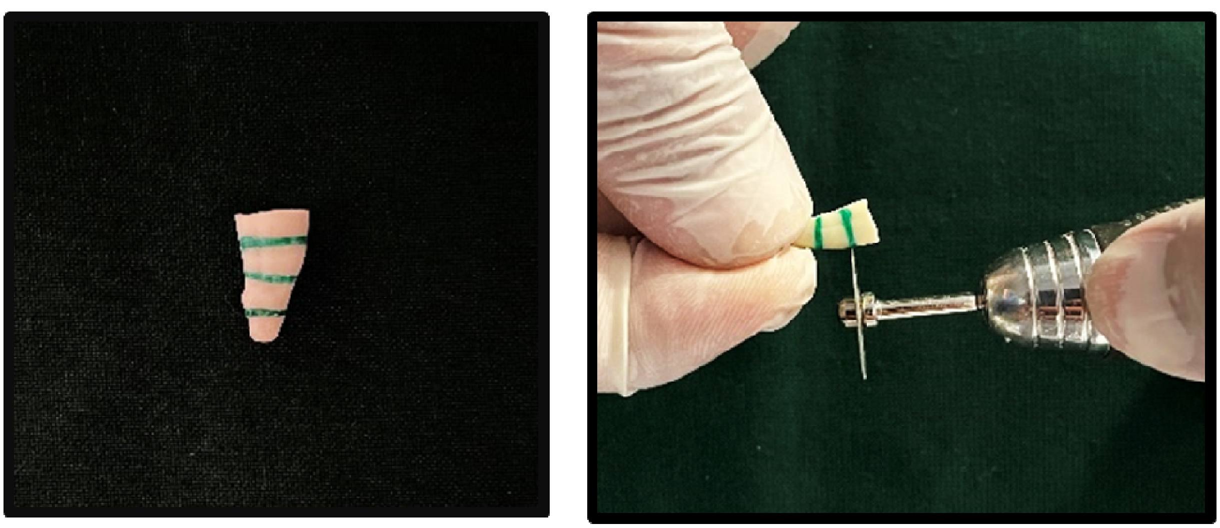


Fig 13: Sectioning of sample at Coronal, Middle and Apical third

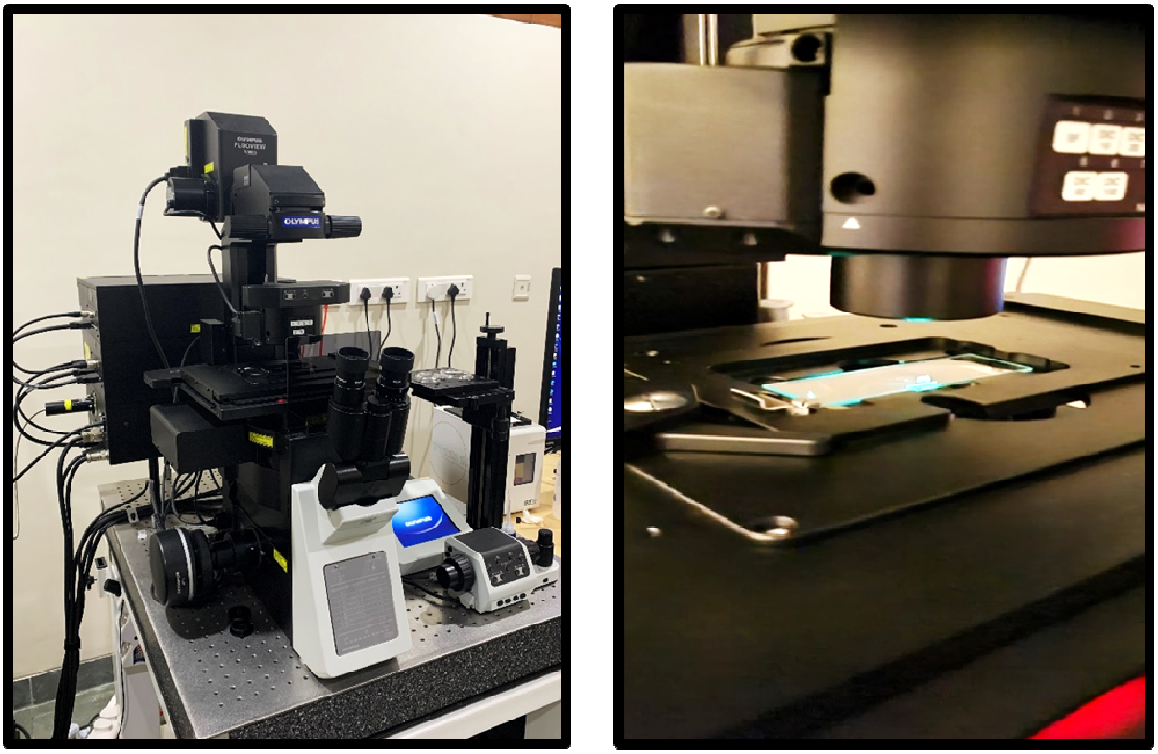


Fig 14: Confocal Laser Scanning Microscope

RESULTS:

Table 1: Summary of dentinal tubule penetration observed in four groups and three regions

Factor	Level of factor	N	Mean	SD	SE	95% CI for mean	
						Lower	Upper
Groups	CI + AH Plus	75	1021.06	295.41	34.11	953.10	1089.03
	CI + Ceraseal	75	1230.60	345.18	39.86	1151.18	1310.02
	PUI + AH Plus	75	1230.18	332.25	38.36	1153.73	1306.62
	PUI + Ceraseal	75	1476.86	321.96	37.18	1402.78	1550.93
Regions	Coronal	100	1506.19	345.94	34.59	1437.54	1574.83
	Middle	100	1223.34	314.04	31.40	1161.03	1285.65
	Apical	100	989.50	199.27	19.93	949.96	1029.04
Group *region	CI + AH Plus with Coronal	25	1256.33	360.12	72.02	1107.68	1404.98
	CI + AH Plus with Middle	25	996.67	117.08	23.42	948.35	1045.00
	CI + AH Plus with Apical	25	810.19	145.19	29.04	750.26	870.12
	CI + Ceraseal with Coronal	25	1591.35	281.07	56.21	1475.33	1707.36
	CI + Ceraseal with Middle	25	1167.51	218.74	43.75	1077.22	1257.80
	CI + Ceraseal with Apical	25	932.95	92.45	18.49	894.79	971.11
	PUI + AH Plus with Coronal	25	1465.19	349.70	69.94	1320.84	1609.54
	PUI + AH Plus with Middle	25	1225.00	302.00	60.40	1100.34	1349.66
	PUI + AH Plus with Apical	25	1000.34	119.52	23.90	951.01	1049.68
	PUI + Ceraseal with Coronal	25	1711.88	210.42	42.08	1625.02	1798.73
	PUI + Ceraseal with Middle	25	1504.19	339.28	67.86	1364.14	1644.24
	PUI + Ceraseal with Apical	25	1214.50	173.81	34.76	1142.76	1286.25

The mean depth of penetration of Ceraseal was highest when irrigated with Chitosan and PUI (Group IIB=1476.86±37.18) than when using CI (Group IB=1230.60±39.86), with AH Plus however, the mean depth of dentinal tubule penetration was lower as compared to Ceraseal irrespective of the irrigation technique used, Groups IA (1021±34.11) and IIA (1230.18±38.36). In addition, PUI produced greater penetration of AH Plus compared to CI.

When the sections of the root were considered, the highest depth of sealer penetration was observed at coronal third (1506.19±34.59) followed by middle third (1223.34±31.40) and least was at apical third (989.50±19.93) among all the groups. The higher tubule penetration values were observed with the Ceraseal groups in all the sections observed as compared to AH Plus, irrespective of the irrigation technique used.

Table 2: Comparison of mean dentinal tubule penetration with four groups and three regions by two way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Group	7816329.77	3	2605443.26	43.5911	0.0001*
Region	13388416.50	2	6694208.25	111.9994	0.0001*
2-way interaction effects					
Group * Region	512068.95	6	85344.83	1.4279	0.2037
Error	17213771.50	288	59770.04		
Total	38930586.72	299			

*p<0.05

When the four groups were compared by two way ANOVA there was a statistical significant difference among the groups (p value 0.0001) and also among the sections of the root (p value 0.0001). However, the 2-way interaction between the irrigants and regions of the tooth did not reveal statistically significant difference. (p value 0.2037).

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

Groups	CI + AH Plus	CI + Ceraseal	PUI + AH Plus	PUI + Ceraseal
Mean	1021.06	1230.60	1230.18	1476.86
SD	295.41	345.18	332.25	321.96
CI + AH Plus	-			
CI + Ceraseal	p=0.0001*	-		
PUI + AH Plus	p=0.0001*	p=1.0000	-	
PUI + Ceraseal	p=0.0001*	p=0.0001*	p=0.0001*	-

*p<0.05

Table 3 compares the dentinal tubule penetration of AH Plus and Ceraseal following CI and PUI using Tukeys multiple posthoc test. All groups showed a statistically significant difference in sealer penetration depth, except for Group IB & IIA, where p=1.0000.

Graph 1: Pair wise comparison of mean dentinal tubule penetration with four groups

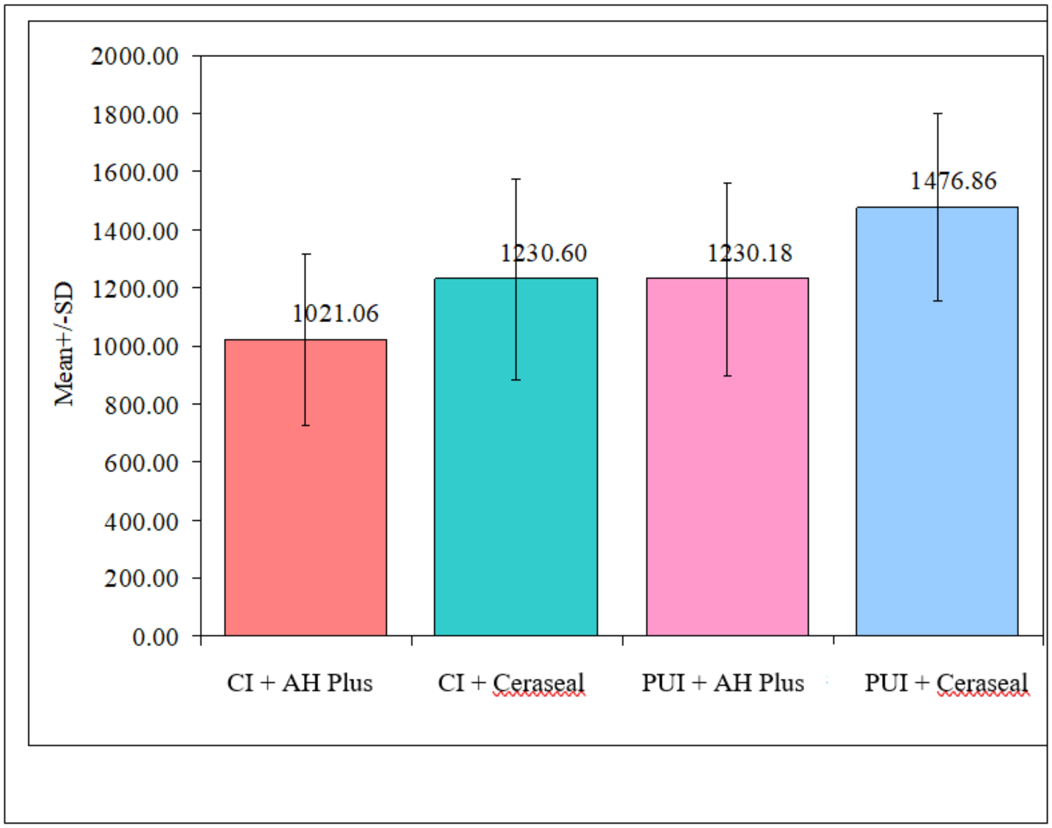


Table 4: Pair wise comparison of mean dentinal tubule penetration with three regions by Tukeys multiple posthoc procedures

Region	Coronal	Middle	Apical
Mean	1506.19	1223.34	989.50
SD	345.94	314.04	199.27
Coronal	-		
Middle	P=0.0001*	-	
Apical	P=0.0001*	P=0.0001*	-

*p<0.05

Graph 2: Pair wise comparison of mean dentinal tubule penetration with three regions

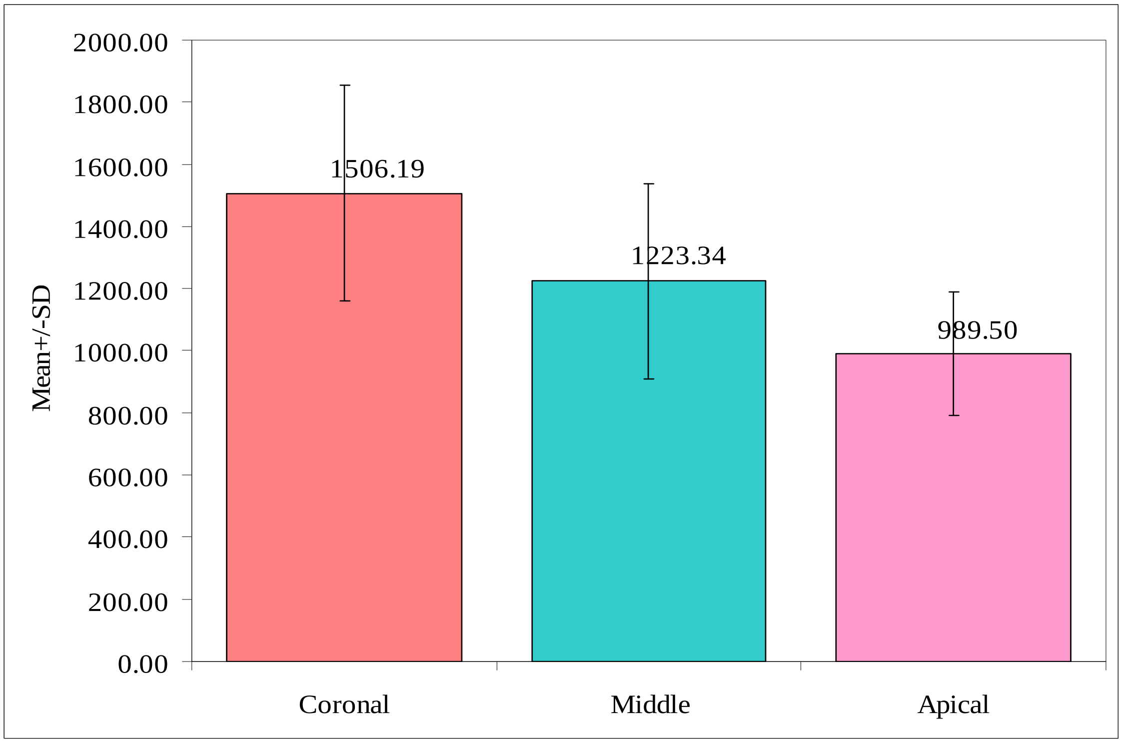


Table 4 and Graph 2 shows pairwise comparison of the mean dentinal tubule penetration within various root sections using Tukey's multiple post hoc test. The analysis revealed a statistically significant difference among all regions (p=0.0001).

Table 5: Comparison of interactions of mean dentinal tubule penetration with four groups and three regions by Tukeys multiple posthoc procedures

Interactions	CI + AH Plus with Coronal	CI + AH Plus with Middle	CI + AH Plus with Apical	CI + Ceraseal with Coronal	CI + Ceraseal with Middle	CI + Ceraseal with Apical
Mean	1256.33	996.67	810.19	1591.35	1167.51	932.95
SD	360.12	117.08	145.19	281.07	218.74	92.45
CI + AH Plus with Coronal	-					
CI + AH Plus with Middle	p=0.0095*	-				
CI + AH Plus with Apical	p=0.0001*	p=0.2276	-			
CI + Ceraseal with Coronal	p=0.0001*	p=0.0001*	p=0.0001*	-		
CI + Ceraseal with Middle	p=0.9812	p=0.3586	p=0.0001*	p=0.0001*	-	
CI + Ceraseal with Apical	p=0.0002*	p=0.9989	p=0.8317	p=0.0001*	p=0.0338*	-
PUI + AH Plus with Coronal	p=0.1026	p=0.0001*	p=0.0001*	p=0.8048	p=0.0010*	p=0.0001*
PUI + AH Plus with Middle	p=1.0000	p=0.0450*	p=0.0001*	p=0.0001*	p=0.9996	p=0.0015*
PUI + AH Plus with Apical	p=0.0115*	p=1.0000	p=0.2020	p=0.0001*	p=0.3939	p=0.9982
PUI + Ceraseal with Coronal	p=0.0001*	p=0.0001*	p=0.0001*	p=0.8482	p=0.0001*	p=0.0001*
PUI + Ceraseal with Middle	p=0.0176*	p=0.0001*	p=0.0001*	p=0.9838	p=0.0001*	p=0.0001*
PUI + Ceraseal with Apical	p=1.0000	p=0.0712	p=0.0001*	p=0.0001*	p=0.9999	p=0.0027*

*p<0.05

Continued…

Table 5: Comparison of interactions of mean dentinal tubule penetration with four groups and three regions by Tukeys multiple posthoc procedures

Interactions	PUI + AH Plus with Coronal	PUI + AH Plus with Middle	PUI + AH Plus with Apical	PUI + Ceraseal with Coronal	PUI + Ceraseal with Middle	PUI + Ceraseal with Apical
Mean	1465.19	1225.00	1000.34	1711.88	1504.19	1214.50
SD	349.70	302.00	119.52	210.42	339.28	173.81
CI + AH Plus with Coronal						
CI + AH Plus with Middle						
CI + AH Plus with Apical						
CI + Ceraseal with Coronal						
CI + Ceraseal with Middle						
CI + Ceraseal with Apical						
PUI + AH Plus with Coronal	-					
PUI + AH Plus with Middle	p=0.0258*	-				
PUI + AH Plus with Apical	p=0.0001*	p=0.0530	-			
PUI + Ceraseal with Coronal	p=0.0187*	p=0.0001*	p=0.0001*	-		
PUI + Ceraseal with Middle	p=1.0000	p=0.0032*	p=0.0001*	p=0.1074	-	
PUI + Ceraseal with Apical	p=0.0152*	p=1.0000	p=0.0829	p=0.0001*	p=0.0017*	-

*p<0.05

Graph 3: Comparison of interactions of mean dentinal tubule penetration with four groups and three regions

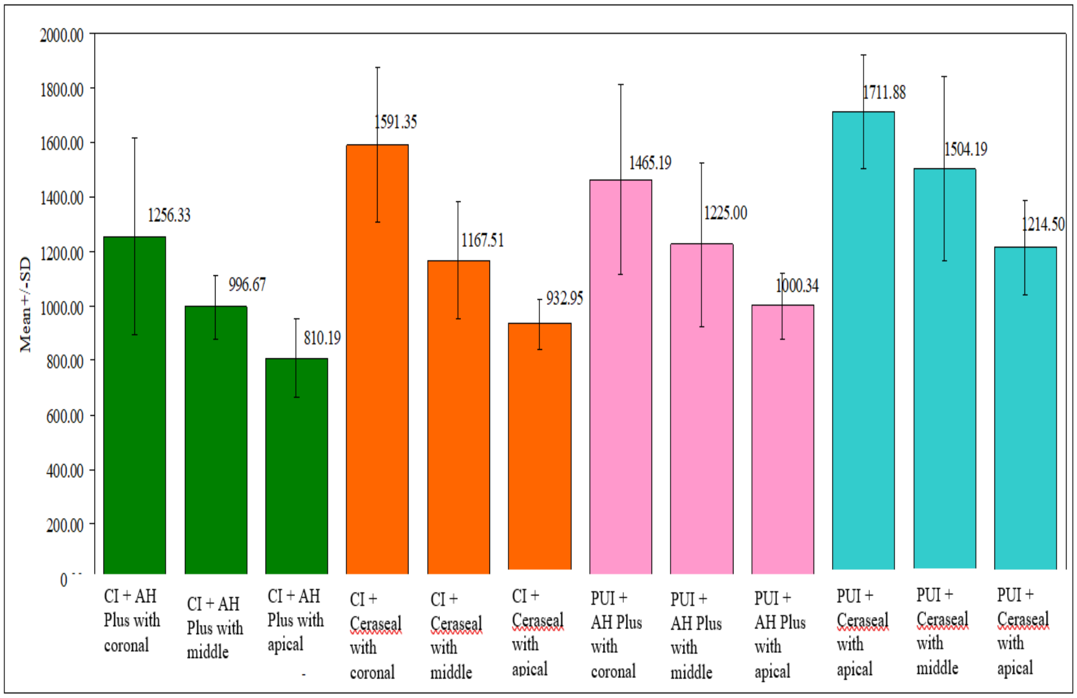


Table 5 and Graph 3 shows pair wise comparisons of mean depth of penetration of sealer among the four groups and three regions by Tukeys multiple posthoc procedure. The mean depth of sealer penetration with agitation technique was highest for IIBa (1711.88±210.42) and least was for IAc (810.19±145.19). Among the 66 interactions analyzed, a significant difference ($p<0.05$) was noted in 43 interactions.

Fig 15: CLSM Images depicting penetration of AH Plus sealer after final irrigation with 0.2% Chitosan using CI technique at coronal, middle and apical sections

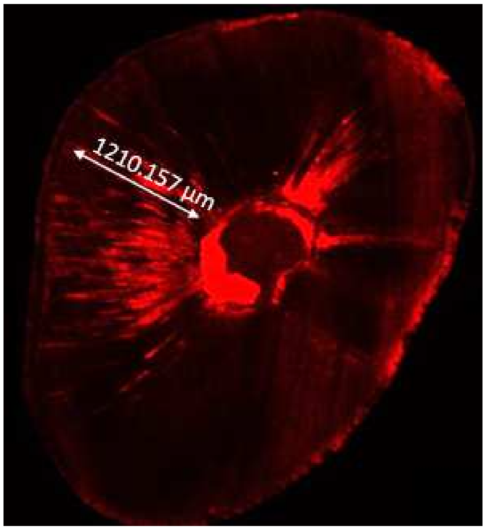


Fig 15 a: CORONAL [Group IAa]

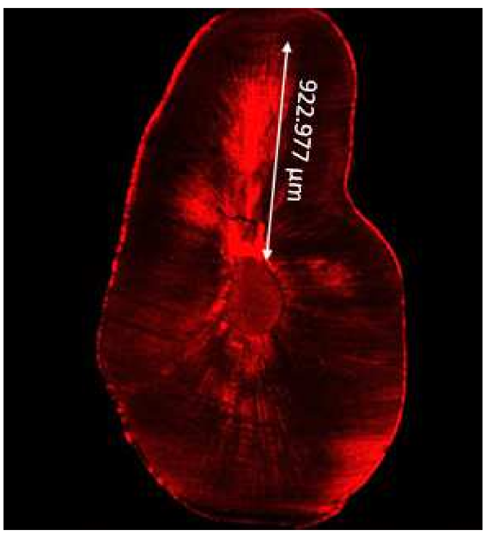


Fig 15 b: MIDDLE [Group IAb]

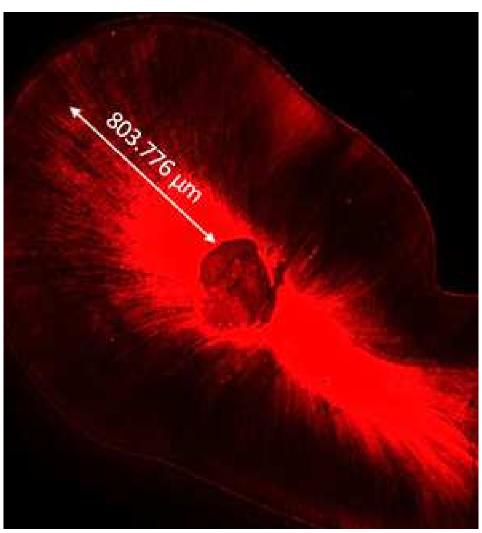


Fig 15 c: APICAL [Group IAc]

Fig 16: CLSM Images depicting penetration of Ceraseal sealer after final irrigation with 0.2% Chitosan using CI technique at coronal, middle and apical sections

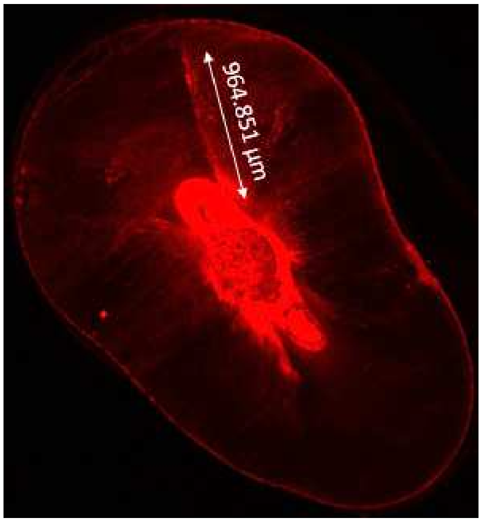


Fig 16 a: CORONAL [Group IBa]

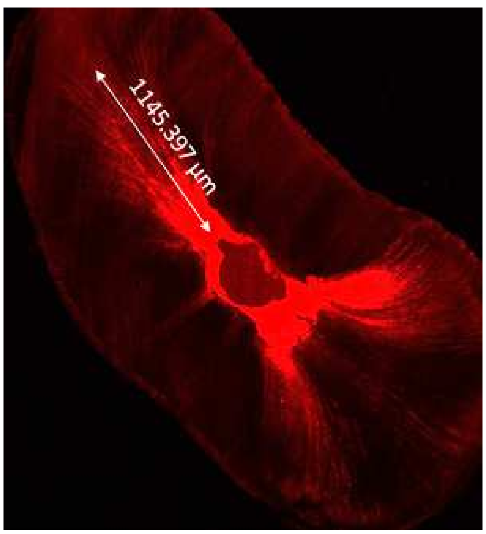


Fig 16 b: MIDDLE [Group IBb]

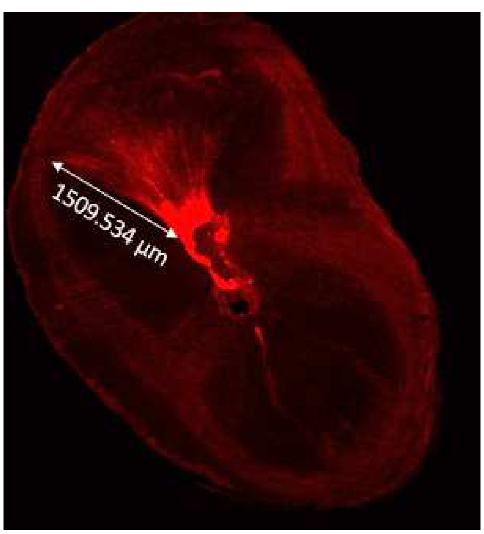


Fig 16 c: APICAL [Group IBc]

Fig 17: CLSM Images depicting penetration of AH Plus sealer after final irrigation with 0.2% Chitosan using PUI technique at coronal, middle and apical sections

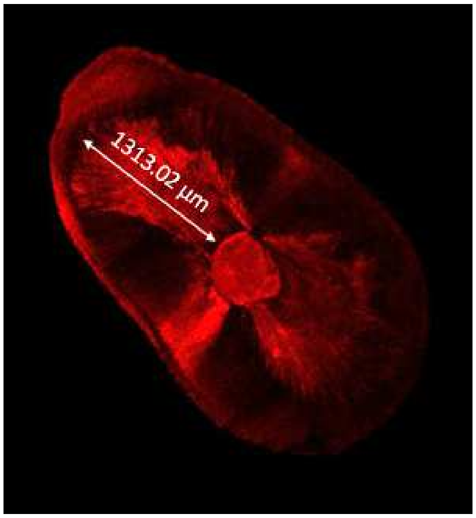


Fig 17 a: CORONAL [Group IIAa]

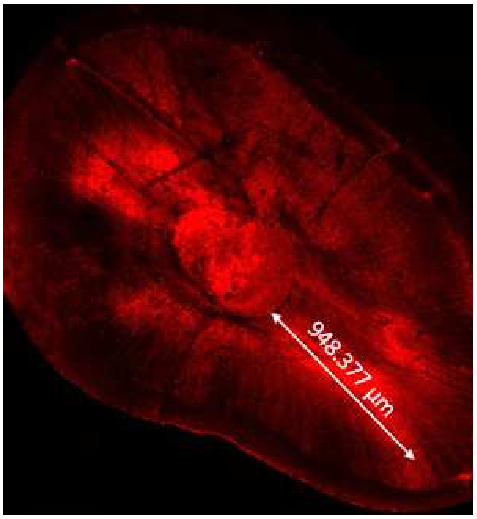


Fig 17 b: MIDDLE [Group IIAb]

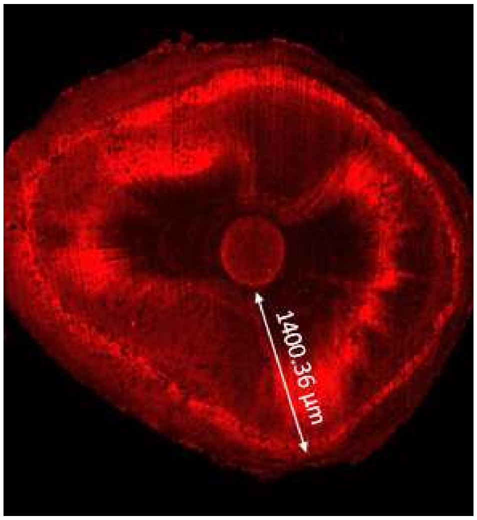


Fig 17 c: APICAL [Group IIAc]

Fig 18: CLSM Images depicting penetration of Ceraseal sealer after final irrigation with 0.2% Chitosan using PUI technique at coronal, middle and apical sections

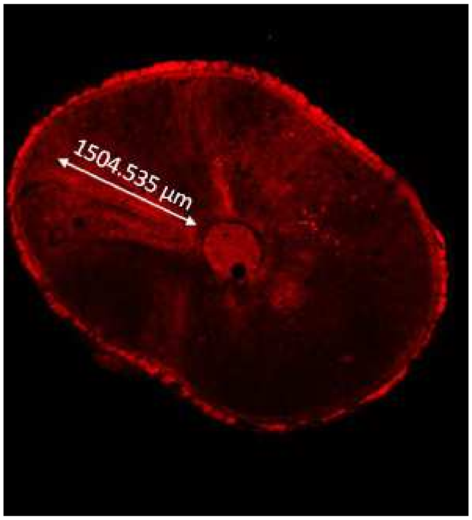


Fig 18 a: CORONAL [Group IIBa]

Fig 18 b: MIDDLE [Group IIBb]

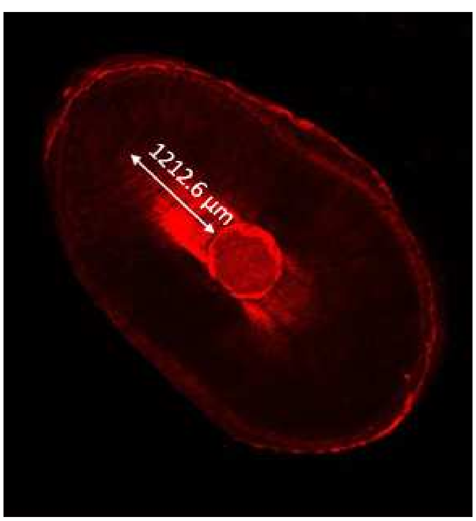


Fig 18 c: APICAL [Group IIBc]

DISCUSSION

Smear layer, an amorphous material (1-2 μm thick) formed during biomechanical instrumentation, acts as a barrier that may prevent root canal irrigants, medicaments and/or sealants from entering the dentinal tubules. Removal of this layer is essential to allow sealer to enter the tubules.[56]

NaOCl serves as primary irrigant in RCT owing to its anti-bacterial properties and ability to dissolve organic tissue. Nevertheless, it has no impact on the inorganic composition.[57] For the successful sealing of dentinal canaliculi, it is recommended to use final irrigation with chelating agents like ethylenediaminetetraacetic acid (EDTA) to eradicate debris and smear matrix, thereby enhancing penetration of sealer into dentin.[9]

EDTA at 15-17% concentration removes calcium from dentine without causing any lethal damage to the apical tissue. Irrigation protocol of NaOCl and EDTA is used to remove smear matrix and debris, leading to successful debridement of canal.[58] According to Calt et al. and Torabinejad, a prolonged EDTA contact time (> 1 min) may result in severe intertubular and peritubular erosion. Acidic nature of EDTA, has a negative effect on hydration properties of cement, preventing adherence to materials. Hence, search for newer alternatives was needed.[59]

Chitosan is a naturally occurring homopolysaccharide derived from Chitin. It consists of monomers of, N-acetyl-D-glucosamine, linked together via β-linkage. It is widely utilized in dentistry owing to notable antimicrobial properties and chelating activity.

Chitosan, as an excipient, possesses another important attribute: its capability to hydrate and create gels in highly acidic aqueous environments. This characteristic makes it valuable in formulating slow-release drug delivery systems.[60] In vitro studies have examined its efficacy as a drug carrier in hydrocolloids and gels. Additionally, it has been suggested that Chitosan can enhance the bioavailability of drugs and facilitate targeted drug distribution to specific regions.[61]

Chitosan has been demonstrated to have chelating capabilities, and maximum effect was observed when solubilized in acetic acid, which is identical to 0.2% Chitosan utilized in this study.[62] It exhibits chelation properties similar to EDTA and citric acid, but with significantly less detrimental effects. Chelating solutions have the potential to remove the smear layer and reveal a significant number of dentinal tubules, which promotes sealer adherence due to greater contact area, resulting in improved adaption between the sealer and dentin.[63]

Apart from irrigants antimicrobial and chelating properties, which are crucial for cleaning and disinfecting the RCS, various tools and strategies for activation of irrigant have been advocated to improve effectiveness and dispersion of final irrigating solution.

Some of the frequently used and studied activation methods include MDA, PUI and SI.[8]

PUI involves activating irrigant through vibration generated by file or wire operating at a frequency of 30 kHz.[64]

Due to acoustic streaming and microcavitation, passive ultrasonic irrigation (PUI) enhances cleansing and disinfection, and allows for irrigant delivery up to the

WL of the root canal.[65] Its effectiveness has been demonstrated by Paragliola et al., who stated that the utilization of ultrasonic agitation led to a noticeably higher rate of irrigant penetration into the tubules compared to sonic agitation. In this study, we used Irrisafe tips (Satelec Acteon Group, Merignac, France) for passive ultrasonic irrigation (PUI).[66]

Another essential objective of root canal therapy after adequate chemomechanical preparation is to achieve a hermetic seal using a biocompatible material. For endodontic therapy to be successful, an endodontic sealer is essential because it provides an impermeable seal, fills in irregularities and inconsistencies between the canal wall and core material, and helps manage bacteria if they persist in the tubules or walls of the root canal.[67] The penetration of sealer into dentinal tubules plays a vital role as it not only enhances the sealing capability but also contributes to better retention of the sealer within the tooth structure. Mamootil et al., and Messer et al., suggest that the physiochemical characteristics of a material, such as surface tension, viscosity, solubility, and size of particle, impact the consistency and depth of tubular penetration.[68]

AH Plus is currently considered as a gold standard sealer for comparison and also exhibits desirable qualities such as low rates of solubility, optimal adhesiveness, and great radiopacity to the root dentin compared to other endodontic sealers.[69]

Zhou HM et al. (2013), stated that the hallmark characteristic of AH Plus sealer is its pseudoplastic behaviour observed during obturation, where flow increases as viscosity decreases, consequently enhancing its penetration into tubules.[70] AH Plus forms a chemical bond with dentin through the creation of a covalent bond between the epoxy group of the resin and the amine group of collagen within dentin.[71]

According to Neto et al., AH Plus sealer's high bond strength can be linked to both, its enduring dimensional stability and low polymerization stress.[72] Dem et al., investigated the bond strength of AH Plus, GF 2 and GF-B and stated that bond strength of AH Plus was highest compared to other groups, possibly due to development of covalent bond.[73]

However, it has short coming, such as possible mutagenic potential, cellular toxicity when freshly mixed, immune response, polymerisation shrinkage and post operative pain. In addition, complete filling of the canal is hindered due to its hydrophobic nature.[74] To overcome these problems, new calcium silicate sealers have been developed.

Calcium silicate sealers have osteogenic potential, high tissue compatibility and low cellular toxicity.[25] Since they are highly biocompatible their rejection by the surrounding tissue is minimal. These bioceramic materials contains calcium phosphate which results in a crystal structure that is similar to teeth and bone apatite materials resulting in improved bonding between the sealer and the root dentin.[28]

Ceraseal, a premixed novel bioactive, calcium silicate-based endodontic sealer. Its pH of 12 is responsible for antimicrobial activity.[30]

These sealers undergo hydration reaction by reacting with water residing in the dentinal tubules which initiates the setting reaction. Upon hydration, calcium silicates generate calcium hydroxide and calcium silicate gel. Subsequently, this calcium hydroxide interacts with phosphate ions from dentin to form hydroxyapatite crystals which may be responsible for the formation of chemical bond between sealer and dentin.[75] Jasrotia et al., also concluded that formation of hydroxyapatite crystals and expansion of sealer on setting was responsible for high bond strength of Ceraseal with dentin.[76]

Another critical aspect of sealers is their pH level. A higher, alkaline pH is significant as it can potentially boost the antibacterial properties of a sealer, facilitate the healing process, counteract the acidity resulting from osteoclastic activity, and promote the deposition of mineralized tissue.[77]

Elfaramawy et al., stated that Ceraseal had the highest release of hydroxyl ions compared to Epoxy-resin and ZOE-based sealers which was responsible for high alkaline pH and antimicrobial activity.[78]

Thus, the purpose of this study was to assess and compare the tubular penetration of Bioceramic sealer and Epoxy resin based sealers following a final rinse using 0.2% Chitosan with or without PUI.

Different techniques are employed for filling the root canal system, among which are warm vertical compaction, lateral compaction, carrier-based obturation and single cone obturation techniques.[79]

Cold lateral compaction can potentially result in spaces between GP, sealer and canal walls. Additionally, vertical root fractures can occur during compaction. To address these drawbacks, obturation with single-cone was introduced. Single-cone obturation technique offers advantages such as reduced treatment time while delivering treatment outcomes that are either better or comparable to those achieved with conventional techniques.[80] According to Bhatia et al., obturation with single-cone in combination with bioceramic sealer showed better sealing contrast to carrier-based obturation and cold lateral condensation techniques.[81]

Therefore, single-cone obturation is the most often utilized technique due to its simplicity, lack of technique sensitivity, low cost and shortest processing time. This method makes use of a gutta-percha cone whose diameter is comparable to that of the last instrument used to shape the root canal. Nevertheless, because this method requires more sealer, the flowability and other physicochemical characteristics of the sealer are crucial to the endodontic treatment's success. [80]

In this research, both AH Plus and Ceraseal was employed with single cone obturation technique.

Various techniques like Scanning Electron Microscopy (SEM), Light Microscopy (LM) and Confocal Laser Scanning Microscopy (CLSM) have been used to assess the sealers sealing ability. CLSM, unlike SEM, creates less artifacts and does not cause specimen dehydration. CLSM utilizes non-decalcified or hard tissue samples that do not require a sputter coating. It uses fluorescence to provide a thorough view of the interfacial adaption and sealer dispersion. LM makes it impossible to distinguish between the sealer and the dentin. CLSM has high contrast, allowing for accurate analysis of sealer in the tubules.[82] Therefore, CLSM emerged as a method for evaluating sealer penetration in this study.

The present study focused on penetration depth of resin-based sealer and BC sealer, following a final flush with 0.2% Chitosan with or without passive ultrasonic activation. Depth of Ceraseal (Group IB=1230.60±39.86 and Group IIB=1476.86±37.18) penetration was higher when compared with AH Plus sealer (Group IA=1021.06±34.11 and Group IIA=1230.18±38.35) regardless of the irrigation technique employed (Table 1 and Graph 1). Similar to the results of this study, Wang et al. (2018), El Hachem R et al. (2019), and Akcay et al. (2016), have demonstrated that, bioceramic root canal sealers have superior tubular penetration when compared to AH Plus. This may be due to their diminutive particle size, hydrophilic nature, and fluidic properties. [83,84,85]

Chitosan's hydrophilic property aids in its close contact with dentin and removes smear layer. [20] The choice of using Chitosan (0.2%) over EDTA (15%) was based on a study conducted by Silva et al. (2012), who investigated the impact of Chitosan's concentration on dentin and smear layer elimination. The authors confirmed that employing a 0.1% Chitosan solution for three min effectively eradicates smear layer but does not eliminate the inorganic plug. Chitosan at a concentration of 0.2% for the same duration resulted in open tubules, visible dentin and mild disruption of dentin. Chitosan at 0.37% cleaned the dentinal walls comparably to 0.2%, albeit with notably greater erosive effects.[62] Hence 0.2% Chitosan was the chosen concentration.

Mathew et al., also stated Chitosan as an effective chelating agent and less erosive than EDTA at 17%.[20]

The action of Chitosan can be explained by two mechanisms. First one being the bridge model in which, multiple amino groups from Chitosan bind to a single metal ion and the second one is pendant model in which a single amino group facilitates binding, with the metal ion forming a pendant structure attached to it.[86] A study by Mekahlia et al. concluded, chitosan at a concentration of 0.2% effectively eliminates the smear layer and opens dentinal tubules, exhibiting a comparable efficacy to that of EDTA.[87]

Moreover, Silva et al., confirmed that Chitosan (0.2%) was as effective as higher concentrations of EDTA (15%) and CA (10%) at removing the smear layer.[62]

Filho et al., evaluated maleic acid (7%) and EDTA (17%) in eradicating smear layer and concluded that in the apical region 7% maleic acid exhibits greater effectiveness, but the main disadvantage is that maleic acid causes more dentin erosion and reduces microhardness of the tooth.[88]

In a study by Scelza et al., time periods of EDTA, EDTA – T, Citric acid irrigation were evaluated for smear layer removal and concluded that all three irrigants were effective at the 3 min interval, however saturation occurred beyond 3 min, resulting in organic content's precipitation.[89]

Irrigants delivered by conventional needles typically penetrate nearly 0 to 1.1 mm beyond the needle tip, leading to the production of gas particles that can become entrapped in the apical region, resulting in a vapor-lock and obstructing the effectiveness of irrigant debridement.[9] PUI eliminates vapor-lock, thereby enhancing efficiency of the solution. Safety of conventional irrigation is also scrutinized due to the +ve pressure employed to agitate the solution, which leads to its extrusion in the apical area, resulting in tissue injury and pain.[5,90]

Shaker et al., evaluated the tubular penetration of Epoxy resin (Adseal) and Bioceramic (Ceraseal) sealers with or without PUI, and concluded that Bioceramic sealer exhibited greater dentinal tubule penetration with PUI than other group.[91]

These findings align with the present study, which observed enhanced smear removal when incorporating passive ultrasonic irrigation (PUI) during the final irrigation process (Group IB=1230.60±39.86, and Group IIB=1476.86±37.18).

Zhou et al., concluded that PUI was more effective than XP-Endo Finisher (XPF) for medicament removal and its efficacy of cleaning may be influenced by the irrigation protocol, fins and deltas, and intracanal medicament's time.[92] Plaza et al., stated that 20 s of 3 cycles (NaOCl-EDTA-NaOCl), 60 s of 2 cycles (EDTA-NaOCl), or 60 s of 1 cycle (NaOCl) with PUI, were equally efficacious at removing dentinal debris and opening of dentinal tubules.[67] This may be due to the flow generated by ultrasound with EDTA, which resulted in superior effects. This underscores the significance of physical methods in enhancing canal irrigation.

When the areas of root canals where considered, greater sealer penetration was seen in cervical and middle thirds irrespective of irrigant and technique used. In terms of penetration between Ceraseal and AH Plus, significant difference was seen (p=0.0001). Ceraseal proved better than AH Plus in terms tubule penetration, with both CI (1230.60±39.86) and PUI (1476.86±37.18). These findings align with earlier studies indicating that irrigating solutions were less effective in the apical third (Table 4, Graph 2).

Camilleri et al., McMichael et al., Wang et al., and Eymirli et al., outlined that depth of penetration of sealer was superior in coronal, followed by middle and least in apical region. [93,83,94] This may be due to the histological features of the apical root dentine, which is known to be sclerotic and poorly permeable, with fewer dentinal tubules compared to dentin in the middle and cervical thirds.[95]

However, in the apical section, the Ceraseal + PUI irrigation resulted in enhanced removal of debris. This finding holds significance as the apical region is regarded as a crucial area of the tooth, contributing to a higher quantity of secondary root canal complexities. The traditional chemomechanical preparation is unable to address these complexities, which permits the retention of residual bacteria and their byproducts and ultimately results in the failure of endodontic therapy.[96]

We can conclude that 0.2% Chitosan was highly effective in terms of dentinal tubular penetration, irrespective of the irrigation activation technique used. PUI further improved the tubular penetration of both the sealers. However, cleaning the apical third of the root canal still remains an area of concern due to the reasons clearly discussed.

CONCLUSION

Within the limitations of the present research, it can be concluded that:

1. Bioceramic sealer (Ceraseal) demonstrated greater depth of dentinal tubule penetration contrast to Epoxy resin (AH Plus). This could be due to fine particle size, high flow and hydrophilic nature of bioceramic sealers which led to greater penetration into the dentinal tubules.

2. Activation of irrigating solution with ultrasonics was more effective method than conventional method of delivery of irrigant with syringe needle regardless of the sealer used.

3. Higher depth of sealer penetration was seen in the coronal region of the root canal and lowest in the apical region due to its complex anatomy.

SUMMARY

During endodontic therapy, a smear layer, made up of dentine, pulp tissue, and bacteria, forms on canal walls, posing therapeutic challenges by preventing irrigants from penetrating dentin and hindering adhesion of root filling materials.

Sodium hypochlorite is the primary irrigant in endodontic procedures due to its antimicrobial efficacy and ability to dissolve organic tissue, but it doesn't affect inorganic content. Hence, chelating agents serve as the primary irrigants to effectively remove debris and the smear layer, enhancing the permeability of the root canal system.

Chelating solutions, including organic acids like Citric and Maleic acid, and inorganic acids like EDTA and Phosphoric acid, are used to remove the smear layer.

EDTA is a popular root canal irrigant due to its chelating properties, which dissolve inorganic dentin components, enhancing root canal disinfection and sealer penetration. However, prolonged exposure can cause erosion and decrease microhardness of dentin.

Chitosan, a naturally occurring homopolysaccharide derived from Chitin, is widely used in dentistry due to its antimicrobial properties and chelating activity. It can hydrate and create gels in acidic environments, making it useful in drug delivery systems. Chitosan also has chelating capabilities, with the highest effect when solubilized in acetic acid. This enables better sealer adherence and better adaption between the sealer and root canal dentin. Various tools and irrigant activation strategies have been developed to enhance the effectiveness of solutions during final irrigation.

Studies have shown that 0.2% Chitosan irrigation is as effective as 15% EDTA and 10% Citric acid in smear layer removal with less of toxic effects.

Ultrasonic energy is used in endodontics for root canal cleaning and disinfection. It improves cleaning by creating a higher speed and flow volume of irrigant, eliminating debris, and allowing better penetration of sealer into dentinal tubules for antibacterial activity.

AH Plus, an epoxy resin-based root canal sealer, is often regarded as the gold standard for comparision. However, it has limitations like mutagenicity and cytotoxicity.

New bioceramic sealers are being developed to overcome these issues. Ceraseal is a new calcium silicate-based bioceramic sealer with advantages like short setting time, high flow rate, alkalinization ability, radio-opacity, and volumetric expansion on setting.

There are very few studies that have evaluated and compared the effect of Chitosan on radicular dentin and the penetration of different sealers into dentinal tubules.

Keeping these rationales in mind, this study aimed to explore the effects of final irrigation with 0.2% Chitosan, with and without the use of passive ultrasonic agitation on dentinal tubular penetration of root canal sealers.

The study was conducted in the Department of Conservative Dentistry and Endodontics, Viswanath Katti Institute of Dental Sciences, KAHER Belagavi.

Hundred extracted human mandibular premolar teeth were used and handled according to OSHA guidelines. All the teeth were evaluated using radiographs and magnification for the inclusion and exclusion criteria. The teeth were decoronated using a diamond disc under copious water spray to acquire a standardized root length of 14 mm. Samples were randomly divided into two groups (n=50) based on irrigating technique used.

Group I: 3% NaOCl and 0.2% Chitosan + Conventional Irrigation for 2 minutes.

Group II: 3% NaOCl and 0.2% Chitosan + Passive Ultrasonic Irrigation for 1 minute.

In both the groups, 5 ml of 3% NaOCl was used as an initial rinse for 1 min followed by 5ml of Chitosan as a final rinse for 2 min. Each group was further divided into two subgroups each depending on the type of root canal sealer used (n=25 per group)

Group I A: Epoxy resin based sealer (AH Plus)

Group I B: Bioceramic sealer (Ceraseal)

Group II A: Epoxy resin based sealer (AH Plus)

Group II B: Bioceramic sealer (Ceraseal)

A Fluorescent dye (Rhodamine B isothiocynate) was added to the sealer during manipulation at an approximate ratio of 0.1% (weight). The endodontic sealer was applied using no. 30 lentulospiral and then a master cone coated with sealer was placed using single cone obturation technique. Excess GP was sheared off at the orifice. Teeth were then sealed with Cavit (3M, ESPE) and incubated at 37°C and 100% humidity for a week to simulate clinical conditions.

Specimens (1mm thickness each) were sectioned horizontally with a diamond disc at coronal (8mm from apex), middle (5mm from apex) and apical thirds (2mm from apex) of each root and these sections were categorized as **a**, **b** and **c** respectively. All specimens were mounted onto glass slides and the specimens were examined under a Confocal Laser Scanning Microscope (CLSM).

Statistical analysis of the data obtained was done by Two-way ANOVA and Tukey's Multiple Post hoc test. The results indicated a significant difference between all the four groups (p value 0.0001).

In conclusion, based on the sealer and irrigation technique used, the mean depth of penetration of Ceraseal sealer, following irrigation with Chitosan activated by PUI was the highest followed by conventional irrigation technique, with AH Plus however, lower depth of sealer penetration was observed irrespective of irrigation techniques used (Table 1). Passive Ultrasonic Irrigation was more effective as compared to Conventional irrigation, interms of dentinal tubule penetration of both the sealers used.

Considering the sections of the root, depth of sealer penetration was highest at coronal third followed by middle third and least at the apical third.

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ANNEXURE – I

ETHICAL CLEARANCE LETTER

Research and Ethics Committee
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Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
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Sl. No. : 1600

CERTIFICATE

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

This is to Certify that the synopsis titled

Comparative Evaluation of the effect of 0.2% Chitosan with or without Passive Ultrasonic activation used as a final rinse on the tubule Penetration of Epoxy resin based and Bioceramic Sealers on radicular dentin - An in-vitro Confocal laser scanning microscopic Study - *Submitted by*

Dr. ______________________ *P. G. Student /*

Staff, Guided by ______________________ *from Department of*

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

Date : 3/4/24

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

ANNEXURE – II

BIOSTATISTIC CLEARANCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
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Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of this dissertation/ Thesis work of post-graduate student, under the guidance of M.D.S Professor, **Department of Conservative Dentistry and Endodontics, entitled "Comparative evaluation of the effect of 0.2% Chitosan with or without Passive Ultrasonic activation used as a final rinse on the tubule penetration of Epoxy resin based and Bioceramic sealers on radicular dentin: An In-vitro Confocal laser scanning microscopy study."** has been done under my guidance and completed satisfactorily.

Place: Belagavi
Date: 23-04-2024

Name & Signature of Biostatistician

Dr. S. B. JAVALI Ph.D.
Sr. Associate Professor in Statistics
Department of Community Medicine
USM KLE International Medical Programme
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ANNEXURE – III

PLAGIARISM CHECK CERTIFICATE

Scientific Correspondence and Review Committee
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Date : 23. 4. 24 **Serial No. :** 195

PLAGIARISM CHECK REPORT

Name of the Applicant :
UG / PG / Ph.D / Staff : POST GRADUATE
Batch & Year : 2021- 2024
Department : CONSERVATIVE DENTISTRY AND ENDODONTICS

The soft copy of Research Work / Manuscript by .. .. entitled "COMPARATIVE EVALUATION OF THE EFFECT OF 0.2% CHITOSAN WITH OR WITHOUT PASSIVE ULTRASONIC ACTIVATION USED AS A FINAL RINSE ON THE TUBULE PENETRATION OF EPOXY RESIN BASED AND BIOCERAMIC SEALERS ON RADICULAR DENTIN : AN IN VITRO CONFOCAL LASER SCANNING MICROSCOPY 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 ....5..........%, 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