

**“Evaluation of effect of N-acetyl cysteine
intra canal medicament on dentinal tubule
penetration and percentage of an Epoxy Resin
based sealer and a Bioceramic based sealer
using Confocal Laser Scanning Microscopy -
An in-vitro study”**

By

REG.NO. IE0221005

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Of the requirements for the degree of**

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(BRANCH – IV)

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ENDODONTICS**

**KAHER VK INSTITUTE OF DENTAL SCIENCES,
BELAGAVI, KARNATAKA**

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KLE Academy of Higher Education & Research, Belagavi
Karnataka

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ABSTRACT

AIM: To evaluate the effect of N- acetyl cysteine intracanal medicament on dentinal tubule penetration and percentage of an Epoxy resin based sealer and a Bioceramic based sealer using Confocal Laser Scanning Microscopy - An in-vitro study

METHODOLOGY: Seventy eight extracted human maxillary anterior teeth were selected and decoronated to acquire a standardized root length of 14 mm. Working length was established using a 10 k file and Chemomechanical preparation was done using Protaper universal till master apical file size F₄ and specimens were irrigated with 2ml of 5.25% sodium hypochlorite between successive files and final irrigation with 5ml of 17% EDTA for 3 minutes. All the specimens were then dried with paper points and randomly divided into 3 groups.

Group 1: Experimental group (NAC) with Epoxy resin sealer

Group 2: Experimental group (NAC) with Bioceramic based sealer

Group 3: Negative control group.

N- acetyl cysteine medicament was placed in Group I and Group II specimens followed by incubation at 37°C for 15 days after which the medicament was removed using Passive Ultrasonic irrigation and obturation was done for all 3 groups with respective Gutta percha coated with rhodamine mixed sealer. Incubation was done for 7 days after which samples were sectioned at different levels (3, 7, 11 mm from the apex) and examined under the confocal laser scanning microscope for dentinal tubule penetration and percentage of sealers. Images obtained were analysed using Image J software. Statistical analysis was done using Two way ANOVA and Tukey's multiple post-hoc tests.

RESULTS: On intragroup comparison, the results showed difference in sealer penetration was statistically significant among all the three sections i.e, coronal, middle and apical section among all the three groups. i.e, NAC + Ceraseal, Ceraseal, NAC + AH Plus, AH Plus. On intergroup comparison, the depth of sealer penetration was statistically significant in Group II (NAC + Ceraseal), Group III B (Ceraseal).

On intragroup comparison, the results showed difference in sealer percentage/ adaptation was statistically significant among all the three sections i.e, coronal, middle and apical section among all the three groups. i.e, NAC + AH Plus, AH Plus, NAC + Ceraseal, Ceraseal. On intergroup comparison, the percentage/adaptation of sealer penetration was statistically significant in Group I (NAC + AH Plus), Group III B (Ceraseal).

CONCLUSION:

- Maximum sealer penetration was noted in the coronal third, followed by middle third and least in apical third for all tested groups.
- Higher penetration of sealer was observed with NAC + Ceraseal (Group II) followed by Ceraseal (Group III B), NAC + AH Plus (Group I) and least by AH Plus (Group III A).
- Higher percentage/ adaptation of sealer was observed with NAC + AH Plus (Group I) followed by AH Plus (Group III A), NAC + Ceraseal (Group II) followed by Ceraseal (Group III B).

KEY WORDS: N-acetyl cysteine, Passive ultrasonic irrigation, AH Plus, Ceraseal, sealer penetration and percentage.

LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	AAE	American Association of Endodontics
2	ANOVA	Analysis of variance
3	Ca (OH) ₂ , CH	Calcium Hydroxide
4	CHX	Chlohexidine
5	CLSM	Confocal laser scanning microscope
6	DAP	Double Antibiotic Paste
7	E. faecalis	Enterococcus Faecalis
8	EDTA	Ethylene diamine tetra acetic acid
9	Er: YAG	Erbium- Doped yttrium- Aluminium- garnet
10	et al	Additional persons involved in the same study
11	gm	Gram
12	GP	Gutta Percha
13	i.e.	That is
14	LAI	Laser Assisted Irrigation
15	LM	Light Microscope

16	mg/mL	Milligram/millilitre
17	min	Minutes
18	mL	Milliliter
19	mm	Millimeter
20	n	Number of specimens
21	NAC	N- acetyl cysteine
22	NaOCl	Sodium Hypochlorite
23	Ni-Ti	Nickel Titanium
24	PDT	Photodynamic Therapy
25	PG	Propylene Glycol
26	PUI	Passive ultrasonic irrigation
27	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
28	RCS	Root Canal Sealers
29	REP	Regenerative Endodontic Procedure
30	SC	Single Cone
31	Sec	Seconds

32	SEM	Scanning Electron Microscope
33	SHED	Stem cells from human exfoliated deciduous teeth
34	SVE	Single Visit Endodontics
35	TAP	Triple Antibiotic Paste
36	UAI	Ultrasonically activated irrigation
37	VSP	Very Short Pulse
38	WL	Working Length
39	ZOE	Zinc Oxide Eugenol
40	%	Percentage
41	°	Degree
42	°C	Degree Celsius
43	µm	Micrometers

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INTRODUCTION

Long term prognosis of root canal therapy depends on thorough eradication of microorganisms. However, it is not always possible in the first visit to eliminate all the microorganisms due to various complexities of root canals.^{1,2} Hence, along with chemomechanical debridement, intracanal medications such as Ca(OH)₂, TAP, DAP, CHX etc. have been widely employed for disinfection of infected root canals to reduce the flare up and to improve the prognosis of endodontic treatment.³

Calcium hydroxide is one of the most widely used medicament which has antimicrobial activity and alkaline pH but its disadvantages are it is resistant to *E. faecalis* and its difficulty associated with removing it from the canals which prevents sealer penetration.⁴ Among the array of intra canal medicaments used in endodontic treatments, N-acetyl cysteine (NAC), a novel medicament has proven to suppress the growth of *E. faecalis* and destroy its biofilm.⁵ It acts against all endodontic pathogen and also provides immense protection of apical stem cells for Regenerative Endodontic Procedure for modern endodontics.^{6,7} Hence, N-acetyl cysteine (NAC) is an antimicrobial, anti-biofilm, anti-inflammatory medicament.⁸

However, N-acetyl cysteine (NAC) has some limitations such as its difficulty in complete removal from the canal and according to literature, complete removal of these medicaments are impossible but the removal can be enhanced by various adjunctive aids like manual agitation with rotary instrument, passive ultrasonic irrigation, sonic irrigation, endoactivator, laser etc.¹¹ These remnants in turn influence the tubular penetrability of sealers and their sealing ability. Thus, complete removal of medicament before root canal filling is necessary which could affect the prognosis of the endodontic therapy.^{1,9,10}

The most preferable obturation material is gutta percha which is imperative for a hermetic seal along with root canal sealer.¹² Dentinal tubule penetration depth and microgap are major factors which decides the success of endodontic therapy.¹³

A root canal sealer's effectiveness is gauged by the depth of dentinal tubule penetration. The advantages of deeper tubular penetration are that it increases the contact area and improves the retention between GP and dentinal walls, entombs remaining bacteria and also increases the fracture resistance of the tooth.^{13,14}

Various physiochemical properties of sealer has impact on dentinal tubule penetration depth. Hence, it is necessary to evaluate the tubular penetration for various sealers used.¹⁵ AH Plus, a gold standard root canal sealer, has better handling characteristics and excellent physical properties. The various advantages are better bonding, low shrinkage, high radioopacity, low solubility and good biocompatibility¹⁶ but their disadvantages are hydrophobicity and mutagenicity. To overcome these problems, calcium silicate based sealers have been advocated.

Bioceramic based sealers like Ceraseal has its major advantages like crystallization and chemical reaction thereby enhancing the seal to radicular dentin, unique stability, antimicrobial activity and short setting time.¹⁷ Besides these advantages, there are certain limitations in these calcium silicate based sealers which are difficulty in retreatment and low sealing ability/ adaptation.⁴

Till date, there is no literature evidence comparing the depth and percentage of penetration of Ceraseal sealer with other sealer.

Hence, our aim of the present study is comparative evaluation of effect of N-acetyl cysteine (NAC) intracanal medicament on percentage and depth of penetrability of an Epoxy resin based sealer and a bioceramic based sealer in coronal, middle, apical areas using confocal laser microscopy.

AIM AND OBJECTIVES

AIM: To assess and compare the effect of N-acetyl cysteine intracanal medicament on percentage and depth of tubular penetrability of an Epoxy resin sealer and a Bioceramic root canal sealer using Confocal Laser Scanning Microscopy.

OBJECTIVES

- To assess the effect of N-acetyl cysteine intracanal medicament on percentage and depth of tubular penetrability of an epoxy resin based root canal sealer using confocal laser scanning microscopy.
- To assess the effect of N-acetyl cysteine intracanal medicament on percentage and depth of tubular penetrability of a bioceramic based root canal sealer using confocal laser scanning microscopy.
- To compare the effect of dentinal tubule penetration depth and percentage of an epoxy resin based root canal sealer and a bioceramic based root canal sealer using confocal laser scanning microscopy.

HYPOTHESIS

NULL HYPOTHESIS: -

There was no difference in percentage and depth of tubular penetrability of epoxy resin sealer and the bioceramic root canal sealer after use of N-acetyl cysteine intracanal medicament.

ALTERNATE HYPOTHESIS: -

There was a difference in percentage and depth of tubular penetrability of epoxy resin sealer and the bioceramic sealer after use of N-acetyl cysteine intracanal medicament.

REVIEW OF LITERATURE

1. Utilizing confocal microscopy, Deebah Choudhary et al investigated the tubule penetrability and durability in obturated canals using 3 distinct bioceramic sealers. The depth of penetration of CeraSeal RC sealer was much higher than that of Bio-C and MTA Fillapex. Nevertheless, after retreatment, none of the sealers was entirely removed from the tubules.¹⁸
2. Using a scanning electron microscope (SEM), Nasr Rashad Hashem et al. examined the interfacial adaptation of CeraSeal and Bio-C Sealer in relation to AH Plus. Additionally, CLSM is used to check the penetrability of CeraSeal, Bio-C Sealer with AH Plus. While Bio-C Sealer demonstrated the greatest penetration, AH Plus demonstrated superior adaptability.¹⁹
3. The penetration of epoxy resin-based sealers and bioceramic sealers following ultrasonic agitation and Er: YAG laser activation of the irrigant was assessed and compared in this study done by Twinkle Talreja et al. It was discovered that the maximum penetration in all tooth portions is achieved by the Er: YAG laser with AH Plus sealer, followed by the CeraSeal sealer.²⁰
4. Mohmed Isaqali Karobari et al. examined the pattern of adhesion, penetration of 6 sealers. When compared to other sealers, One-Fil was shown to have the maximum dentinal tubule penetration; in contrast, BioRoot RCS have a stronger push-out bond and a sticky adhesive pattern.²¹
5. Using the dye penetration method, Diksha Batra et al. evaluated leakage of 4 different sealers in extracted teeth. Sealapex exhibited highest penetration of

dye, whereas Ceraseal endodontic sealer demonstrated the least amount of microleakage.²²

6. In comparison to an epoxy resin sealer, Soo Teng Chew et al. investigated the adaptability and penetrability of 3 distinct BC sealers in oval root canals. Using a SC obturation technique in an oval canal, it was found that Nishika Canal Sealer BG had greater adaptability and penetrability and that Endoseal has the lowest adaptation and penetration depth.²³
7. Riccardo Tonini et al. assessed the clinical endodontic procedures and the limitations of irrigating solutions in which he compared activation techniques to traditional needle irrigation techniques and it concluded that activation technique reveals a noticeably greater biofilm removal.²⁴
8. In root canal retreatment, Blanca Ortiz-Blanco et al. investigated the penetration of 3 bioceramic sealers. The depth of penetration and percentage between AH + and Ca-Si sealers were found to be non-significantly lower.²⁵
9. In a study published recently, Fausto Zamparini et al. investigated the physiochemical properties and bioactivity of 3 premixed BC sealers that were newly released: Ceraseal, AH Plus Bioceramic, AH Plus, Neosealer Flo. Calcium silicates (CaSi) in the sealers varied in quantity. The findings showed that Ceraseal and AH Plus Bioceramic have shorter final setting time, AH Plus and NeoSealer Flo have the longest. Only AH Plus Bioceramic and NeoSealer Flo exhibited a little covering of a CaP phase. Ceraseal has no CaP deposit, but it did have the highest calcium release of any CaSi-containing sealer tested.²⁶

10. Tara Haji et al. looked at the sealing quality and compatibility of two bioceramic sealers, BioRoot RCS and CeraSeal RCS. Scanning Electron Microscope was utilized to appraise the adhesion. The outcomes were contrasted with a control of ZnO Eugenol sealer. Compared to other two Bioceramic sealers, BioRoot and CeraSeal sealers exhibited good sealing adaptability, biocompatibility, and fast recovery of the soft tissues. ZOE sealers have less sealing adaptation and a slow recovery of inflammation.²⁷

11. In order to compare the physical characteristics and cytocompatibility of four modern calcium silicate sealer with an epoxy resin sealer, Min-Gyu Park et al. conducted this study. It was discovered that calcium silicate-based sealers characterized radiopacity, clinically acceptable flow, and favorable cytocompatibility.²⁸

12. Using the dye penetration method, Ankush Jasrotia et al. examined the sealing capabilities of three distinct sealer types: Ceraseal bioceramic sealer, AH Plus sealer and Epiphany sealer. The study's findings indicated that AH + had the highest levels of vertical and horizontal dye penetration, indicating that Ceraseal bioceramic sealer and epiphany sealed the root canal more effectively than AH + sealer.²⁹

13. Manoel E.L. Machaado et al conducted a study in which samples were medicated with calcium hydroxide after which epoxy based sealer was tested for penetrability and concluded that Ca(OH)_2 had an impact on penetration into the tubules.³⁰

14. Using CLSM, Khullar S et al. evaluated the penetrability of Adseal, Sealapex, and BioRoot RCS into the tubules in apical area. The findings indicated that Adseal showed reduced tubular penetration at all root locations, BioRoot RCS demonstrated more penetration.³¹

15. Abu Hasna et al. investigated the best ways to remove *E.faecalis* infected biofilm using various adjuncts like NAC, PDT, and NAC + PDT in vitro. According to this in vitro investigation, NAC exhibited antibacterial activity which is comparable to CH.³²

16. In conjunction with a single cone obturation procedure, Eid BM et al assessed the penetrability of 3 different sealers: AH +, Apexit +, and Smart paste bio in the apical, middle, and coronal thirds. Among the three studied groups, the apical demonstrated the least sealer penetration. There is no statistically significant difference seen in coronal and middle thirds.³³

17. To maintain the regeneration capability of SHED during in vitro cultivation, Martacic et al. conducted a study to examine if N-acetyl-L-cysteine (NAC) might shield the cells from oxidative damage. In SHED, the degree of oxidative damage was examined following a 48-hour exposure to varying NAC doses. The results showed that a low dose of NAC changed the fatty acid composition in a way that increased PUFA and greatly reduced lipid peroxidation. A stronger correlation between enhanced SHED survival in vitro and decreased oxidative damage to cellular lipids may exist.³⁴

18. Using confocal laser scanning microscopy, Uzunoglu-Özyürek E et al investigated the impact of Ca(OH)₂ medicament on tubular penetrability of two distinct root canal sealers, Bioroot-RCS & AH 26. Bioroot-RCS even after Ca(OH)₂ was found to have a greater penetration rate than AH 26.³⁵
19. The purpose of this study by Yahui Wang et al. was to appraise the quality of filling and penetration of BC sealer (iRoot SP) using Protaper Universal instrumentation and filling techniques such as AH Plus SC technique, iRoot SP warm vertical technique, and iRoot SP SC technique. It was discovered that the frequency of holes and gaps was not statistically significantly affected by filling methods or sealer types. It was discovered that iRoot SP can produce superior penetration and comparable filling quality compared to AH Plus, regardless of the technique employed.³⁶
20. In order to assess how well four distinct sealer adapt to root canal walls and to measure their tubular penetration depth in all thirds of the root canal, Chen H et al. conducted a study. The findings showed that RealSeal SE had the greatest penetration. The best adaptability to root canal walls is exhibited by AH-Plus.³⁷
21. Using a file, brush, or passive ultrasonic irrigation (PUI), Zorzin J et al. assessed the amount of Ca(OH)₂ eliminated by irrigation with varying volumes and activation methods. It was discovered that a considerable drop in residual Ca(OH)₂ occurred when the irrigation amount was increased. However, no irrigation technique could totally eliminate Ca (OH)₂, the most successful activation technique was PUI.³⁸

22. Palaniswamy et al. investigated a study to determine antibacterial effects of 2% CHX and NAC to determine if they would operate as an antagonist or synergist as an intracanal medication against *E. faecalis* cells using an agar diffusion test. Of all the tested groups that were evaluated, inhibition zone for 2% CHX and NAC were nearly identical, but the combination of 2% CHX and NAC exhibited the highest level of inhibition, indicating a synergistic effect.³⁹
23. Quah et al. carried out a study to assess N-acetylcysteine's (NAC) antibacterial and biofilm-eradication capabilities against *Enterococcus faecalis*. He discovered that at pH 11, NAC is the most bactericidal. Furthermore, prolonged (up to three weeks) NAC was mixed with dentin powder and incubated which did not substantially lessen its antibacterial effectiveness against *E. faecalis*.⁴⁰
24. Marciano MA et al. conducted a study to assess the root canal wall adaptability, radiopacity, flow, solubility, film thickness, and setting time of three resin-based sealers: Adseal, Acroseal, and AH Plus. The results showed comparable root canal adaptability, solubility, flow, and film thickness of all sealers.⁴¹
25. Sequeira et al. performed an analysis to verify intracanal bacterial decrease using instrumentation and irrigation using saline solution or sodium hypochlorite (NaOCl) at concentrations of 1%, 2.5%, and 5.25%. There was no discernible variation in the concentration of the NaOCl solutions, although all test solutions considerably decreased the amount of bacterial cells in the root canal. All NaOCl solutions, however, were noticeably superior to saline

solution in terms of their ability to lower the quantity of bacterial cells in the root canal.⁴²

26. T. OKSAN et al. investigated how smear layer affected tubular penetration of sealers in all thirds of the root canal. Penetration of Diaket, N2, and SPAD into the tubules was shown to be superior to that of Forfenan. This difference in penetration may be attributed to the physiochemical characteristics of the filling materials.⁴³

MATERIAL AND METHODS

SOURCE OF DATA:

- Extracted human maxillary anterior teeth were collected from Department of Oral and Maxillofacial Surgery, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi.
- The study was conducted in the Department of Conservative Dentistry and Endodontics, KLE VK Institute of Dental Sciences, Belagavi, KLE Academy of Higher Education & Research and the laboratory procedures will be undertaken in Dr. Prabhakar Kore's Basic Science Research Laboratory, KAHER, Belagavi.
- Specimens were evaluated under the confocal laser scanning microscope at National Centre for Biological Sciences, Bangalore.

INCLUSION CRITERIA

- Human maxillary anterior teeth with single root and single straight canal with closed apex.
- Teeth with apical width # 20 k or less.

EXCLUSION CRITERIA

- Teeth with radicular resorption, cracks or fracture line.
- Root canal treated teeth.
- Teeth with calcified canals.
- Teeth with root caries.
- Teeth with multiple canals/Anatomic variation.
- Tooth with apical width more than #20 k.

MATERIALS USED FOR THE STUDY:

- Human extracted maxillary anterior teeth
- 0.1% thymol
- 0.9% saline (Aishwarya Lifescience, Solan)
- 5.25 % Sodium Hypochlorite (Vishal Dentocare, Ahmedabad)
- 17% Ethylene diamine tetra acetic Acid (EDTA) (Canalarge)
- Paper points (Diadent Color coded paper points, South Korea)
- Rhodamine B dye (Sigma Aldrich)
- Distilled water
- N- acetyl cysteine (MolyChem Chemicals Ltd, Mumbai)
- Propylene glycol (MolyChem Chemicals Ltd, Mumbai)
- AH Plus sealer (Dentsply)
- Ceraseal sealer (Meta Biomed)
- Gutta-percha points (Diadent, South Korea)
- Cavit (3M, ESPE)

ARMAMENTARIUM USED FOR THE STUDY

- Micromotor handpiece (NSK, Marathon)
- K Files (10-40) (Mani Inc, Japan)
- ProTaper universal nickel-titanium files (Dentsply Maillefer, Switzerland)
- Endomotor (X- Smart, Dentsply)
- 27 gauge syringe (Dispovan)
- Ultrasonic system handpiece and files (Ultra X)
- Lentulo spirals (Mani Inc, Japan)
- Diamond disks
- Confocal laser scanning microscope

SAMPLE SIZE ESTIMATION:

95 % confidence interval & 95% power

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (SD_1^2 + SD_2^2)}{(x_1 - x_2)^2}$$

Where SD1 = 50.76

$$SD2 = 225.06$$

$$x_1 = 201.33$$

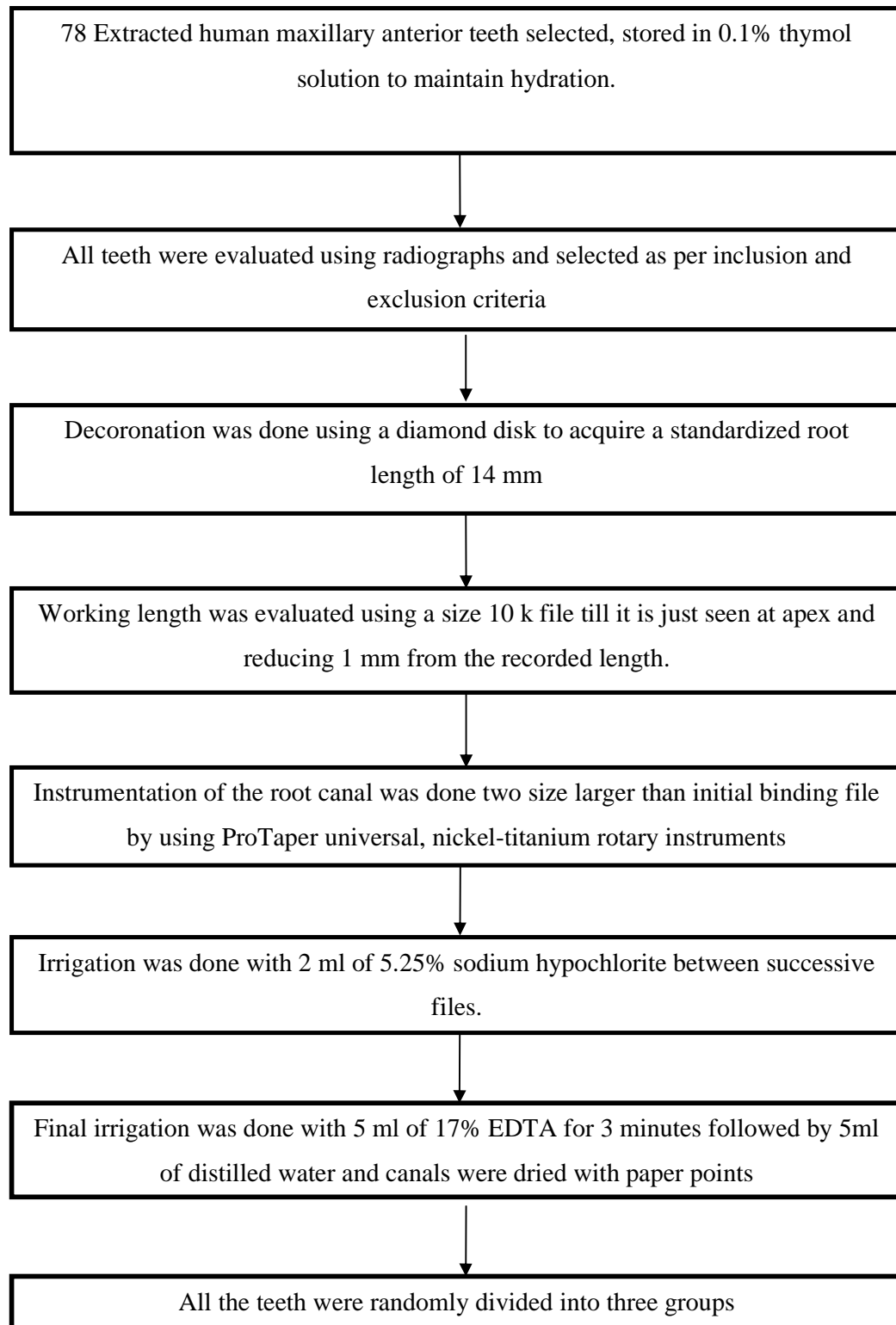
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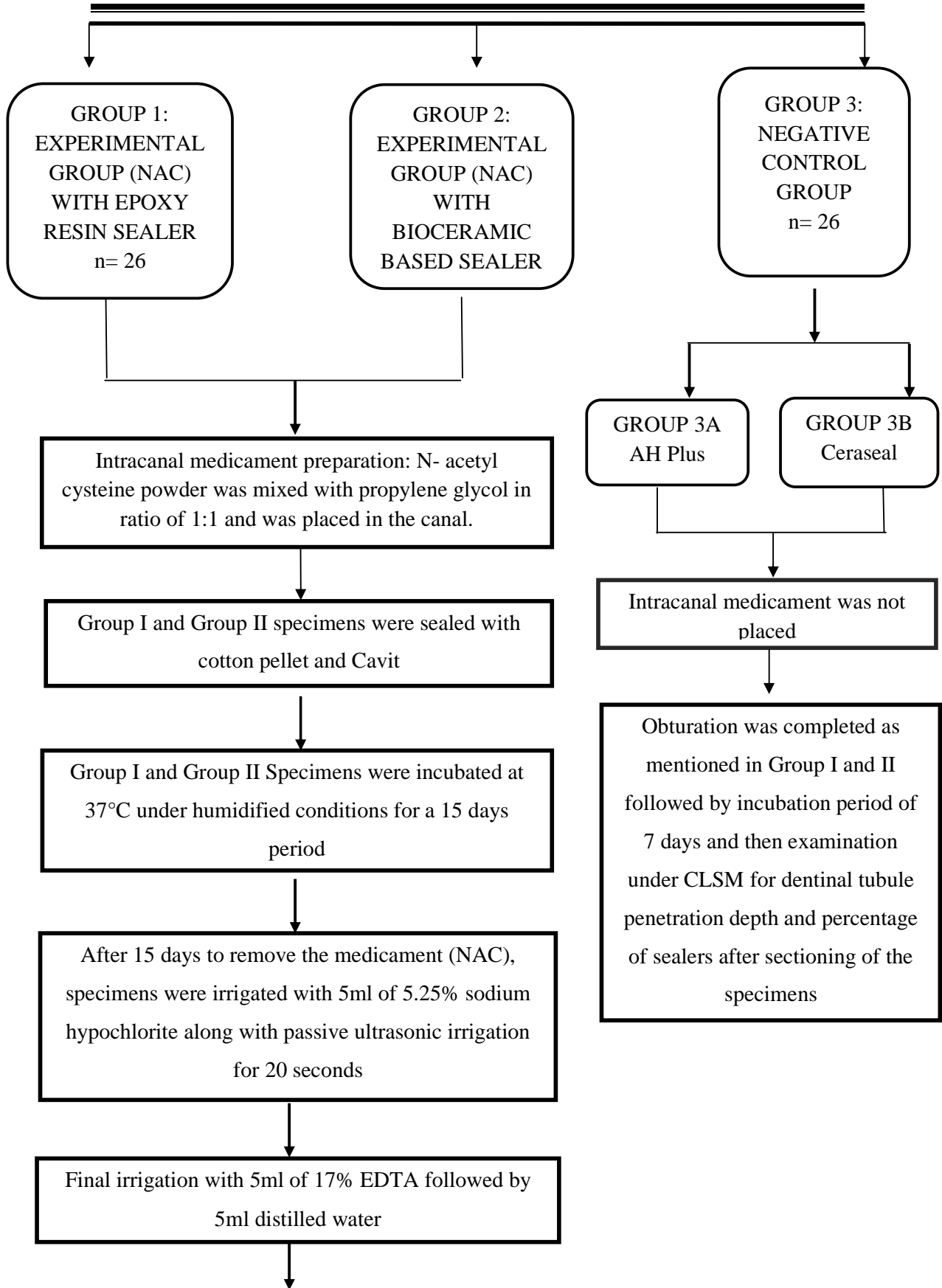
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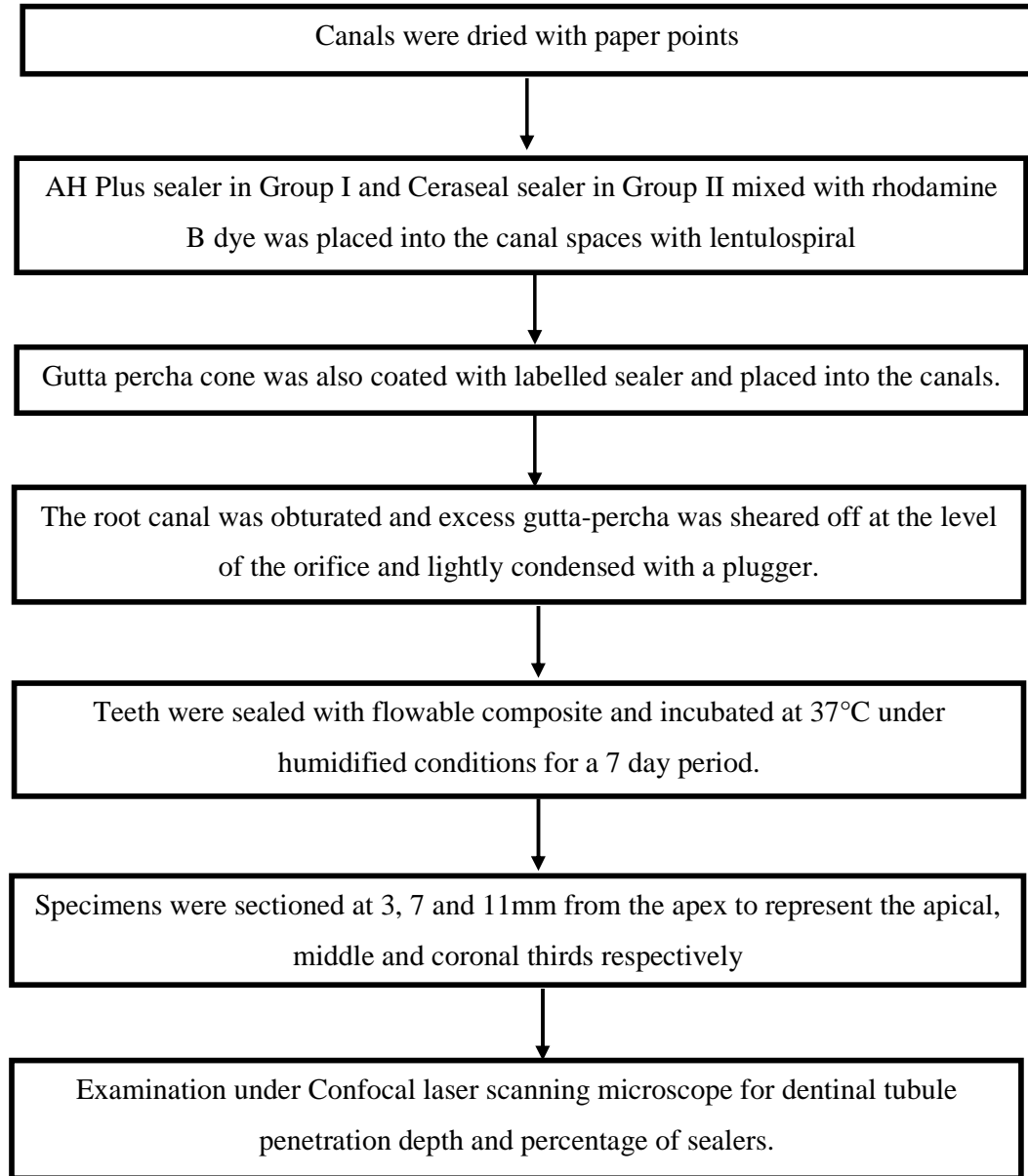
SAMPLE SIZE PER GROUP: 26

TOTAL SAMPLE SIZE: 78

FLOWCHART DEPICTING THE STUDY DESIGN







FLOWCHART DEPICTING STUDY DESIGN OF SEM PILOT STUDY

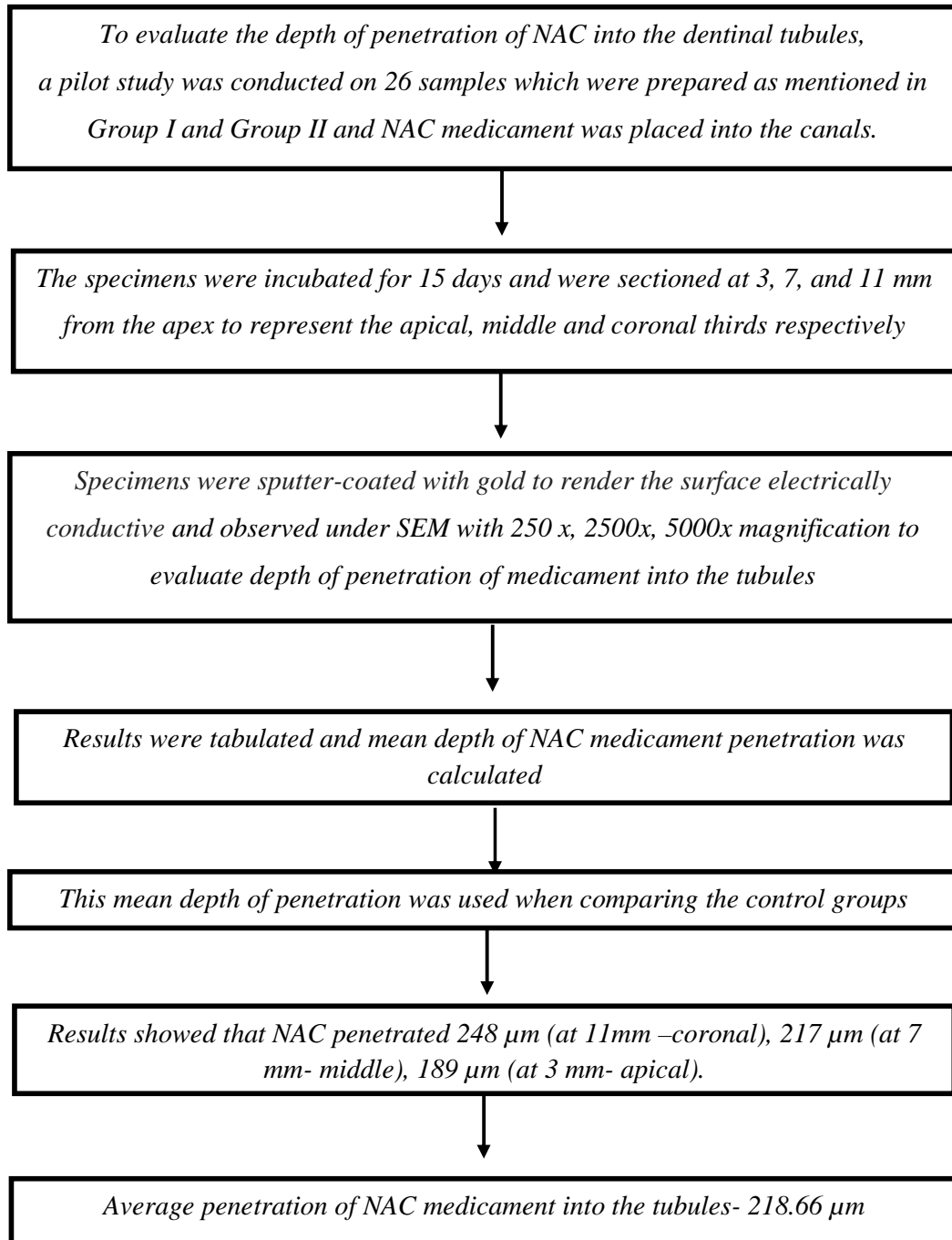


FIGURE 1: SEM analysis shows the presence of NAC medicament in the **coronal third** of the canal

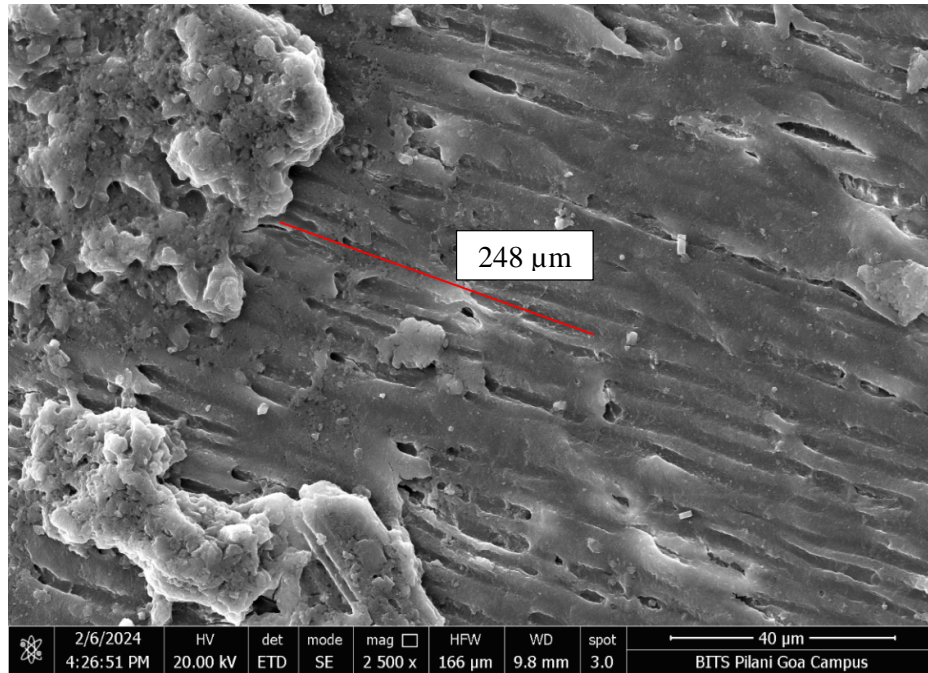


FIGURE 2: SEM analysis shows the presence of NAC medicament in the **middle third** of the canal

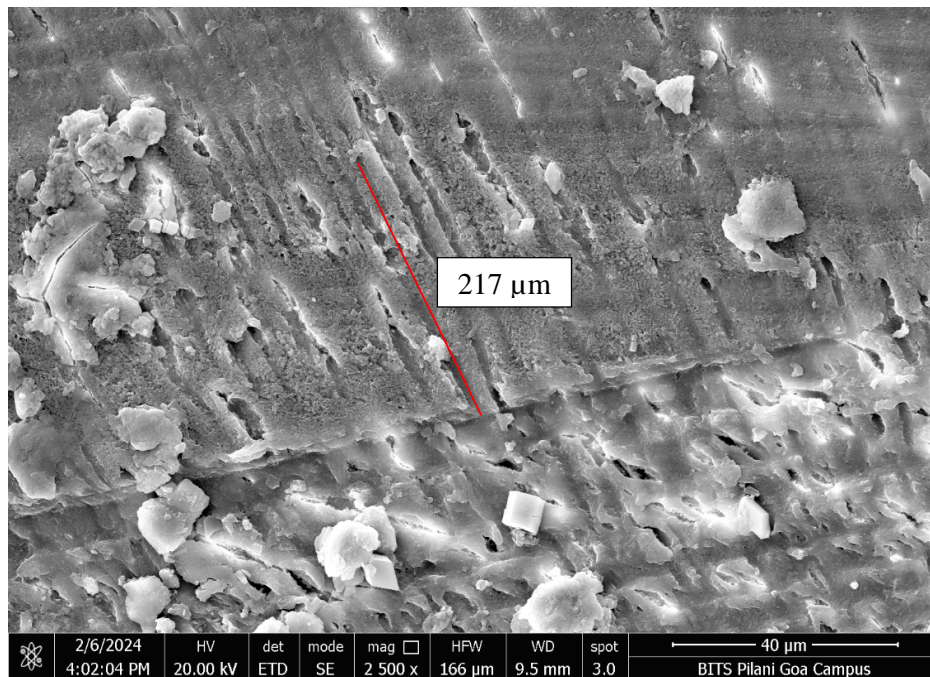
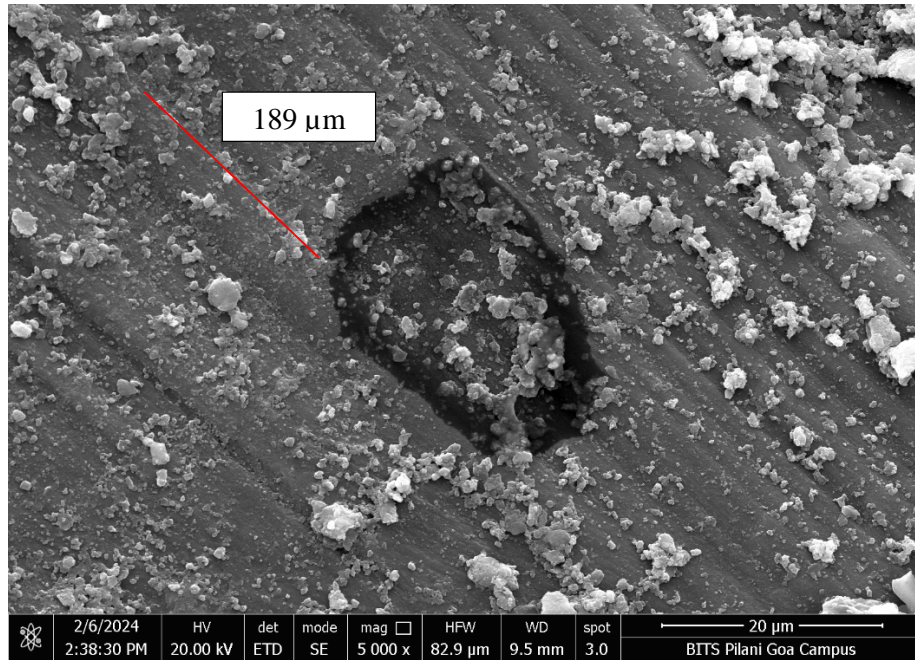


FIGURE 3: SEM analysis shows the presence of NAC medicament in the **apical third** of the canal



STEPS OF THE PROCEDURES DURING THE RESEARCH

- 78 human maxillary anterior teeth were used for the study. According to OSHA guidelines, debris were cleaned and kept in 0.1% thymol and selected as per the criteria.
- Decoronation was done to a standard length of 14 mm and WL was assessed after which instrumentation was done two sizes larger than initial binding file by ProTaper universal, Ni- Ti rotary and 2 ml of 5.25% NaOCl was irrigated inbetween successive files and final flush of 5 ml of 17% Ethylene diamine tetraacetic acid for 3 minutes followed by 5ml of distilled water and canals were dried with paper points.
- Teeth was randomly divided into 3 groups

Group 1: Experimental group (NAC) with Epoxy resin sealer

Group 2: Experimental group (NAC) with Bioceramic based sealer

Group 3: Negative control group

- 26 specimens were chosen randomly to represent the three groups.
- Intracanal medicament was prepared by mixing N- acetyl cysteine powder with propylene glycol in the ratio of 1:1 and was placed in Group I and Group II specimens. Teeth were sealed with Cavit and specimens were incubated at 37 °C for 15 days then irrigated to remove the medicament with 5ml of 5.25% NaOCl along with PUI for 20 seconds, final flush of 5ml of 17% EDTA followed by 5ml of DW and canals were dried with paper points.
- To promote fluorescence, sealer was manipulated with rhodamine B dye at a ratio of 0.1% (weight). Using lentulospiral, sealers were coated and GP was placed in the canal and excess gutta-percha was sheared and condensed with a

plugger and sealed with Cavit and incubation was done at 37°C for 7 days. Then samples were sectioned horizontally at the coronal (11mm from root tip), middle (7mm from root tip) and apical third (3mm from root tip) to obtain 1mm section. The specimens were examined in Confocal laser scanning microscope for dentinal tubule penetration and percentage of sealer.

- The negative control group was used to test the sealer penetration without N-acetyl cysteine in order to see the difference in sealer penetration after using N-acetyl cysteine medicament.
- Group 3 was randomly divided into 2 subgroups.

Group 3A: AH Plus

Group 3B: Ceraseal

- Obturation was done as mentioned in Group I and Group II followed by incubation period of 7 days and then examination under the confocal laser scanning microscope for testing penetration and percentage of sealers after sectioning of the specimens.
- Calculation of dentinal tubular penetrability: Images analyzed with Image J software and longest depth and area adaptation of sealer penetration for each sample was measured. The penetration depth measured from canal wall to point of maximum sealer penetration using measuring tool in the Image J software. Area/ Percentage was calculated by measuring the canal perimeter and area adapted by the sealer using Image J software.

SEM PILOT STUDY:

To assess penetration depth of NAC into the tubules, a pilot study was conducted on 26 samples which were prepared as mentioned in Group I and Group II and medicament placed into the canals. The specimens were incubated for 15 days and were sectioned at 3mm, 7mm, and 11 mm from the root tip to represent the apical, middle and coronal thirds respectively and then examined under Scanning Electron Microscope (SEM) with 250 x, 2500 x, 5000 x magnification.

STATISTICAL TEST

Depending upon whether we obtain normal distribution or not, Statistical analysis were performed using Two way ANOVA and Tukey's multiple post hoc test.

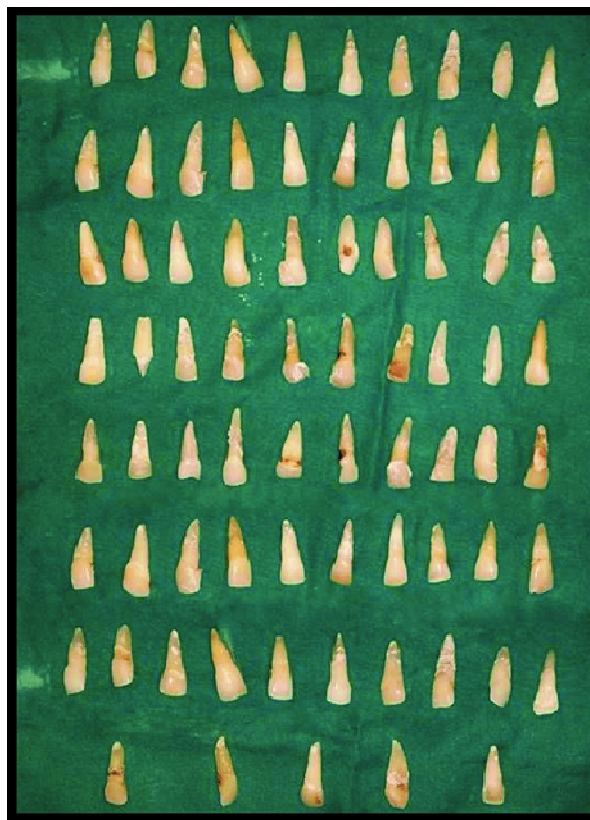


FIGURE 4: Human maxillary anterior selected for study



FIGURE 5: Debris removal with ultrasonic scaler



FIGURE 6: Materials used for the study



FIGURE 7: Armamentarium used for the study

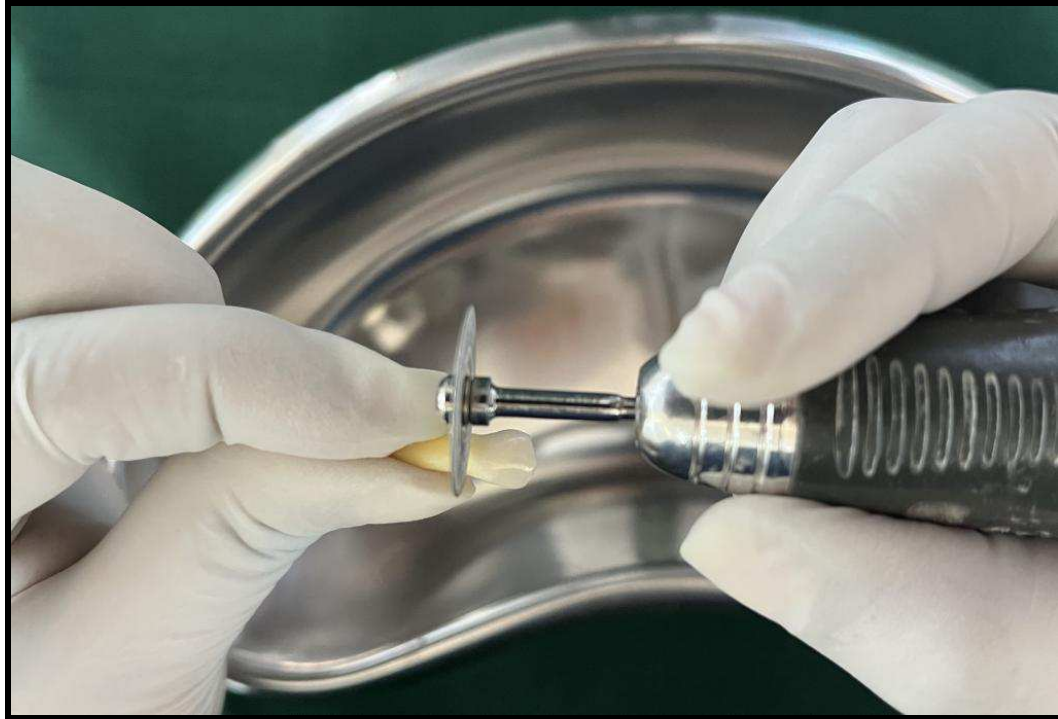


FIGURE 8: Decoronation of the samples with diamond disk

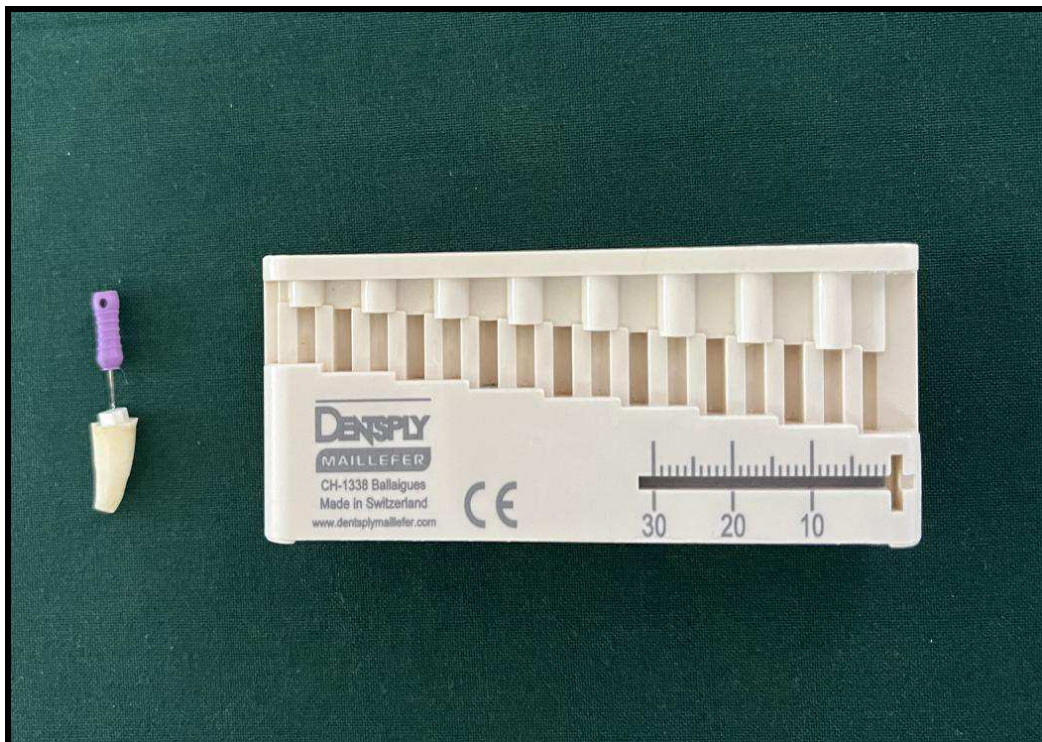


FIGURE 9: WL Determination

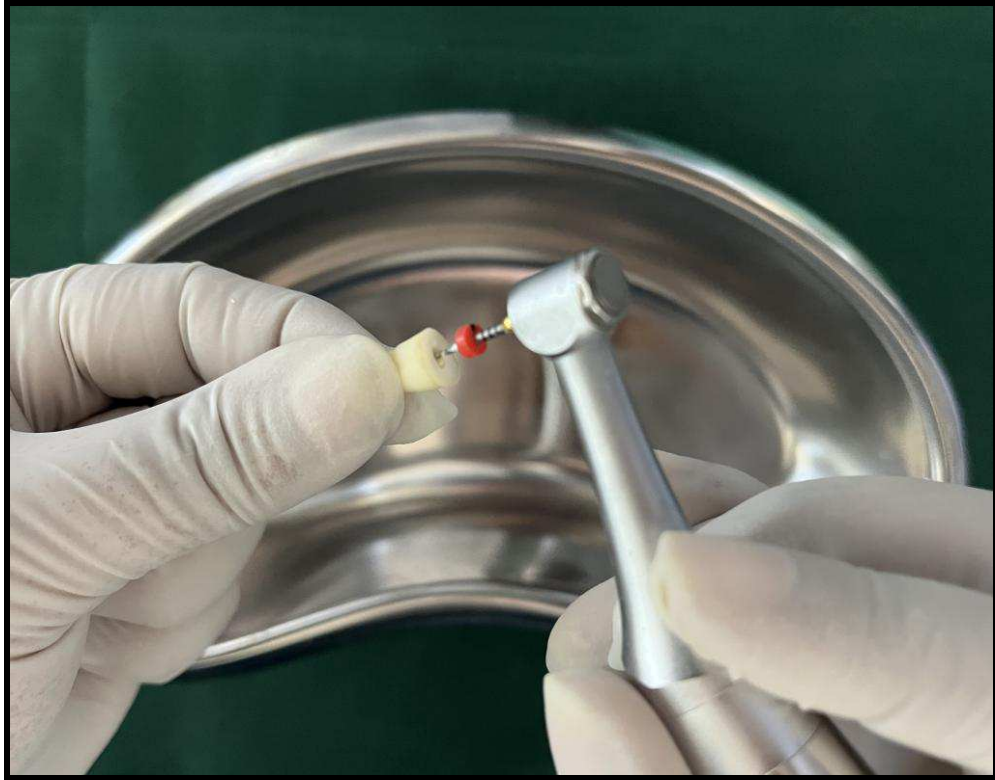


FIGURE 10: Biomechanical Preparation



FIGURE 11: Irrigation of samples



FIGURE 12: Materials used for preparation of NAC medicament

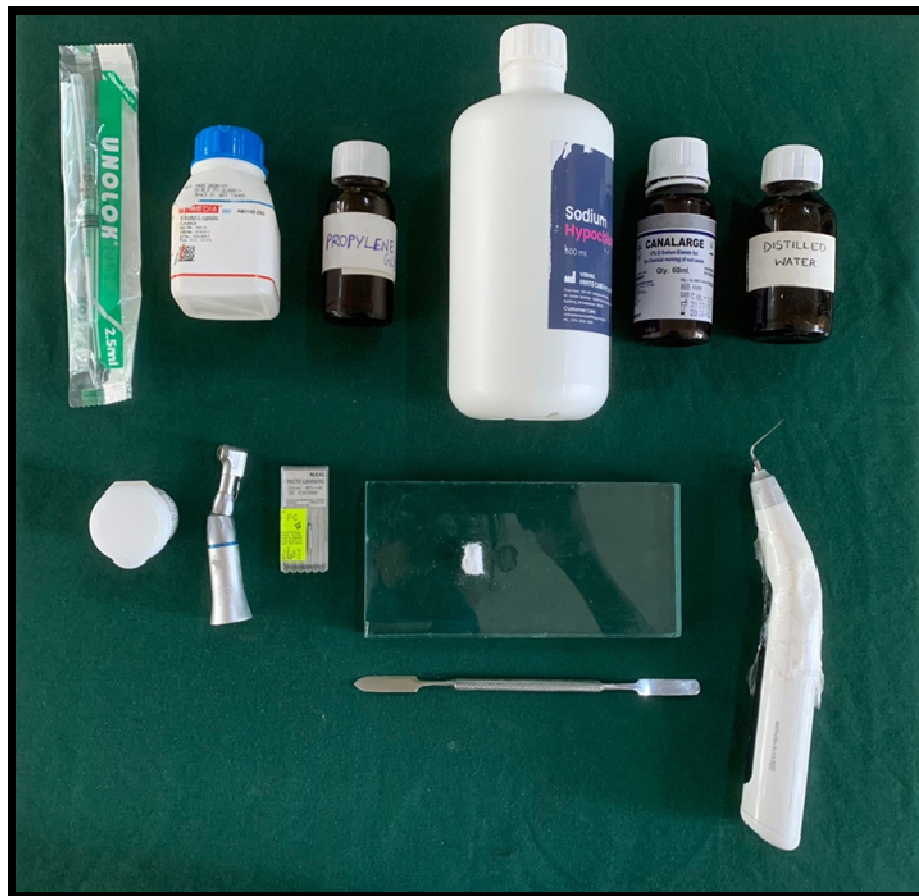


Figure 13: Materials for placement and removal of NAC medicament

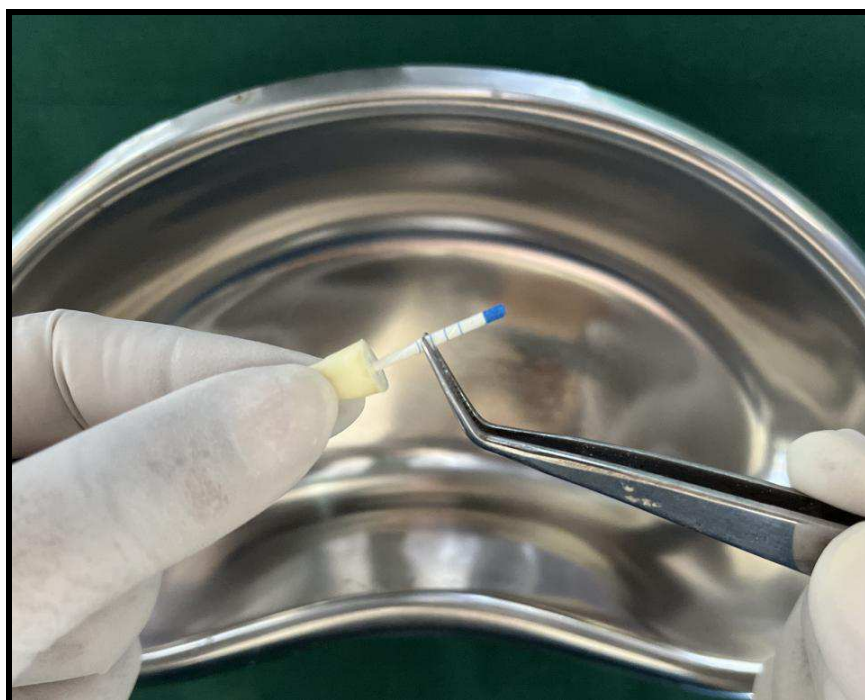


FIGURE 14: Drying canals with paper point

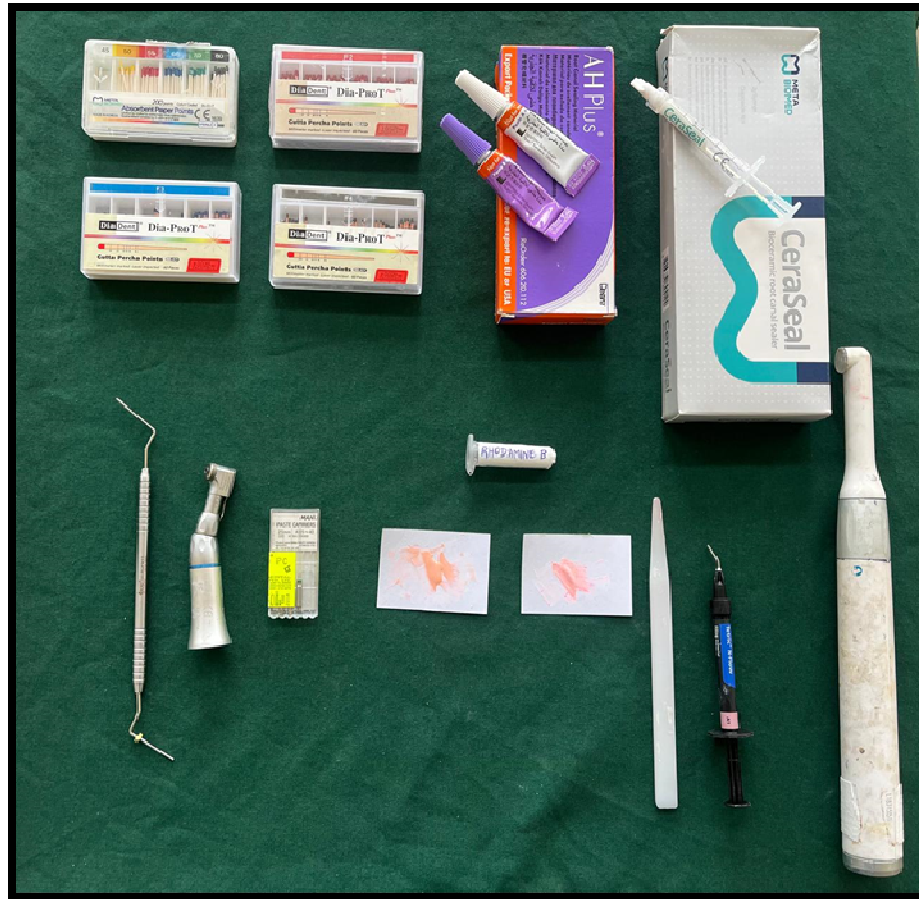


FIGURE 15: Materials used for obturation



FIGURE 16: Placement of sealer with lentulospiral

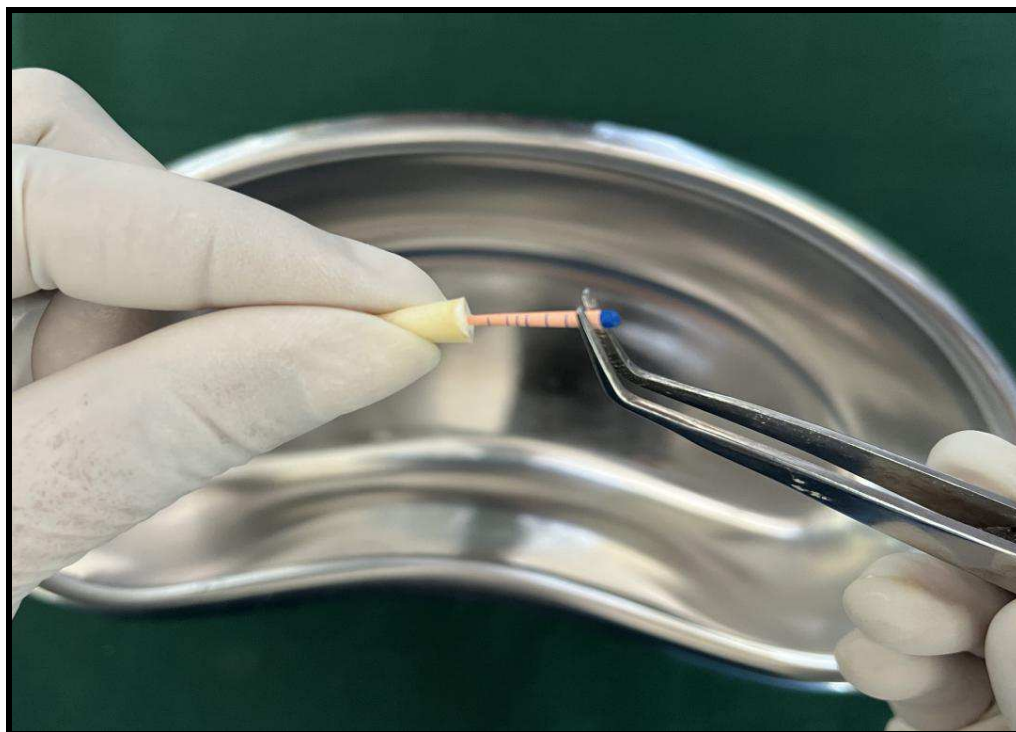


FIGURE 17: Obturation

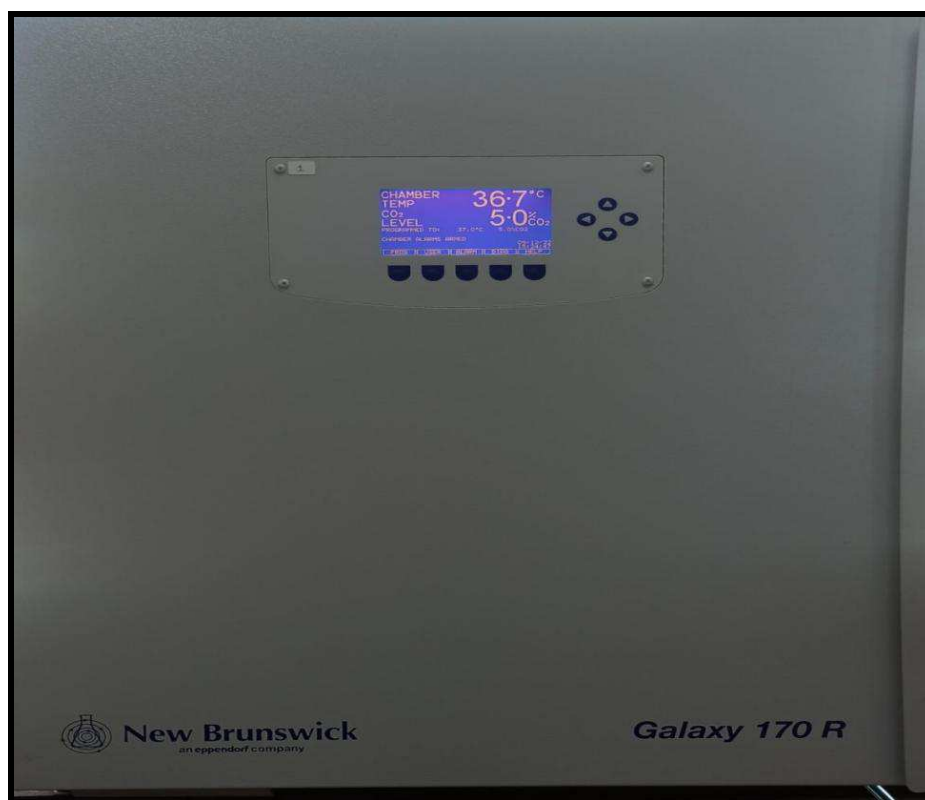


FIGURE 18: Incubator



FIGURE 19: Sectioning

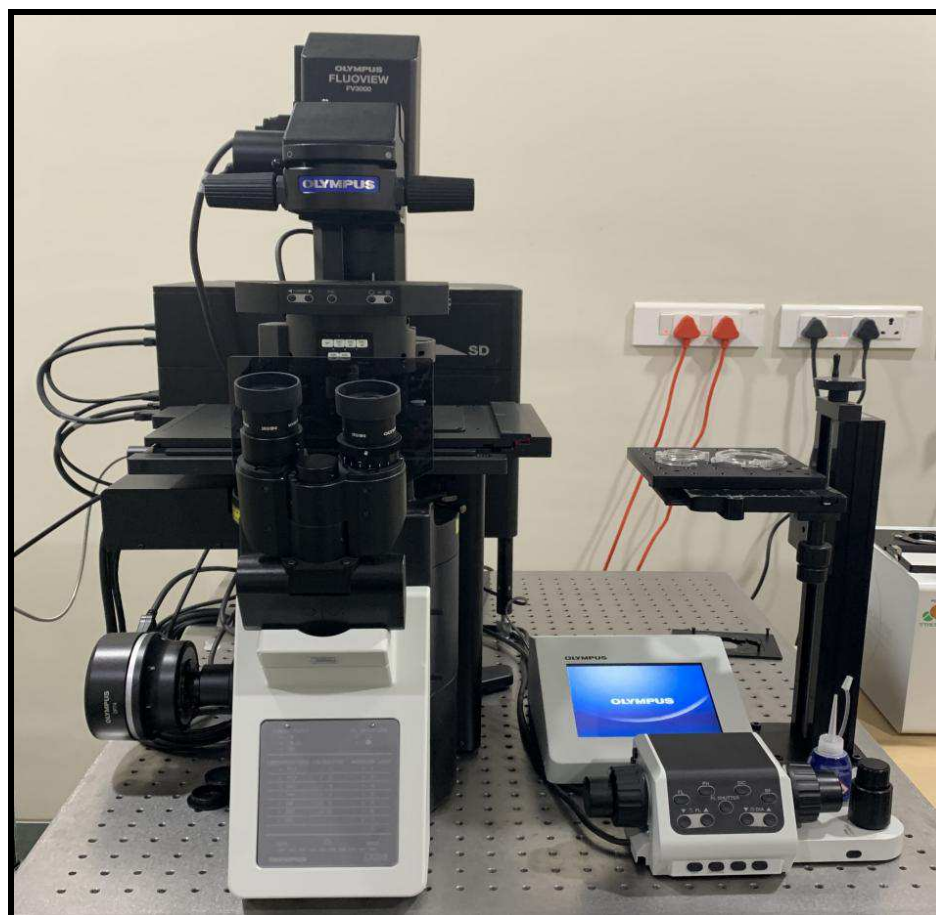


FIGURE 20: Confocal laser scanning microscope

RESULTS**Table 1: Summary of length values (penetration depth) in four groups and three regions**

Factor	Level of factor	N	Mean	SD	SE	95% CI for mean	
						Lower	Upper
Groups	Group 1	78	1354.22	238.97	27.06	1300.34	1408.10
	Group 2	78	1546.76	270.98	30.68	1485.67	1607.86
	Group 3A	39	1333.69	248.40	39.78	1253.17	1414.21
	Group 3B	39	1448.89	292.80	46.89	1353.98	1543.81
Regions	Coronal	78	1738.96	123.02	13.93	1711.22	1766.69
	Middle	78	1419.46	147.42	16.69	1386.22	1452.70
	Apical	78	1133.86	72.76	8.24	1117.46	1150.27
Group* region	Group 1 with Coronal	26	1634.49	76.09	14.92	1603.75	1665.22
	Group 1 with Middle	26	1346.48	94.23	18.48	1308.42	1384.54
	Group 1 with Apical	26	1081.71	47.27	9.27	1062.62	1100.80
	Group 2 with Coronal	26	1845.80	71.51	14.03	1816.91	1874.68
	Group 2 with Middle	26	1583.01	88.11	17.28	1547.42	1618.59
	Group 2 with Apical	26	1211.49	44.94	8.81	1193.34	1229.64
	Group 3A with Coronal	13	1651.32	89.92	24.94	1596.98	1705.66
	Group 3A with Middle	13	1261.65	70.70	19.61	1218.92	1304.37

Group 3A with Apical	13	1088.10	45.27	12.56	1060.75	1115.46
Group 3B with Coronal	13	1821.85	46.54	12.91	1793.73	1849.98
Group 3B with Middle	13	1396.13	52.21	14.48	1364.59	1427.68
Group 3B with Apical	13	1128.69	42.61	11.82	1102.94	1154.44

Table 2: Comparison of four groups and three regions with mean length values by two way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Group	1886834.90	3	628944.97	132.0836	0.0001*
Region	12996433.60	2	6498216.81	1364.6784	0.0001*
2-way interaction effects					
Group * Region	302595.53	6	50432.59	10.5913	0.0001*
Error	1057101.91	222	4761.72		
Total	16242965.94	233			

*p<0.05

Table 2 shows statistically significant difference between both the groups and regions with a p value of 0.0001*

Table 3: Intergroup (Pair wise) comparison of four groups with mean length values by Tukeys multiple posthoc procedures

Groups	Group 1	Group 2	Group 3A	Group 3B
Mean	1354.22	1546.76	1333.69	1448.89
SD	238.97	270.98	248.40	292.80
Group 1	-			
Group 2	0.0001*	-		
Group 3A	0.4270	0.0001*	-	
Group 3B	0.0001*	0.0001*	0.0001*	-

*p<0.05

Table 3, Graph 1 revealed intergroup comparison using Tukeys multiple posthoc procedure which showed statistically significant difference in all groups except 1 and 3A.

The mean depth of penetration was highest for NAC + Ceraseal with a value of 1546.76 followed by Ceraseal with value of 1448.89. However, NAC +AH Plus showed least penetration depth with mean value of 1354.22 followed by AH Plus with a value of 1333.69.

Table 4: Intergroup (Pair wise) comparison of three regions with mean length values by Tukeys multiple posthoc procedures

Region	Coronal	Middle	Apical
Mean	1738.96	1419.46	1133.86
SD	123.02	147.42	72.76
Coronal	-		
Middle	0.0001*	-	
Apical	0.0001*	0.0001*	-

*p<0.05

Table 4, Graph 2 According to the sections/region of the tooth, highest depth of penetration of sealer was observed at coronal third (1738.96) followed by middle third (1419.46) and least at apical third (1133.86).

Table 5: Comparison of interactions of four groups and three regions with mean length values by Tukeys multiple posthoc procedures

Interactions	Group 1 Coronal	Group 1 Middle	Group 1 Apical	Group 2 Coronal	Group 2 Middle	Group 2 Apical
Mean	1634.49	1346.48	1081.71	1845.80	1583.01	1211.49
SD	76.09	94.23	47.27	71.51	88.11	44.94
Group 1 Coronal	-					
Group 1 Middle	0.0001*	-				
Group 1 Apical	0.0001*	0.0001*	-			
Group 2 Coronal	0.0001*	0.0001*	0.0001*	-		
Group 2 Middle	0.2310	0.0001*	0.0001*	0.0001*	-	
Group 2 Apical	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	-
Group 3A Coronal	0.9999	0.0001*	0.0001*	0.0001*	0.1356	0.0001*
Group 3A Middle	0.0001*	0.0156*	0.0001*	0.0001*	0.0001*	0.5938
Group 3A Apical	0.0001*	0.0001*	1.0000	0.0001*	0.0001*	0.0001*
Group 3B Coronal	0.0001*	0.0001*	0.0001*	0.9973	0.0001*	0.0001*
Group 3B Middle	0.0001*	0.6095	0.0001*	0.0001*	0.0001*	0.0001*
Group 3B Apical	0.0001*	0.0001*	0.6907	0.0001*	0.0001*	0.0211*
Interactions	Group 3A Coronal	Group 3A Middle	Group 3A Apical	Group 3B Coronal	Group 3B Middle	Group 3B Apical
Mean	1651.32	1261.65	1088.10	1821.85	1396.13	1128.69
SD	89.92	70.70	45.27	46.54	52.21	42.61
Group 1 Coronal						
Group 1 Middle						
Group 1 Apical						

Group 2 Coronal						
Group 2 Middle						
Group 2 Apical						
Group 3A Coronal	-					
Group 3A Middle	0.0001*	-				
Group 3A Apical	0.0001*	0.0001*	-			
Group 3B Coronal	0.0001*	0.0001*	0.0001*	-		
Group 3B Middle	0.0001*	0.0001*	0.0001*	0.0001*	-	
Group 3B Apical	0.0001*	0.0001*	0.9412	0.0001*	0.0001*	-

*p<0.05

Table 5, Graph 3 When the interactions between the medicament and sealers and various sections of the tooth was analysed, the mean depth of tubular penetration was highest for NAC + Ceraseal with coronal (1845.80) and least for NAC +AH Plus with apical (1081.71).

Therefore, the null hypothesis stating that there was no difference in dentinal tubule penetration depth of Epoxy resin sealer and Bioceramic based sealer after use of N-acetyl cysteine intracanal medicament was rejected.

Table 6: Summary of area values in four groups and three regions

Factor	Level of factor	N	Mean	SD	SE	95% CI for mean	
						Lower	Upper
Groups	Group 1	78	9560.38	1586.90	179.68	9202.59	9918.17
	Group 2	78	8425.12	1691.58	191.53	8043.72	8806.51
	Group 3A	39	8692.51	1803.92	288.86	8107.75	9277.28
	Group 3B	39	7919.71	1593.84	255.22	7403.04	8436.37
Regions	Coronal	78	10720.01	867.47	98.22	10524.43	10915.60
	Middle	78	8643.29	817.53	92.57	8458.96	8827.61
	Apical	78	6928.30	798.03	90.36	6748.37	7108.23
Group *region	Group 1 with Coronal	26	11412.06	731.02	143.37	11116.79	11707.32
	Group 1 with Middle	26	9419.58	541.04	106.11	9201.04	9638.11
	Group 1 with Apical	26	7849.50	546.25	107.13	7628.87	8070.13
	Group 2 with Coronal	26	10493.50	708.75	139.00	10207.23	10779.77
	Group 2 with Middle	26	8164.27	564.99	110.80	7936.06	8392.47
	Group 2 with Apical	26	6617.58	271.47	53.24	6507.93	6727.23
	Group 3A with Coronal	13	10743.81	526.49	146.02	10425.65	11061.96
	Group 3A with Middle	13	8753.85	659.28	182.85	8355.45	9152.24
	Group 3A with Apical	13	6579.88	441.84	122.55	6312.88	6846.89
	Group 3B with Coronal	13	9765.15	504.35	139.88	9460.38	10069.93
	Group 3B with Middle	13	7938.19	473.77	131.40	7651.90	8224.49
	Group 3B with Apical	13	6055.77	334.56	92.79	5853.60	6257.94

Table 7: Comparison of four groups and three regions with mean area values by two way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Group	86426581.70	3	28808860.6	93.3763	0.0001*
Region	508650098.0	2	254325049.0	824.3270	0.0001*
2-way interaction effects					
Group * Region	3525097.40	6	587516.23	1.9043	0.0812
Error	68492433.80	222	308524.48		
Total	667094210.9	233			

*p<0.05

Table 7 reports a statistical difference between the main groups and regions of the tooth with a p value of 0.0001*. However, the two way interaction between the groups and regions did not give statistically significant difference with p value of 0.0812.

Table 8: Intergroup (Pair wise) comparison of four groups with mean area values by Tukeys multiple posthoc procedures

Groups	Group 1	Group 2	Group 3A	Group 3B
Mean	9560.38	8425.12	8692.51	7919.71
SD	1586.90	1691.58	1803.92	1593.84
Group 1	-			
Group 2	0.0001*	-		
Group 3A	0.0001*	0.0672	-	
Group 3B	0.0001*	0.0001*	0.0001*	-

*p<0.05

Table 8, Graph 4 shows intergroup comparison using Tukeys multiple posthoc procedures revealed statistically significant difference between all groups except Group 2 and 3A.

The mean area of penetration of NAC + AH Plus was highest with a value of 9560.38 followed by AH Plus with mean value of 8692.51. However, NAC + Ceraseal showed lowest mean area adaptation with value of 8425.12 followed by Ceraseal with a mean value of 7919.71.

Table 9: Intergroup (Pair wise) comparison of three regions with mean area values by Tukeys multiple posthoc procedures

Region	Coronal	Middle	Apical
Mean	10720.01	8643.29	6928.30
SD	867.47	817.53	798.03
Coronal	-		
Middle	0.0001*	-	
Apical	0.0001*	0.0001*	-

*p<0.05

Table 9, Graph 5 According to the sections/region of the tooth, highest area/ adaptation of sealer was observed at coronal third (10720.01) followed by middle third (8643.29) and least at apical third (6928.30).

Table 10: Comparison of interactions of four groups and three regions with mean area values by Tukeys multiple posthoc procedures

Interactions	Group 1 Coronal	Group 1 Middle	Group 1 Apical	Group 2 Coronal	Group 2 Middle	Group 2 Apical
Mean	11412.06	9419.58	7849.50	10493.50	8164.27	6617.58
SD	731.02	541.04	546.25	708.75	564.99	271.47
Group 1 Coronal	-					
Group 1 Middle	0.0001*	-				
Group 1 Apical	0.0001*	0.0001*	-			
Group 2 Coronal	0.0001*	0.0001*	0.0001*	-		
Group 2 Middle	0.0001*	0.0001*	0.6635	0.0001*	-	
Group 2 Apical	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	-
Group 3A Coronal	0.0204*	0.0001*	0.0001*	0.9758	0.0001*	0.0001*
Group 3A Middle	0.0001*	0.0214*	0.0001*	0.0001*	0.0766	0.0001*
Group 3A Apical	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.0000
Group 3B Coronal	0.0001*	0.8007	0.0001*	0.0064*	0.0001*	0.0001*
Group 3B Middle	0.0001*	0.0001*	1.0000	0.0001*	0.9892	0.0001*
Group 3B Apical	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.1150
Interactions	Group 3A Coronal	Group 3A Middle	Group 3A Apical	Group 3B Coronal	Group 3B Middle	Group 3B Apical
Mean	10743.81	8753.85	6579.88	9765.15	7938.19	6055.77
SD	526.49	659.28	441.84	504.35	473.77	334.56
Group 1 Coronal						
Group 1 Middle						
Group 1 Apical						

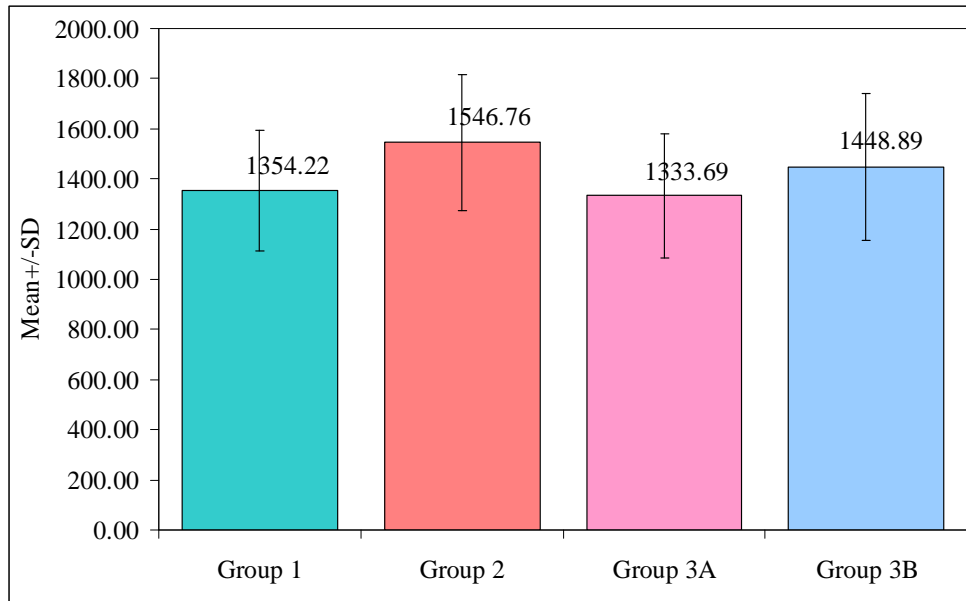
Group 2 Coronal						
Group 2 Middle						
Group 2 Apical						
Group 3A Coronal	-					
Group 3A Middle	0.0001*	-				
Group 3A Apical	0.0001*	0.0001*	-			
Group 3B Coronal	0.0004*	0.0002*	0.0001*	-		
Group 3B Middle	0.0001*	0.0099*	0.0001*	0.0001*	-	
Group 3B Apical	0.0001*	0.0001*	0.4020	0.0001*	0.0001*	-

Table 10, Graph 6 When the interactions between the medicament and sealers and various sections of the tooth was analysed, the mean area/ adaptation of tubular penetration was highest for NAC + AH Plus with coronal (11412.06) and least for Ceraseal with apical (6055.77).

Therefore, the null hypothesis stating that there was no difference in percentage /Area adaptation of Epoxy resin based sealer and the bioceramic based sealer after use of N-acetyl cysteine intracanal medicament was rejected.

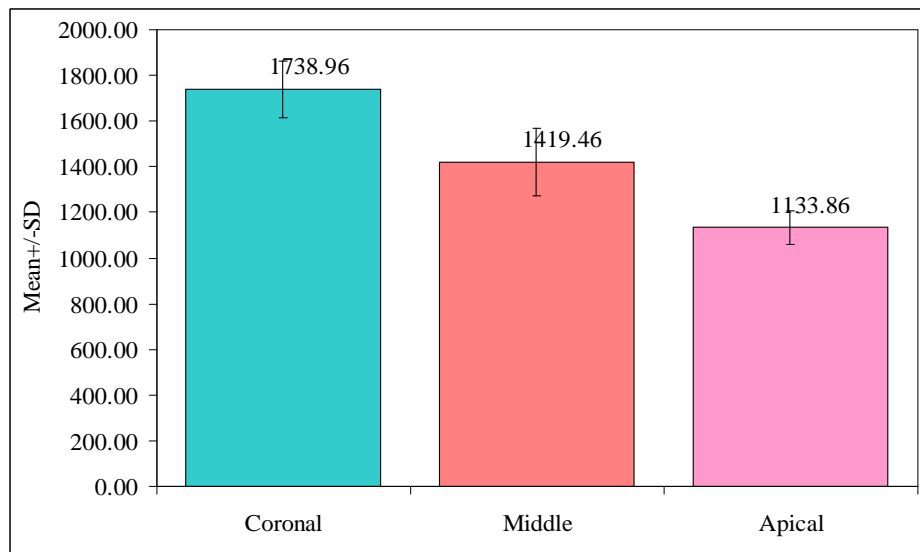
GRAPH 1

Intergroup (Pair wise) comparison of four groups with mean length values by Tukeys multiple posthoc procedures



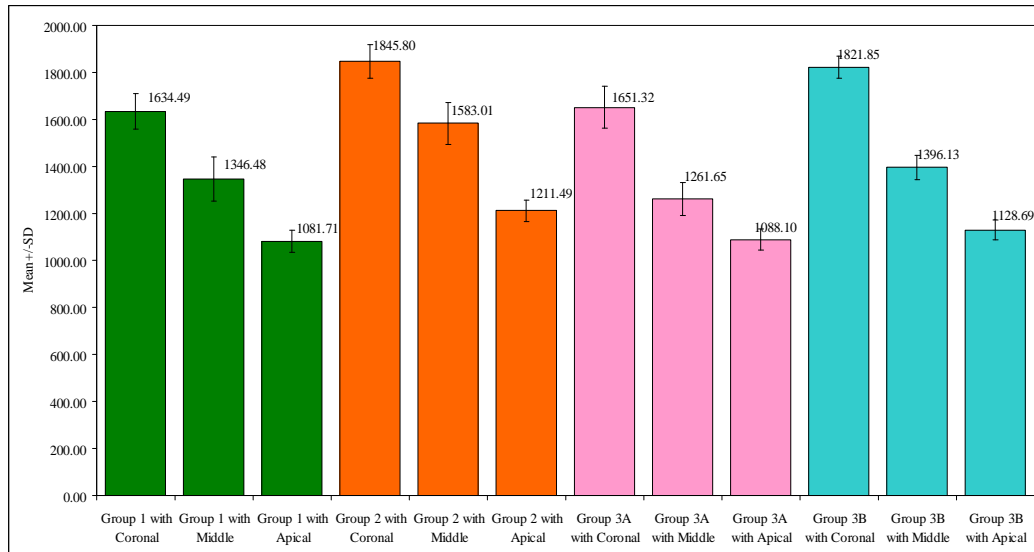
GRAPH 2

Intergroup (Pair wise) comparison of three regions with mean length values by Tukeys multiple posthoc procedures



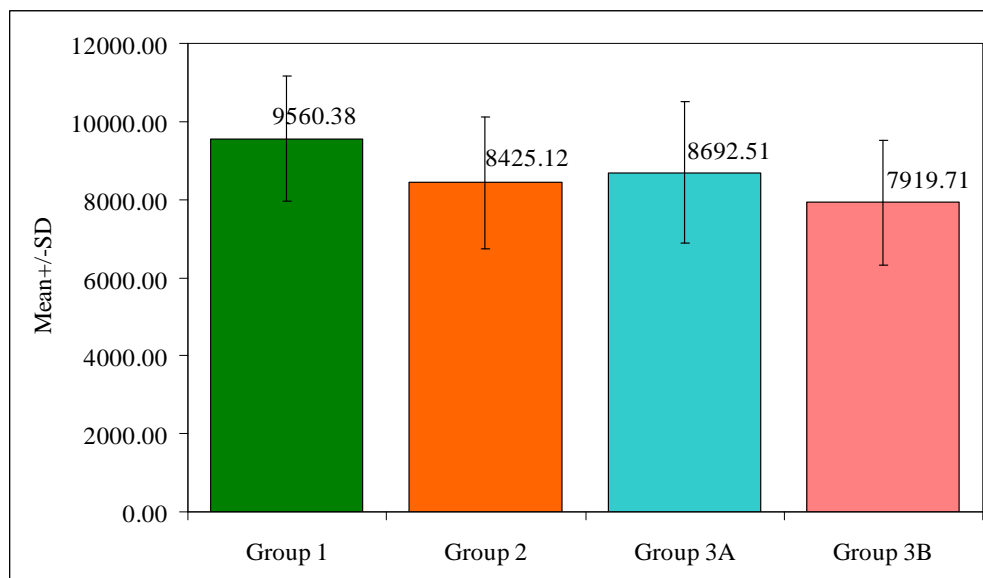
GRAPH 3

Comparison of interactions of four groups and three regions with mean length values by Tukeys multiple posthoc procedures



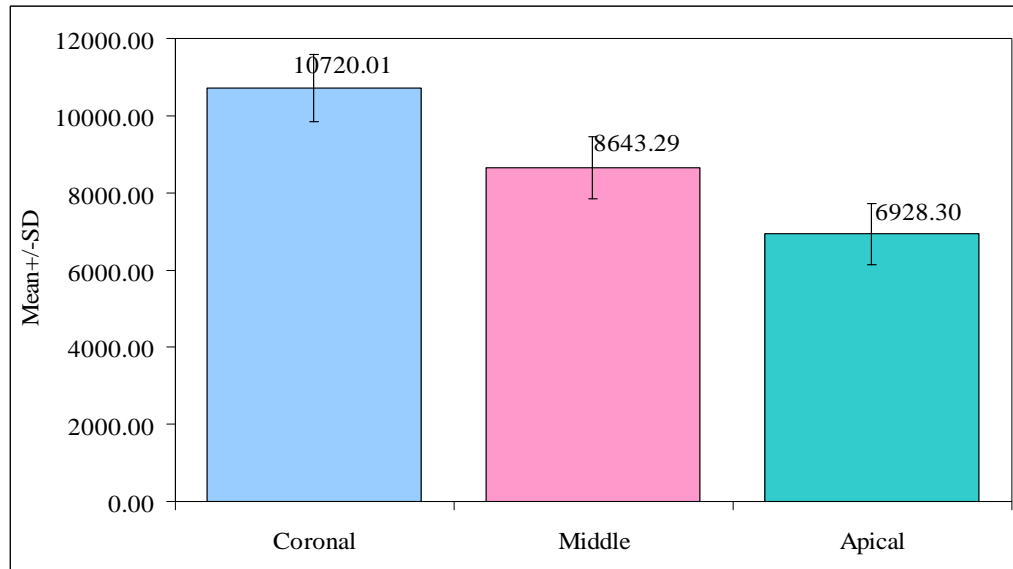
GRAPH 4

Intergroup (Pair wise) comparison of four groups with mean area values by Tukeys multiple posthoc procedures



GRAPH 5

Intergroup (Pair wise) comparison of three regions with mean area values by Tukeys multiple posthoc procedures



GRAPH 6

Comparison of interactions of four groups and three regions with mean area values by Tukeys multiple posthoc procedures

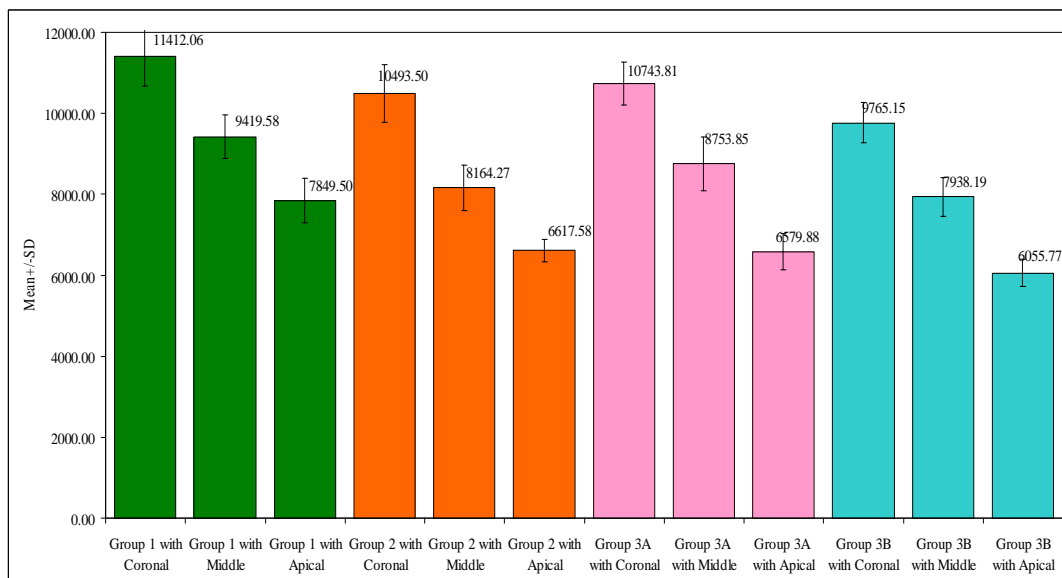
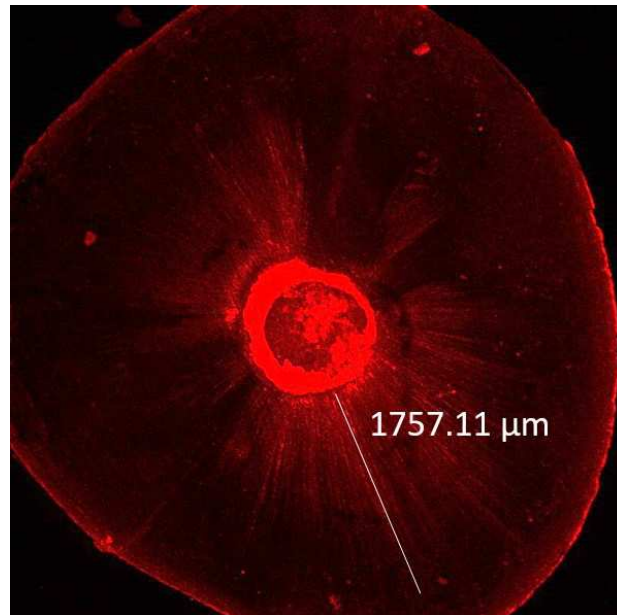


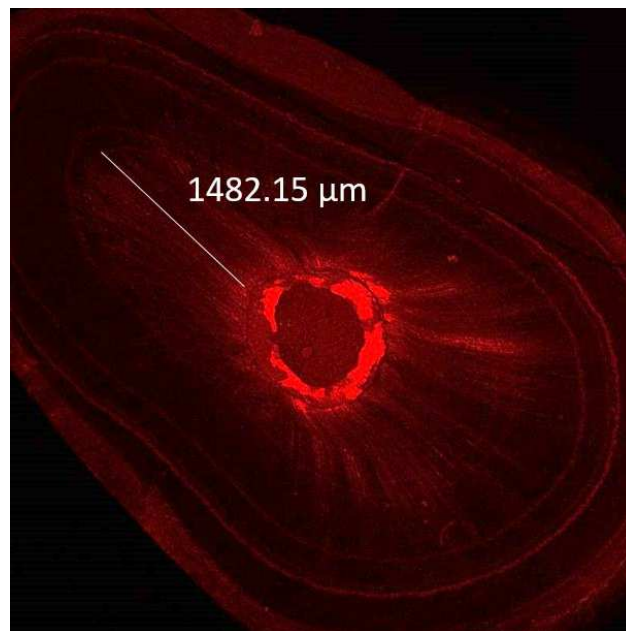
Figure 21: Shows dentinal tubule penetration of NAC+ AH Plus sealer (Group I).

Maximum infiltration was exhibited in coronal area followed by middle and least in

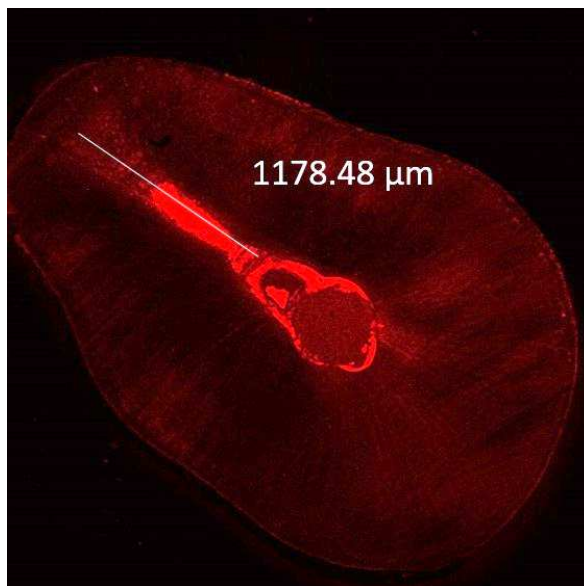
apical part.



CORONAL



MIDDLE

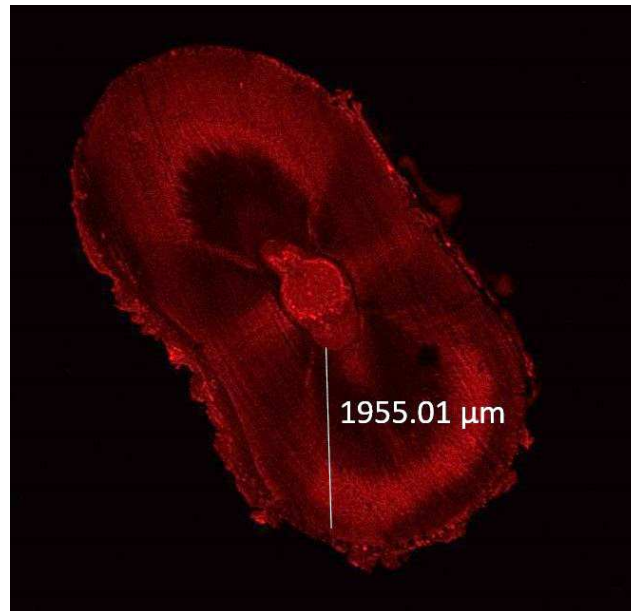


APICAL

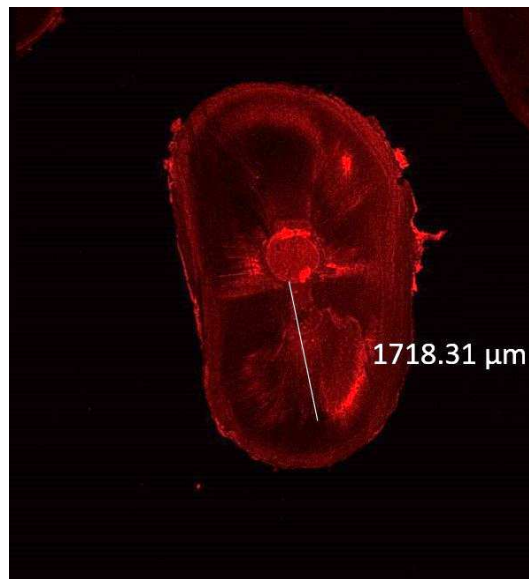
Figure 22: Shows dentinal tubule penetration of NAC+ Ceraseal sealer (Group II).

Maximum infiltration was exhibited in coronal area followed by middle and least in

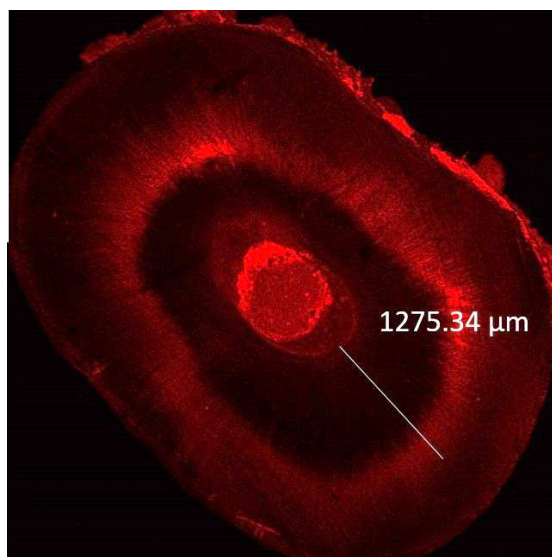
apical part



CORONAL

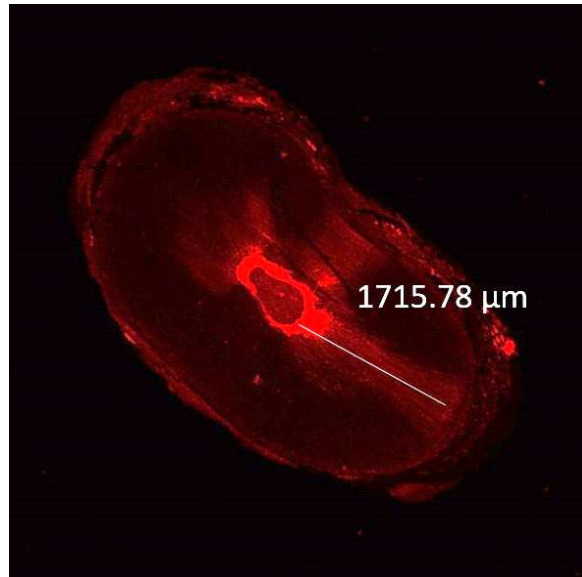


MIDDLE

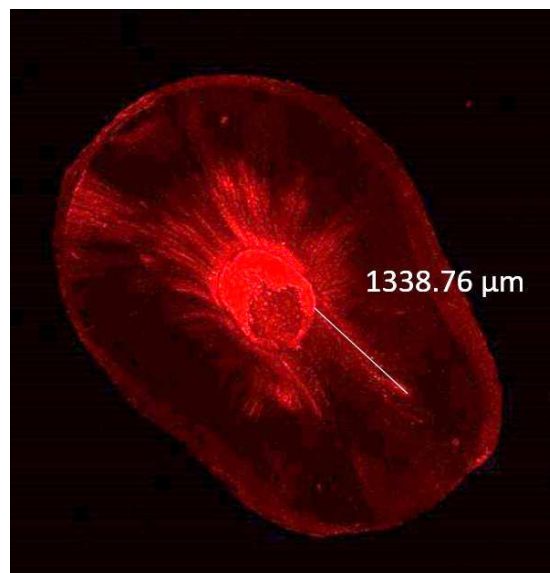


APICAL

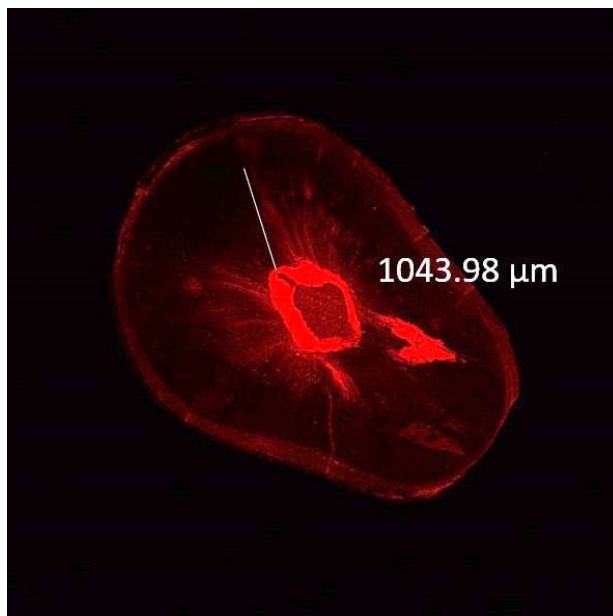
Figure 23: Shows dentinal tubule penetration of **AH Plus sealer (Group III A)**.
Maximum infiltration was exhibited in coronal area followed by middle and least in
apical part.



CORONAL

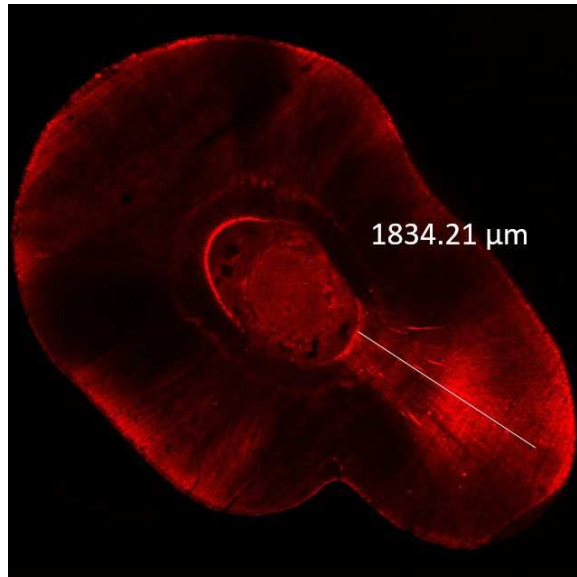


MIDDLE

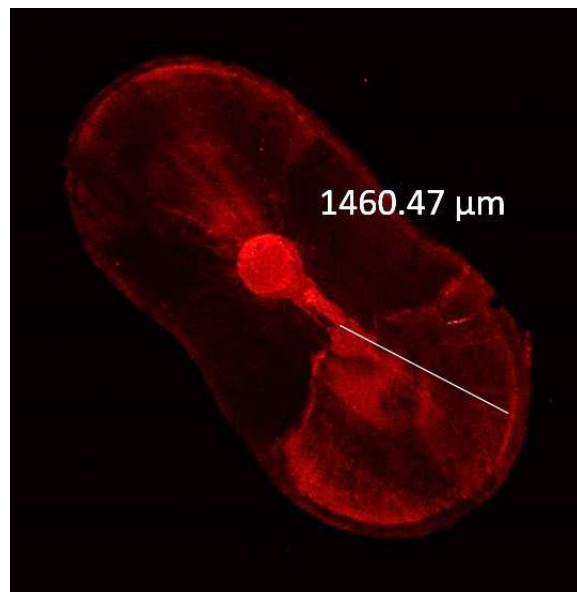


APICAL

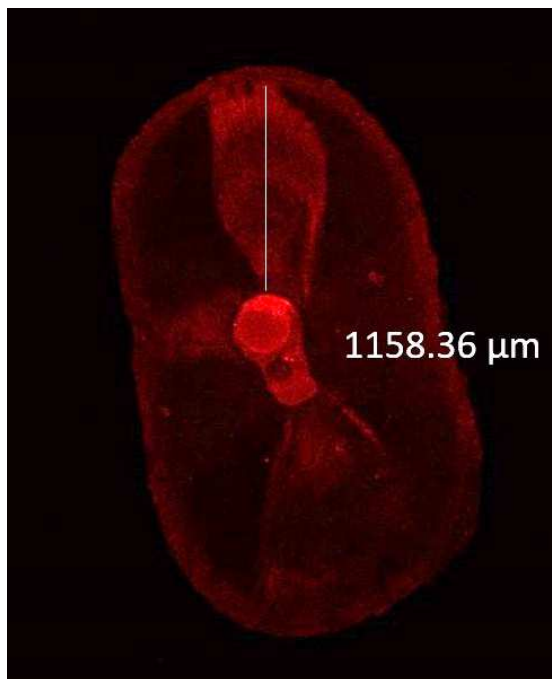
Figure 24: Shows dentinal tubule penetration of **Ceraseal sealer (Group III B)**.
Maximum infiltration was exhibited in coronal area followed by middle and least in apical part.



CORONAL

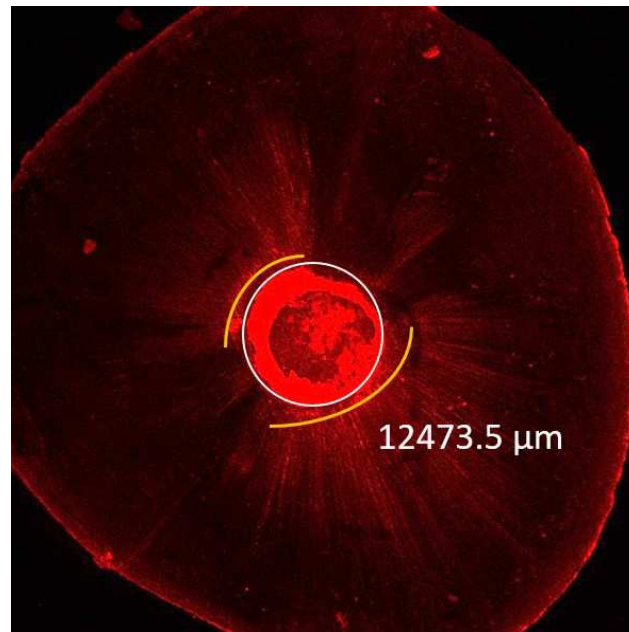


MIDDLE

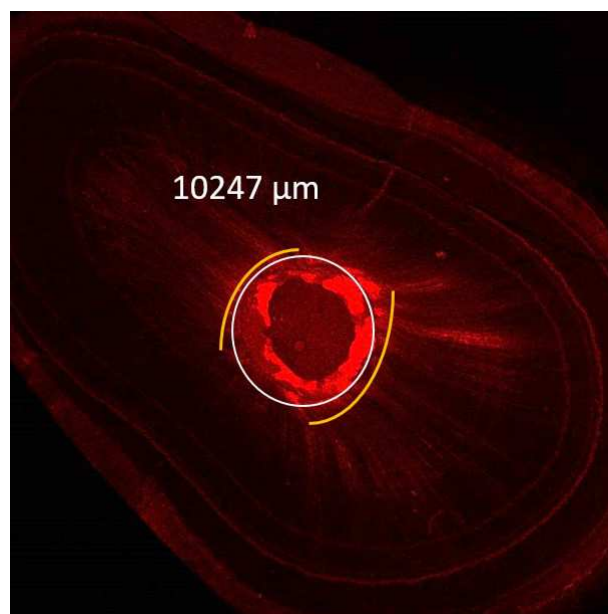


APICAL

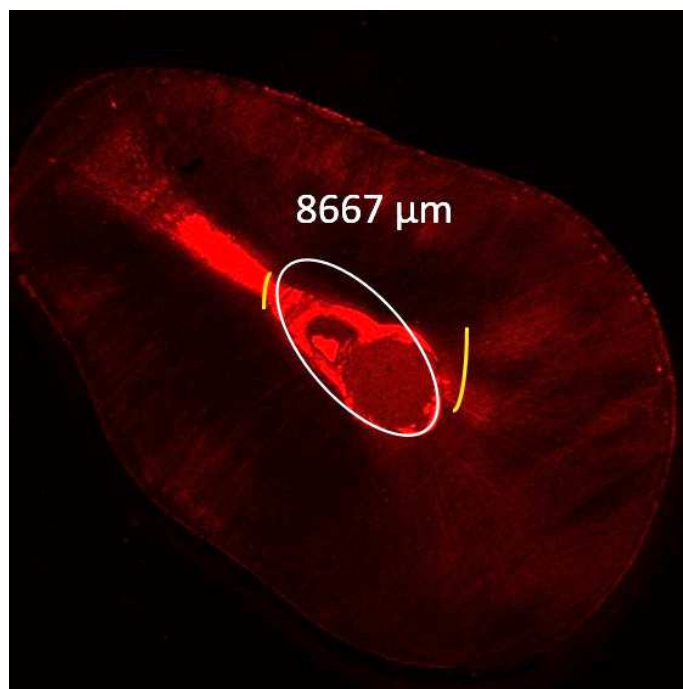
Figure 25: Shows dentinal tubule percentage/ area adaptation of **NAC+ AH Plus sealer (Group I)**. Maximum infiltration was exhibited in coronal area followed by middle and least in apical part



CORONAL

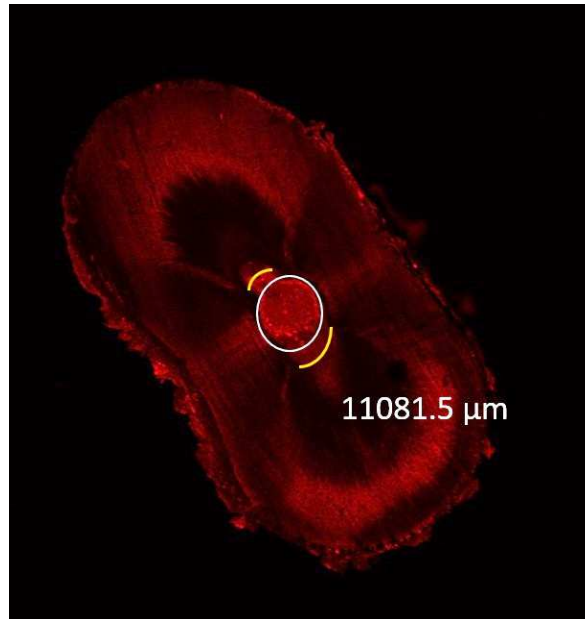


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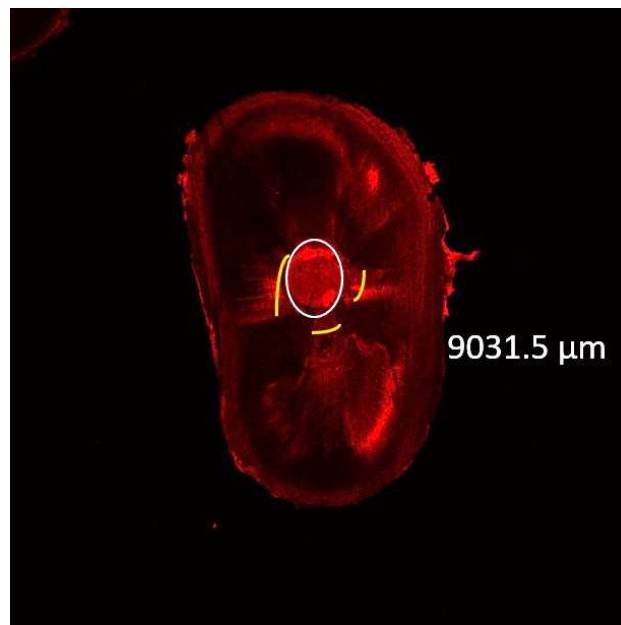


APICAL

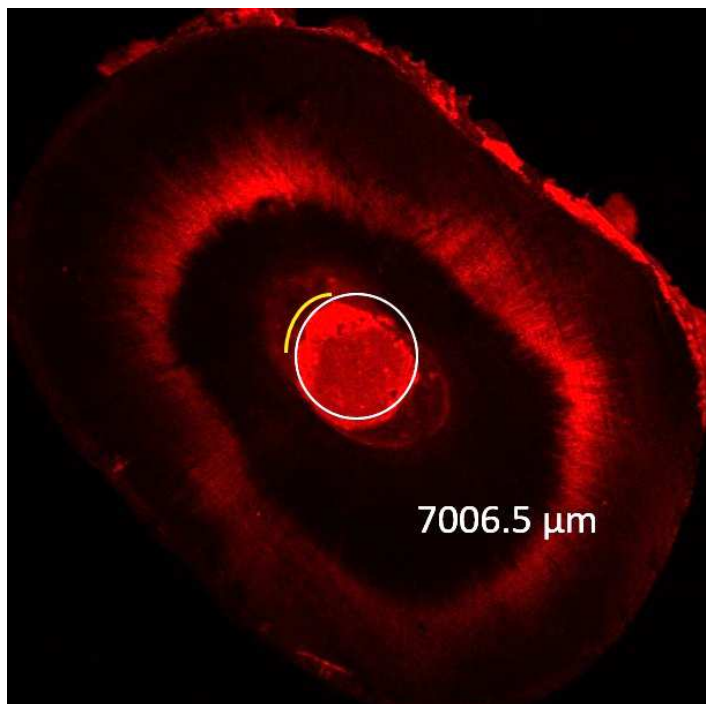
Figure 26: Shows dentinal tubule percentage/ area adaptation of NAC+ Ceraseal sealer (Group II).Maximum infiltration was exhibited in coronal area followed by middle and least in apical part



CORONAL

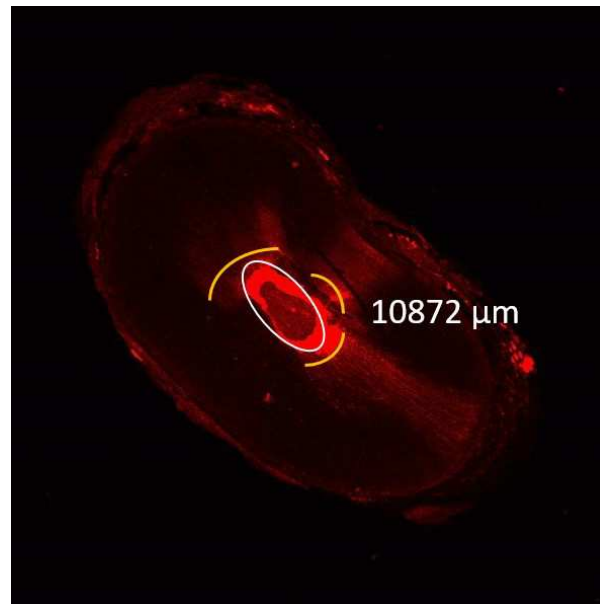


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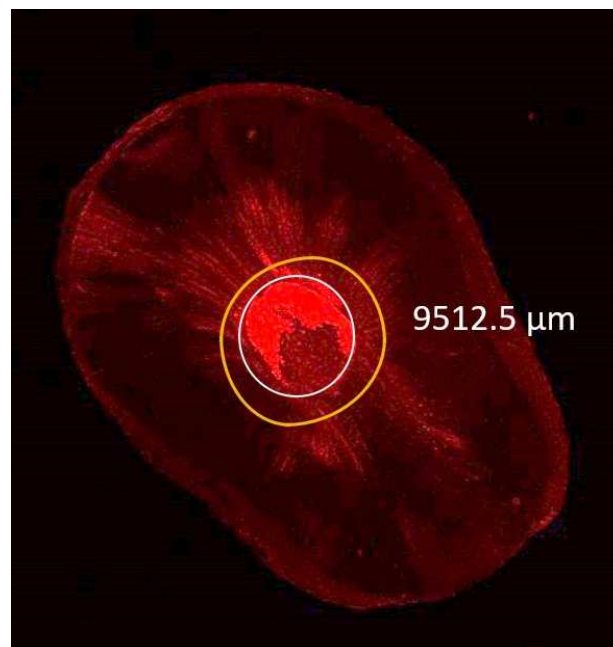


APICAL

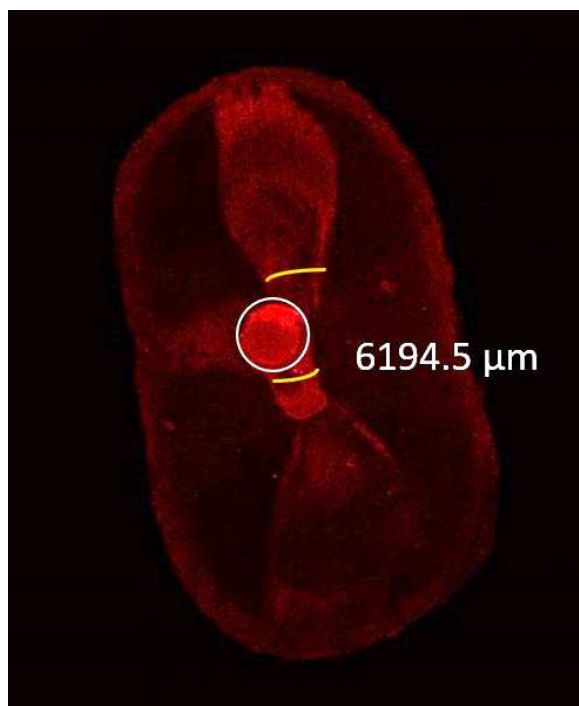
Figure 27: Shows dentinal tubule percentage/ area adaptation of **AH Plus sealer** (**Group III A**).Maximum infiltration was exhibited in coronal area followed by middle and least in apical part.



CORONAL

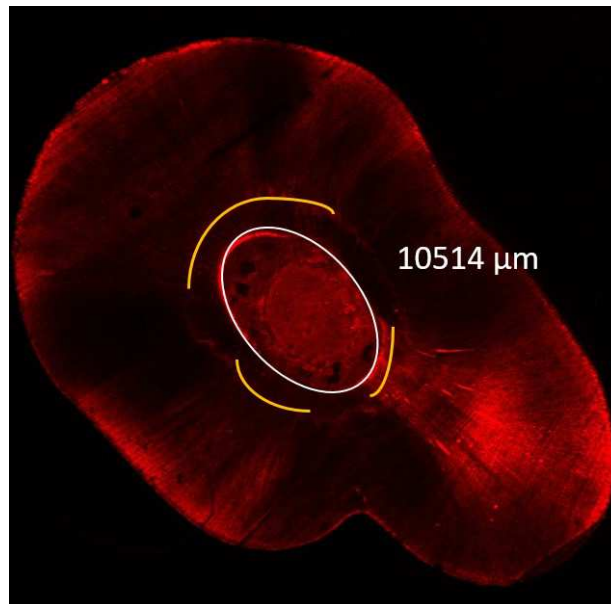


MIDDLE

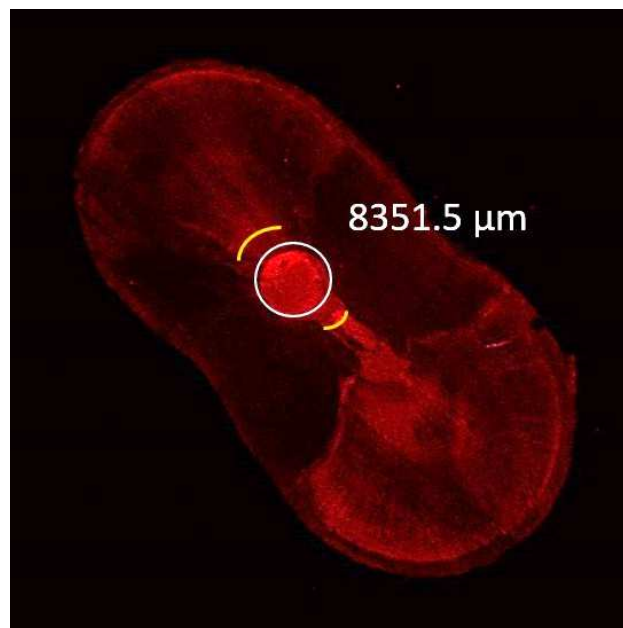


APICAL

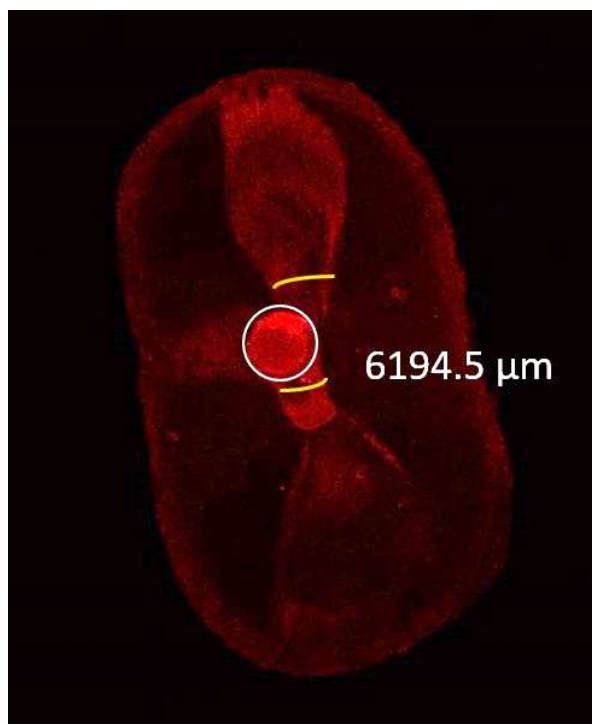
Figure 28: Shows dentinal tubule percentage/ area adaptation of **Ceraseal sealer** (**Group III B**).Maximum infiltration was exhibited in coronal area followed by middle and least in apical part.



CORONAL



MIDDLE



APICAL

DISCUSSION

The ultimate goal of successful root canal therapy is sealer penetration and interfacial adaptation to dentinal walls.⁴⁴ The amount of sealer penetrating the tubules and the way in which the sealer adapts to the dentine wall are the key elements which are correlated with the quality of the root canal therapy.⁴⁵ Increased depth of sealer penetrability entombs the microorganisms and maintain an adverse micro environment.^{30,45}

The amount of penetration depth is influenced by various factors such as tubule density, diameter, removal of the smear layer, solubility, viscosity, surface tension, and particle size of sealers.³⁵ The sealer must have high flowability and sufficient thickness to enter various complexities in the apical third of the canal.²⁶

Resin sealers are commonly used sealers because of its low solubility and better adaptation. AH Plus considered gold standard as it has superior adaptation and penetration but their chief disadvantages are cytotoxicity, hydrophobicity and mutagenicity. To subside these problems, newer sealers like calcium silicate, calcium phosphate, silicone based sealers were introduced. In our study, we used calcium silicate based sealers.⁴⁴

Bioceramic sealers major advantages are great biocompatibility to surrounding tissues that permit chemical reaction with hydroxyapatite of tooth structure improving the bond of sealer-to-root dentin. CeraSeal, a premixed flowable sealer which sets in presence of moisture have excellent cytocompatibility, mineralisation activity and osteogenic potential.^{28,44}

One of the key factor for sealer penetration is removal of smear layer by chemomechanical preparation which also eradicates microorganisms.⁴⁶ Since large sections of the root canal walls remain intact even after mechanical instrumentation, eliminating microorganisms from infected root canal system is a challenging operation. *E. Faecalis* has the ability to multiply in deeper layers of dentine. Therefore, penetration of medications into the dentinal tubules is necessary.⁴⁶

In Conventional endodontic therapy multiple visits are necessary, whereas some practitioners believe that single-visit treatment is preferable. Both single-visit endodontic therapy (SVE) and multiple-endodontic therapy offer benefits and drawbacks. Careful case selection is essential to the outcome of endodontic treatment. Before starting single-visit endodontic treatment, Ashkenaz recommended taking into account the clinicians experience, clinical procedures, restorative point of view and pulpal state.⁴⁷

Carrotte suggested that after the dentist has completed the chemomechanical debridement, if periapical lesions and pus exudates are not seen it would be okay to move forward with obturation. The patient may require multiple appointments/sessions of intracanal medicament if the periapical lesions or prolonged apical exudates prevent the root canals from drying out.⁴⁷

Calcium hydroxide (Ca(OH)₂) is extensively utilized medicament in root canal therapy because of its properties of antimicrobial activity, tissue dissolving ability and alkaline environment. However, there are certain limitations, it is resistant to *E.faecalis* and complete removal is highly unlikely leading to hampering of sealer penetration that influence the prognosis of the tooth.³⁵ Till date, no technique is able to completely remove the medicament. However, it was noted by Calt and Selper that

the remnants does not infiltrate into the tubules but produces a thin layer that blocks the entry of sealers.³⁵

A novel medicament N-acetyl cysteine, a non-antibiotic drug having antibacterial properties, anti-inflammatory activity stops the growth of *E. faecalis* and removes its biofilm.³⁹ Quah et al.'s research has shown that NAC's antimicrobial properties are unaffected by dentin, which is one way that NAC differs from CHX.⁴⁰

NAC is a chelating agent which effectively removes smear layer leading to increase in the depth and area of sealer penetration. N-acetyl cysteine has been shown by Rajakumaran et al. to be just as successful as EDTA at removing smear layer from the root canal.⁴⁸ Hence, NAC can be used as both irrigant and intracanal medicament. N-acetyl cysteine medicament removes smear layer opens the dentinal tubules which has been confirmed in our SEM pilot study. Therefore, sealer penetration behaves as a gauge for the amount of smear layer removal.⁴⁹

There are few documented evidences on the effect of N-acetyl cysteine irrigant on sealer penetration. Nevertheless, to the best of our search there was no research done on effects of N-acetyl cysteine intracanal medication.

Thus, the current study evaluated the effect of N-acetyl cysteine intracanal medicament on dentinal tubular penetrability of an Epoxy resin sealer and a Bioceramic based sealer using CLSM.

Human maxillary anterior teeth were used in this study to eliminate any anatomical complexities that could lead to bias in the study. As a result, single-rooted incisors and canines were included.⁵⁰ Teeth were stored in 0.1% thymol solution to inhibit fungal growth. After radiographing teeth, 78 teeth were chosen in accordance

with inclusion and exclusion standards. Using a diamond disc, teeth were decoronated to provide a uniform length of 14 mm. Root length was standardized in order to create a flat surface that would serve as a consistent and reliable reference point for chemomechanical preparation and to remove any difference in access cavity preparation.⁵¹ WL was measured up to the apex and then reduced by 1 mm from the recorded length. Using a hand instrument, canals were expanded to size 20-K file.

Van der Vyver has stated 15 or 20-K hand file should reach the working length easily to establish a glide path. K- files offer certain advantages over rotary Ni-Ti files for glide path preparation such as better tactile sensation and less potential for instrument separation.⁵² ProTaper Universal Ni-Ti rotary files were utilized for biomechanical preparation of the canals following standard protocol as per manufacturer instructions. ProTaper instruments uses less files and reduces chair side time over stainless steel instruments. Furthermore, Ni-Ti instruments have superior properties such as super-elasticity and enhanced cutting efficiency.⁵³

Irrigation plays a crucial role in disinfecting the root canal system. In the current study, 2 ml of 5.25% NaOCl solution was used between successive instrumentation due to its potent antimicrobial activity, lubricating action, and ability to dissolve pulp remnants.⁵⁴ Final flush done with 5 ml of 17% Ethylene Diamine Tetraacetic Acid for 3 minutes. The pH, volume of solution, application time, and concentration all contribute to the effectiveness of a chelating agent.⁴⁹ With 17 % EDTA after 3 mins, there is a greater reduction in microhardness. Dentin decalcification can occur upto 20–30 μm if irrigation of EDTA is done more than 5 minutes which inturn will reduce the microhardness and fracture resistance of the tooth.^{55,56}

A study conducted by Mai S et al also showed that 17% EDTA for more than 3 minutes have significant microhardness reduction⁵⁷ and excessive EDTA (volume and time) and extended period of NaOCl as an initial rinse causes dentinal erosion and decrease in flexural strength.⁵⁸ According to AAE, EDTA can be used for stipulated time (within 4 minutes) and concentration (17%) for removing smear layer and to dissolve inorganic debris. This agent has the ability to soothe the dentine and maintain a fluid chelating effect.⁵⁵ Periera et al also concluded that 17% EDTA removed smear layer and medicament in all thirds of the canal than 2.5% NaOCl.⁵⁹

To eliminate the impact of the residual oxygen in the NaOCl on the sealer polymerization^{60,61}, a final flush was carried out with five milliliters of distilled water and canals dried with sterile PP.

Teeth was randomly divided into 3 groups ie. N-acetyl cysteine with Epoxy resin based sealer (Group I), N-acetyl cysteine with Bioceramic based sealer (Group II), negative control (Group III). Group III was further divided into 2 subgroups: III A – AH plus, III B – Ceraseal. Obturation was done with Gutta percha coated with labelled sealer for these subgroups.

NAC medicament was prepared according to Quah et al. One milliliter of sterile distilled water was used to dissolve 0.2 grams of N-acetyl cysteine to create a concentration of 200 mg/ml.⁴⁰ In this study, 0.2 gm of N- acetyl cysteine was crushed into powder then mixed with 1 ml of propylene glycol. Olitzky observed that because of the remarkable germicidal activity of propylene glycol in concentrated solutions, using it as a carrier may offer a potential for preventing or treating microbial infections. Even though, Propylene Glycol is more viscous it can flow through dentinal tubules which offers advantage over pure water.⁶² Research has demonstrated

that the dentinal tubules are penetrated by intense bacteria up to a depth of 300 μm .³⁵ Our SEM pilot investigation revealed that N-acetyl cysteine could reach depths of 200 to 250 μm .

PILOT STUDY:

A pilot study was conducted to assess the depth of penetration of medicament into the tubules. Twenty six human extracted single rooted maxillary anterior teeth were selected for this study which were prepared as mentioned in Group I and Group II and medicament was placed in the canals. The specimens were incubated for 15 days and were sectioned at 3, 7, and 11 mm from the apex to represent the apical, middle and coronal thirds respectively examined under Scanning Electron Microscope (SEM). Specimens were coated in gold by sputter coating to make their surface electrically conductive⁶³ and observed with 250 x, 2500x, 5000x magnification.⁶⁴ The SEM study's findings indicate that NAC medication opens the dentinal tubules and eliminates the smear layer and penetrated 248 μm deep at 11 mm (coronal), 217 μm deep at 7 mm (middle) and 189 μm deep at 3mm (apical). Average penetration of NAC into the tubules are 218.66 μm . The results of this pilot study were used to compare the mean depth of penetration of Control groups.

N-acetyl cysteine was placed in all the canals of Group I and II using lentulospirals as it has 360 ° centrifugal action, it pushes the medicament inside the dentinal tubules.⁶⁵ Cavit was used as a temporary restorative material over medicament in order to prevent microleakage.⁶⁶ Incubation was done at 37°C for a 15 days period. In a study by Julian et al., E. faecalis biofilm was created in root canals and left there for 21 days, allowing the germs to spread throughout the dentinal tubules and root canal system. Recolonization of the primary root canal was noted

seven days following the removal of medication, indicating that *E. faecalis* had not been entirely eradicated from the root canal system. In 14 and 21 days, *E. Faecalis* was completely eradicated.⁶⁷ Thus, a medication is administered for 15 days in our study.

Numerous studies confirm how challenging it is to completely remove Ca(OH)_2 , particularly from the apical section of the root canal. A systematic review by Nandhini Suresh concluded that Calcium chelators enhances CH removal from root canal when used with agitation techniques such as passive ultrasonic instrumentation and rotary instrument compared to non-calcium chelators.¹¹ According to a recent assessment of the literature, syringe irrigation and apical negative pressure were less effective than PUI in eliminating Ca(OH)_2 from the apical third.³⁵ Zorzin J et al concluded that passive ultrasonic method was most efficacious in removing calcium hydroxide when compared with files, canal brushes.³⁸

Nonetheless, a study by Twinkle Talreja et al. claims that CeraSeal sealer has the second-highest penetration in all tooth regions after Er: YAG laser with AH Plus sealer.²⁰ According to a comprehensive evaluation by Badami et al., LAI is more effective than UAI at removing bacteria, dentin debris and organic material from the root canal system⁶⁸ but did not mention the type of laser.

Nevertheless, there are drawbacks of Er:YAG laser to accelerate treatment. Increasing pulse intensity might cause more side effects, like cracks and leaflets, as well as underlying enamel damage. When the laser power is increased, the thermal side effects are reduced but the mechanical side effects increase.⁶⁹ A laser-abrasive technique employing sapphire powder is utilized to accelerate the speed of ablation in enamel; however, this technology may not be pursued further due to its negative

effects on the surrounding tissues.⁷⁰ When teeth are overheated, the pulp becomes damaged and the pulpal tissue becomes inflamed either during or after laser treatment. According to studies by Sikaew et al., there hasn't been any discernible difference between laser-treated and conventionally treated root canals when it comes to microleakage.⁶⁹

Hence in this study, Passive Ultrasonic Irrigation was used as it is mostly widely used and it serves its purpose.

To promote fluorescence, sealer was manipulated with rhodamine B dye at a ratio of 0.1% (weight).³⁵ According to ADA specifications, the sealer flow did not alter due to addition of 0.2% rhodamine B.⁴⁵ Lentulospiral's centrifugal action forces the sealer against the root canal walls and it has been found to have improved sealer penetration into the dentinal tubules.⁶⁵ Obturation was performed via the single cone (SC) method. Obturation aims at elimination of canal space and sealing of apical foramen to acquire greater area of GP and lesser area of sealer.⁷¹ According to Rodrigues et al, SC has shown a higher percentage of GP in the apical area compared to lateral condensation, thus providing a viable alternative⁷² and also according to manufacturer instructions, Protaper Universal files used in this study also advocates single cone obturation system. Using the heated hand plugger, excess GP was sheared off and the access cavity was sealed with cavit for all the specimen. Incubation was done at 37° C in 100% humidity for 7 days for complete set of the sealer and to simulate oral conditions.

Sectioning of the teeth were done for all 3 groups to obtain 1-mm-thick sections from all three levels (3, 7, 11 mm from the root tip) using a diamond disc and used for CLSM scanning. Using silicon carbide abrasive paper, all parts were polished

to provide a clean surface and remove any dentinal debris created during the cutting process.⁷³ Moreover, these silicon carbide paper produces smear layer which can affect the CLSM examination.

Confocal Laser Scanning Microscope was preferred for evaluation of tubular penetration depth and area. Other evaluation techniques utilized to assess the sealer penetration are light microscope⁷⁴ and scanning electron microscope.⁴⁷ The drawback of LM is the inability to differentiate between sealer and the radicular dentin.⁷⁵

By enabling the visualization of sealer penetration beneath the dentine's surface, CLSM removes the requirement for smear removal or disruptive specimen preparation, both of which increase the risk of sealer loss.⁴⁷ SEM is not very useful for measuring penetration depth. Samples for SEM analysis must be well polished, dry, and free of surface smear layers. The sealer may be lost as a result of this. This could account for some of the SEM investigations' reported penetration depths, which appear to be modest.⁴⁷ The advantage that CLSM provides over the other techniques is that it does not need additional sample processing and produces fewer artifacts.

Current research data was analysed using ImageJ software. Statistical analysis was done with Two-way ANOVA and Tukey's multiple post hoc test. Intragroup comparisons performed with Two-way ANOVA while intergroup comparisons were done by utilizing Tukey's multiple post hoc test.

Analysis for sealer penetration depth:

Results of the current study showed difference in penetration of the sealer was statistically significant among all the three sections i.e, coronal, middle and apical section among all the three groups. i.e, NAC + Ceraseal, Ceraseal, NAC + AH Plus,

AH Plus. The intragroup comparison results showed that Ceraseal sealer with use of NAC showed highest tubular penetration in coronal, middle and apical thirds of root canal than without NAC. Intragroup comparison for AH Plus sealer showed highest penetration in coronal, middle thirds of root canal with NAC and highest penetration in apical thirds without use of NAC.

This may be explained by the following: tapering root canal morphology, tubular obliteration in the apical third, difficulty in completely eliminating debris and the smear layer, and medication, particularly in the apical third.³⁵ Babb et al. proposed that the endodontic sealer's adhesiveness cannot be changed by variations in tubular density within the canal.³⁵ Due to the morphological and histological variations, there is a reduction in the effectiveness of removal of smear layer in apical region. Furthermore, the lesser the diameter of apex more difficult it is to access this part of the canal.⁷⁶ Moreover, the apical root dentin is poorly permeable due to the sclerotic dentin⁷⁷ as compared to the coronal and middle third dentin. Besides the properties of sealer, dressing may have caused modifications in the permeability of the dentin.³⁵ These findings are consistent with Emel Uzunoglu-Ozy €urek, Thota, as coronal thirds of root canals were found to have greater smear layer removal than apical thirds. This is because the coronal third of the canal has more and large diameter dentinal tubules than the apical portion.⁴⁹

Another reason can be due to inefficacious irrigant delivery and ineffectual smear layer removal in apical region.⁷⁷ Nonetheless, sealer penetration in apical third of a research by Machado et al. showed a comparable results with the middle and coronal thirds.³⁰ The study's filling method, which increased the sealer's penetrability into the tubules by pressing it up against the root canal walls due to lateral and vertical condensation stresses, may account for these contentious findings.⁷⁸

Results of the current study on Intergroup comparison using Tukeys multiple posthoc procedure showed statistically significant difference in all groups ie. NAC +Ceraseal, Ceraseal except NAC +AH Plus, AH Plus. The reason is that Premixed Calcium silicate-based sealer has high flowability²⁶ and the expansion of sealer due to hygroscopic effect, mineralisation activity which produces mechanical interlocking ability to bond with dentin and osteogenic potential.^{26,28} N- acetyl cysteine, a crystalline structure also a chelating agent which removes the smear layer and inhibits biofilm formation³⁹, reduced demineralisation and microhardness due to its soft chelating nature.⁴⁸

The results of NAC +AH Plus, AH Plus were not statistically significant in depth of penetration due to high viscosity, their low solubility which also reduces its compatibility within hydrophilic canals.^{44,59} Another reason might be the crystalline structure and hydrophilic nature of NAC and the hydrophobic penetration of AH Plus sealer.⁷⁹ Sealer types also promotes different penetrations in the tubules as concluded by Mamootil and Messer.³⁵ In order to achieve optimal penetration, Aktener et al. also found that sealers must have low surface activity.⁴³

AH Plus sealer has larger particle size, so polymerization shrinkage or problems during mixing might diminish its permeability into the dentinal tubules.⁴⁴ Moreover, its film thickness and flow are affected in Epoxy resin-based sealers as it contains calcium hydroxide³⁰ which is also in accordance with Duarte et al. This explains the lesser penetration of sealer as also observed by Veintimilla Lozada et al.³⁰

According to Emel et al, pH of the sealer also influences the penetration. Epoxy resin sealers have pH 7 or less, calcium silicate sealers have pH 7 or more.⁸⁰ A Study by Emel et al shows that calcium silicate based sealer have a higher sealer penetrability and adaptation.³⁵ Although studies have shown Ca(OH)₂ remnants block the tubules and hamper sealer penetration, Bio-C sealer in the study showed an even more enhanced penetration after the use of Ca(OH)₂.³⁵ One of the reasons could be because of the alkaline environment created by Ca(OH)₂. Calcium silicate based sealers have a high pH and hence greater penetration in such an environment.⁸¹

Ozyurek in his study assessed the impact of Ca(OH)₂ on AH Plus and BioRoot RCS penetration, also demonstrated similar results with BioRoot RCS having deeper penetration after Ca(OH)₂ than AH Plus.^{35,82,83}

In the current study, N- acetyl cysteine, a chelating agent was used as intracanal medicament and results showed highest depth of tubular penetration in NAC + Ceraseal (Group II) followed by Ceraseal (Group III B), AH Plus (Group III A) followed by NAC +AH Plus (Group I).

Analysis of sealer penetration percentage:

The intragroup comparison results of the study showed AH Plus sealer with use of NAC showed highest area adaptation/ percentage of tubule penetration in coronal, middle and apical thirds respectively of root canal than without NAC. Intragroup comparison for Ceraseal sealer showed highest area adaptation in coronal, middle and apical thirds respectively of root canal with NAC.

Adhesion, penetration of sealers into dentin is influenced by some factors as physio chemical properties of the used sealer, dentin permeability, obturation method, and removal of smear layer.⁴⁴

According to Cergneux et al (1987) and Aya wayel (2024) suggests that Sealer penetrability and adaptation to the dentinal walls are necessary properties⁴⁴ which increases surface contact that improves apical seal.^{23,44} In the same way, interfacial adaptation between root canal filling material and dentinal walls is a crucial issue of concern, as most failures occur at core-sealer interface and dentin sealer interface which leads to failure of endodontic treatment.^{44,84}

Epoxy resin sealer has high viscosity, less flowability and sealer penetrates by capillary action whereas bioceramic sealer has high flowability, less viscous in which sealer penetrates by hydraulic condensation ie. This hydrophilic sealer is been drawn into the dentinal tubules, lateral canals, apical deltas so penetration is high whereas adaptability is less.⁸¹ Good adaptation of AH Plus sealer might be because of chemical bond formation with root dentin. Moreover, minor acidity of sealer might cause self-etching to root dentin, so enhance adaptation and bonding.^{44,84} AH Plus sealer has its pseudoplastic behaviour, the penetrability of this high viscous sealer is difficult but when GP is placed into the canals the sealer spreads and adapts to the canals. This depends upon viscosity, flow, pH, type of obturation technique.^{84,85}

The results of our study is in agreement with study conducted by Chen H et al. which concluded that AH Plus has best adaptation capacity followed by calcium silicate based sealers.³⁷

In single cone (SC) technique, the sealer gets displaced when gutta percha is being inserted. When gutta percha is being plugged into the canal, it improves the adaptation to the canal wall.^{47,78} If cold lateral or warm vertical techniques had been used in this study, the penetrability of sealers might have been improved.⁴³ However, in SC technique, there is more sealer because there is no condensation pressure as compared to cold lateral and warm vertical techniques.³⁶

Results of the current study on Intergroup comparison using Tukeys multiple posthoc procedure showed clinically significant difference in all groups NAC +AH Plus, AH Plus, NAC +Ceraseal, Ceraseal but statistically significant difference is not observed in NAC +Ceraseal and AH Plus.

This non-significant difference may be attributed due to the physiochemical characters of RCS following removal of smear layer.⁴³ Another reason can be due to inefficacious irrigant delivery and ineffectual removal of smear layer in apical region.⁷⁷ High mean gap values at apical area may be due to the cementum like structure and atubular dentin in these regions,^{44,76} Bioceramic Sealers also have profuse open pore which takes up moisture, leading to high solubility ie. Penetrate into the tubules rather than adaptation which is confirmed by Zamparini et al.^{26,84,85}

In the current study, N- acetyl cysteine was used as intracanal medicament and results showed highest adaptation/ percentage of tubular penetration in NAC + AH Plus (Group I), AH Plus (Group III A), NAC + Ceraseal (Group II), followed by Ceraseal (Group III B).

Results of SEM pilot study shows that NAC medicament helps in eliminating smear layer and opening of dentinal tubules and penetrated 248 μm deep at 11 mm (coronal), 217 μm deep at 7 mm (middle) and 189 μm deep at 3mm (apical). Average penetration of NAC into the tubules are 218.66 μm .

Further studies are needed to evaluate sealer penetration with other medicament and obturation methods. Limitation of our study is that the experiment was an in-vitro laboratory set up and has yet to be clinically tested. Clinical studies are required to evaluate effect of the N-acetyl cysteine intracanal medicament on penetration and percentage of various sealers to know the outcome of endodontic treatment.

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn:

- Maximum sealer penetration was noted in the coronal third, followed by middle and least in apical third for all tested groups.
- Higher *penetration* of sealer was observed with NAC + Ceraseal (Group II) followed by Ceraseal (Group III B), NAC + AH Plus (Group I) and least by AH Plus (Group III A).
- Higher *percentage/ adaptation* of sealer was observed with NAC + AH Plus (Group I) followed by AH Plus (Group III A), NAC + Ceraseal (Group II) followed by Ceraseal (Group III B).
- Higher penetration of NAC medicament was seen in coronal (11mm)- 248 μm , middle (7mm)-217 μm followed by apical third (3mm)- 189 μm of root canal. Average penetration of NAC into the tubules are 218.66 μm .

However, persistence of the medicament residues did not hamper the sealer penetration but enhanced the penetration of Ceraseal sealer. Moreover, NAC did not change the properties of both the sealers. Hence, NAC can be a choice of intracanal medicament for further use in clinical practice.

Future studies evaluating the other properties of the NAC intracanal medicament and Ceraseal sealer and long-term studies need to be done in order to establish a strong literature base.

SUMMARY

The purpose of endodontic therapy is to create a micro-free environment in the root canal and a hermetic seal. Novel equipments and materials have been introduced to attain an aseptic environment within the canal resulting in long term successful outcomes. Among the various root canal sealers, bioceramic sealers are being appreciated by many clinicians due to its enhanced properties like a good flow, mineralisation potential. Dentinal tubule penetrability and sealer adaptation are the main feature that aids to establish a fluid impervious seal and enhanced adaptation between root dentin wall and core material.

In this study, dentinal penetration and area adaptation after using NAC medicament has been evaluated as it has been observed that residues of the medicament could interfere with sealer penetrability. However, an association between these residues and the newer bioceramic sealers was found which led to the aim of this study. 78 human maxillary anterior teeth were selected and decoronation was done to a standard length of 14 mm and WL was estimated after which instrumentation was done two sizes larger than initial binding file by ProTaper universal, Ni- Ti rotary and 2 ml of 5.25% NaOCl utilized between successive files and final flush with 5 ml of 17% EDTA for 3 minutes followed by 5ml of distilled water and dried with paper points.

All specimens were randomly divided into 3 groups. Group 1: Experimental group (NAC) with Epoxy resin sealer, Group 2: Experimental group (NAC) with Bioceramic based sealer, Group 3: Negative control group. N- acetyl cysteine medicament was placed in Group I and Group II specimens followed by incubation at 37°C for 15 days after which the medicament was removed using Passive Ultrasonic

irrigation and obturation was done for all 3 groups with respective Gutta percha coated with rhodamine mixed sealer. Incubation was done for 7 days after which samples were sectioned at different levels (3, 7, 11 mm from the apex) and examined under the CLSM for dentinal tubular penetrability and percentage of sealers. Images were analysed with Image J software.

Statistical tests done with Two-way ANOVA and Tukey's multiple post-hoc tests. Results of the intragroup comparison showed difference in sealer penetration was statistically significant among all the three sections i.e, coronal, middle and apical section among all the three groups. i.e, NAC + Ceraseal, Ceraseal, NAC + AH Plus, AH Plus. On intergroup comparison, sealer penetrability was statistically significant in Group II (NAC + Ceraseal), Group III B (Ceraseal). On intragroup comparison, the results showed difference in sealer percentage/ adaptation was statistically significant among all the three sections i.e, coronal, middle and apical section among all the three groups. i.e, NAC + AH Plus, AH Plus, NAC + Ceraseal, Ceraseal. On intergroup comparison, percentage/adaptation of sealer penetration was statistically significant in Group I (NAC + AH Plus), Group III B (Ceraseal).

Maximum sealer penetration was noted in the coronal third, followed by middle third and least in apical third for all groups. Higher penetration of sealer was observed with NAC + Ceraseal (Group II) followed by Ceraseal (Group III B), NAC + AH Plus (Group I) and least by AH Plus (Group III A). Higher percentage/ adaptation of sealer was observed with NAC + AH Plus (Group I) followed by AH Plus (Group III A), NAC + Ceraseal (Group II) followed by Ceraseal (Group III B).

The null hypothesis that after the use of NAC medicament there will be no difference in percentage and dentinal tubule penetration depth of Epoxy resin based sealer and bioceramic based sealer was rejected.

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ANNEXURE – I – ETHICAL CLEARANCE LETTER

**Research and Ethics Committee****KLE VK INSTITUTE OF DENTAL SCIENCES**

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Sl. No. : **1602**

CERTIFICATE

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

This is to Certify that the synopsis titled

*Evaluation of Effect of N-acetylcysteine intracanal
medicament on dentinal tubule penetration of an Epoxy resin
based sealer and Bioceramic based sealer using
Confocal Laser Scanning Microscope
- AN IN-VITRO 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 : 31/7/24

Member Secretary

Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi
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Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman

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KLEVK Institute of Dental Sciences
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Research and Ethical Committee
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ANNEXURE – II – BIOSTATISTICS CLEARANCE CERTIFICATE



KLE V.K. Institute of Dental Sciences

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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
Professor & Head of Department, **Department of
Conservative Dentistry and Endodontics, entitled “Evaluation of effect of N- acetyl
cysteine intracanal medicament on dentinal tubule penetration of an Epoxy resin
based sealer and a Bio ceramic based sealer using Confocal Laser Scanning
Microscopy -An 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
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ANNEXURE – II – PLAGIARISM CHECK REPORT

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences



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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

“ EVALUATION OF EFFECT OF N-ACETYL CYSTEINE INTRACANAL
MEDICAMENT ON DENTINAL TUBULE PENETRATION AND PERCENTAGE
... OF AN EPOXY RESIN BASED SEALER AND A BIOCERAMIC BASED ... ”
SEALER USING CONFOCAL LASER SCANNING MICROSCOPY

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
.....8.....%, which is **within** / **not within** the acceptable limits of 10% as per
the UGC guidelines.

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23/04/2024
Member Secretary

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Chairman

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