"COMPARATIVE EVALUATION OF THE DEPTH OF PENETRATION OF AN EPOXY RESIN BASED SEALER FOLLOWING A FINAL RINSE OF 17% EDTA AND 0.2% CHITOSAN, WITH OR WITHOUT PASSIVE ULTRASONIC ACTIVATION: AN IN-VITRO CONFOCAL LASER SCANNING MICROSCOPY STUDY."

By

REG.NO. IE0220005

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LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	NaOCl	Sodium Hypochlorite
2	CA	Citric Acid
3	EDTA	Ethylene Diamine Tetra-acetic Acid
4	CI	Conventional irrigation
5	PUI	Passive Ultrasonic Irrigation
6	SP	Sealer Penetration
7	+	Plus
8	WL	Working Length
9	ANOVA	Analysis of Variance
10	SD	Standard Deviation
11	n	Number of specimens
12	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
13	<	Less than
14	>	Greater than
15	μm	Micrometers
16	kHz	Kilohertz

17	&	And
18	NMR	Nuclear Magnetic Resonance
19	AASF	Atomic Absorption Spectrophotometry with Flame
20	SEM	Scanning Electron Microscope
21	ml	Milliliter
22	° C	Degree Celsius
23	mm	Millimeter
24	i.e.	That is
25	SE	Standard error
26	CLSM	Confocal Laser Scanning Mircroscope

ABSTRACT

Aim and Objectives: To evaluate and compare the depth of penetration of an Epoxy resin based sealer following a final rinse of 17% EDTA and 0.2% Chitosan, with or without Ultrasonic activation: An In-vitro Confocal laser scanning microscopy (CLSM) study.

Study design: Eighty extracted human mandibular premolar teeth were selected, disinfected and decoronated to obtain a standardized root length of 15 mm. The teeth were prepared with ProTaper Universal rotary files upto F4 (size 40, 0.06 taper). The samples were divided into 4 groups of 20 samples each based on the final irrigant (5ml) and irrigating technique used.

Group 1: 17% EDTA + Conventional Irrigation for 2 min.

Group 2: 0.2% Chitosan + Conventional Irrigation for 2 min.

Group 3: 17% EDTA + Passive Ultrasonic Irrigation for 1 min.

Group 4: 0.2% Chitosan + Passive Ultrasonic Irrigation for 1 min.

The canals were rinsed with 3ml of saline solution and were dried with paper points and obturated with Gutta percha points and AH Plus sealer labelled with fluorescent dye (Rhodamine B isothiocynate), to promote fluorescence under CLSM. 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 of 1mm thickness each and 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 AH Plus sealer when irrigated with Chitosan with PUI was highest followed by Chitosan with conventional irrigation. However, when EDTA was used as a final rinse, both irrigation techniques showed low depth of sealer penetration.

According to the sections of the tooth, highest depth of sealer penetration was observed at Coronal third followed by middle third and least was at the apical third.

However, when the interactions between the various irrigants, activation technique and various sections of the tooth was analysed, the mean depth of sealer penetration was observed to be the highest for Chitosan at the Coronal third with PUI and the least was for EDTA at the apical third with CI.

Conclusion: Within the limitations of the present study, we can conclude that 0.2% Chitosan was highly effective in terms of dentinal depth of sealer penetration, irrespective of the irrigation technique used. PUI further improved the effectiveness of both the irrigants. Cleaning the apical third of root canal still remains an area of concern due to the reasons clearly discussed.

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

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INTRODUCTION

The long term success of root canal treatment depends on complete elimination of micro-organisms and their by-products.[1] This is achieved by chemo-mechanical disinfection and appropriate antimicrobial sealing of the root canal system.[2] Root canal instrumentation and irrigation should be carried out jointly and meticulously. The biomechanical preparation using mechanical instrumentation and antimicrobial solutions aims to shape the root canal and either eliminate or reduce toxic and necrotic contents, including pulp remains and pathogens.

Sodium hypochlorite (NaOCl) is the main irrigating solution used in the endodontic treatment due to its antimicrobial action and solvent capacity on organic tissues, but it does not affect the inorganic content.[3] As a result of instrumentation, a 1 to 2-μm thick smear layer primarily composed of inorganic dentin is formed on the root canal walls. The smear layer might act as a physical barrier that limits the antibacterial effects of intracanal medicaments and adhesion of root canal sealers.[4] This layer prevents intimate contact between the endodontic sealer and dentin walls, impairing sealer adhesion and consequently reducing the bond strength of the sealer mass. Moreover, the removal of the smear layer maximizes the retention of root canal filling and sealer penetration is considered to be an indicator that the smear layer was removed in such an area.

The use of auxiliary chelating solutions for final irrigation of the root canal generally aims to promote the removal of the smear layer. A dentine-free smear layer allows for penetration of the sealer into the dentin tubule. In addition to favoring the sealer's mechanical retention, the penetration of root canal sealer entombs residual

bacteria and also enables to transfer the antibacterial effect of the sealer into the dentin tubules.[5]

Several chelating solutions, including organic acids such as Citric acid (CA), Maleic acid and inorganic acids such as Ethylenediaminetetraacetic acid (EDTA), Phosphoric acid have been used to remove the smear layer.[6] Although EDTA is one of the most widely used chelating molecules, it has some limitations and disadvantages as a root canal irrigant. Many studies have revealed that EDTA is not effective in smear layer removal in the apical third of the root canals.[7] In addition, longer contact time with EDTA may cause loss of dentinal surface and reduction in microhardness of dentinal walls. Therefore, researchers seek an alternative to EDTA solution because of its erosive and toxic side effects on dentinal and periapical tissues.

Chitin is the second-most abundant natural polysaccharide composed of Beta-(1,4)-linked N-acetyl glucosamine units. Partial de-acetylation of chitin results in the production of Chitosan . Chitosan is a natural polysaccharide, obtained from crab and shrimp cells.[8] Previous studies have proven Chitosan has positive biological traits such as biocompatibility, biodegradability, bioadhesion, and lacks toxicity. Chitosan has shown a large number of pharmaceutical applications and has been used in medicine as a drug carrier, wound healing accelerator, and antibacterial and antitumor agent. Ballal et. al. showed that in root canals, prolonged calcium hydroxide ion release occurs through the addition of Chitosan to calcium hydroxide paste.[9] Furthermore, Silva et al. indicated that a 0.2% Chitosan solution was as effective as EDTA and CA with higher concentrations (15% EDTA and 10% CA) at removing the smear layer. [10]

Although the antimicrobial and chelating actions of endodontic irrigants play a critical role in disinfecting and cleaning the root canal system, conventional needle irrigation may not allow these substances to work deep into the dentinal tubules.

The penetrating ability of the irrigants and flushing action created by irrigation are dependent not only on the anatomy of the root canal system but also on the system of delivery, the depth of placement, volume and fluid properties of the irrigants.[11]

Thus, different devices and irrigant activation techniques have been developed and recommended to improve the efficiency and distribution of solutions.

The use of ultrasonic energy for cleaning the root canal and to facilitate disinfection has a long history in endodontics. Ultrasonics together with an irrigant contributes to a better cleaning of the root canal system than syringe irrigation.[12] Passive ultrasonic irrigation (PUI) activates the irrigant solution by acoustic microstreaming transmitted from an oscillating file or smooth wire at an ultrasonic frequency of 30 kHz. The acoustic streaming produces a rapid vortex – like motion of the liquid and cavitation causes the formation of spontaneous cavities throughout the liquid contributing to the better penetration of the irrigants into the dentinal tubules.[13]

The endodontic triad for successful root canal treatment comprises of shaping and cleaning, disinfection and 3D obturation of the root canal system. Root canal sealers play a critical role in three-dimensional obturation of root canal system by eliminating the space between the root canal wall and core filling material.[14] To minimize marginal gaps between sealer and dentin, there has been continuous search for the alternative endodontic sealers which could adhere to dentin.

Resin sealers, compared with zinc oxide- based sealers, have been reported to have greater dentin tubule penetration ability and can adhere to the dentin walls.[15]

AH plus, is an epoxy resin-based root canal sealer, that kills bacteria beyond its penetration depth.[16] The pseudoplastic behaviour of AH Plus is a benefit which allow increased flow by reduction of viscosity during filling procedures thus enhancing its penetration in tubules. These sealers have been known for their desirable properties of greater dimensional stability, low rates of solubility, great radiopacity and optimal adhesiveness to the root dentin than other endodontic sealers and currently serve as gold standard for comparison. Hence, is the sealer of choice for this study.

Locally, further sealer penetration (SP) is considered as an indicator that the smear layer was removed in such an area. The removal of the smear layer maximizes the retention of root canal filling. Although the flow and penetration of the sealer is not completely blocked, the smear layer's effect can be described as limiting. In a previous study, 0.2% Chitosan removed the smear layer as effectively as 15% EDTA and 10% CA from the middle and apical thirds of the canal.

There is a gap in the literature, which does not discuss whether 0.2% Chitosan may provide greater SP than other chelating agents. There are fewer studies that have evaluated and compared the effect of EDTA and Chitosan on radicular dentin and the penetration of sealers into dentinal tubules. [17]

Accordingly, the present study evaluated the effects of final canal irrigation with 0.2% Chitosan and 17% EDTA, with and without passive ultrasonic agitation on dentinal tubule penetration of AH plus sealer.

HYPOTHESIS

NULL HYPOTHESIS-

There is no difference in the depth of dentinal tubular penetration of an Epoxy resin based sealer after the use of 17% EDTA and 0.2% Chitosan as a final rinse with or without Passive Ultrasonic activation.

ALTERNATIVE HYPOTHESIS-

There is a difference in the depth of dentinal tubular penetration of an Epoxy resin based sealer after the use of 17% EDTA and 0.2% Chitosan as a final rinse with or without Passive Ultrasonic activation.

AIM OF THE STUDY:

To evaluate and compare the depth of dentinal tubular penetration of an Epoxy resin based sealer following a final rinse of 17% EDTA and 0.2% Chitosan, with or without Passive Ultrasonic activation.

OBJECTIVES:

1. To evaluate the depth of dentinal tubular penetration of an Epoxy resin based sealer following a final rinse of 17% EDTA and 0.2% Chitosan, with or without Passive Ultrasonic activation using Confocal laser scanning microscopy (CLSM).

2. To compare the depth of dentinal tubular penetration of an Epoxy resin based sealer following a final rinse of 17% EDTA and 0.2% Chitosan, with or without Passive Ultrasonic activation using Confocal laser scanning microscopy (CLSM).

REVIEW OF LITERATURE

1. Kesim et al. investigated the effectiveness of final irrigation with Chitosan, Ethylene diamine tetraacetic acid (EDTA), and Citric acid (CA). Seventy mandibular premolars were obturated with a resin-based sealer using Confocal laser scanning microscopy. The study concluded that Chitosan, EDTA, and CA significantly improved the percentage of SP for coronal thirds.[18]

2. Antunes et. al. assessed to compare the effects of final irrigation with Chitosan and EDTA on dentin microhardness, sealer dentin tubules penetration capacity, and push-out strength. Fifty canine roots were distributed and were obturated (AH Plus) and sectioned in 3 slices per root third. The study concluded that 0.2% Chitosan and 15% EDTA solutions act in a similar manner and the use of Endovac potentiates the effect of these solutions.[19]

3. Arathi et. al. compared the depth of penetration of canal irrigants into the dentinal tubules with and without ultrasonics using light microscope. 40 non-carious mandibular premolars were divided into four groups of ten each and were irrigated with 2% Chlorhexidine (CHX) and agitated ultrasonically, 2% Chlorhexidine, 2% Chitosan groups were agitated similarly. The study concluded that 2% Chlorexidine as irrigant with ultrasonic agitation was found to have maximum depth of penetration into the dentinal tubules when compared with Chitosan .[20]

4. Abraham et. al. investigated the effect of diode laser, EndoActivator, and passive ultrasonics for smear layer removal at the apical third from root canals with 0.2% Chitosan. 40 mandibular premolars were prepared, irrigated with 0.2% Chitosan, 0.2% Chitosan activated with diode laser, 0.2% Chitosan activated with

endoActivator, 0.2% Chitosan activated with passive ultrasonics. The study concluded that Diode laser and endoActivator with 0.2% Chitosan proved better in the removal of the smear layer when compared to passive ultrasonic irrigation.[21]

5. De Souza investigated the effect of different final irrigating solutions on depth of sealer penetration into dentinal tubules. In this study, 20 extracted human mandibular premolar teeth with single canals were randomly divided, irrigated with QMIX, Ethylenediaminetetraacetic acid, obturated and analysed using Confocal laser scanning microscopy. The study concluded that final irrigation with EDTA and Chitosan after the use of sodium hypochlorite affected sealer penetration.[22]

6. Praveen et. al. investigated the intraradicular smear removal efficacy of 2% Chitosan, 4% Chitosan citrate and 10% Citric acid when used as final rinse in irrigation protocols. Sixty single rooted maxillary incisors and canines were prepared and divided into 4 experimental groups (2% Chitosan , 4% C-citrate, 10% CA, and 1% Acetic acid) and 2 control groups (1-17% EDTA, 2- normal saline). The study concluded that 4% Chitosan citrate, 10% citric acid and 2% Chitosan as final rinse showed least amount of presence of smear layer, debris and erosion at the apical, middle, and coronal one-third of root canal respectively.[23]

7. Grande et. al. evaluated the interaction between EDTA and Sodium hypochlorite using a nuclear magnetic Resonance analysis. This study has shown that use of EDTA as a final flush resulted in erosion of dentinal walls. The study confirmed

that the reaction between the sodium hypochlorite and EDTA lead to a very slow but progressive degradation of this compound.[24]

8. Daran et. al. investigated smear layer removal after final irrigation with 17% Ethylenediaminetetraacetic acid (EDTA), 10% citric acid (CA), Biopure MTAD, and 0.2% Chitosan solutions. Fifty extracted maxillary central incisors were prepared irrigated with 17% EDTA, 10% CA, MTAD, 0.2% Chitosan, 2.5% NaOCl. Samples were split longitudinally and the study concluded that 0.2 % Chitosan solution has the lowest mean rank of smear layer scores in the sections followed by MTAD than that of 17% EDTA and 10% CA at apical level. 0.2% Chitosan solution was more effective in smear layer removal suggesting of supreme irrigant.[25]

9. Calt et. al. investigated the time dependent effects of EDTA on dentin surfaces. Six extracted single-rooted teeth were instrumented irrigated using 10 ml of 17% EDTA solution for 1 and 10 min, respectively. All specimens were subjected to irrigation with 10 ml of 5% NaOCl. After irrigation the specimens were evaluated with scanning electron microscope and it was concluded that 10 min application of EDTA caused excessive erosion of peritubular and intertubular dentin. Therefore, irrigation with EDTA should not be prolonged more than 1 min.[26]

10. Silva et. al. evaluated the efficacy of smear layer removal using different chelators. Twenty-five canines were collected and root canals were prepared and the groups were randomly divided into groups 15% EDTA, 0.2% Chitosan , 10% citric acid, 1% acetic acid and control (without final irrigation) analysed by AASF for quantification of calcium ions in the solutions. The study concluded that 15% EDTA, 0.2% Chitosan and 10% citric acid effectively removed smear

layer from the middle and apical thirds of root canal. 15% EDTA and 0.2% Chitosan were associated with the greatest effect on dentine demineralization, followed by 10% citric acid and 1% acetic acid.[10]

11. Vineetha et. al. compared the ability of smear layer removal by ultrasonic activation of ethylenediaminetetraacetic acid (EDTA) and Chitosan analysed using SEM. Forty-five mandibular single-rooted premolars were prepared, divided into three groups control group ultrasonically activated-normal saline, ultrasonically activated EDTA, ultrasonically activated-Chitosan . The study concluded that Ultrasonic activation of EDTA had superior ability of smear layer removal and Chitosan showed significant difference in smear layer removal compared to normal saline and EDTA was found to be better than Chitosan .[30]

12. Ratih et. al. investigated the effect of Chitosan nanoparticle as a final irrigation solution on smear layer removal, micro-hardness and surface roughness of root dentin. Seventy-two premolars prepared randomly assigned into three groups 17% EDTA, 0.2% Chitosan nanoparticle, 2.5% NaOCl. The study concluded that Final irrigation using 0.2% Chitosan nanoparticles had the same effect on smear layer removal compared to 17% EDTA; however, 0.2% Chitosan produced higher micro-hardness and lower surface roughness of root canal dentin than 17% EDTA.[31]

13. Hussein et. al. investigated the efficacy of Chitosan (CS) nanoparticles (CNPs), citric acid (CA), and ethylenediaminetetraacetic acid (EDTA) in removing the smear layer using two different irrigation needles (IrriFlex, ProRinse) and viewed under SEM. Palatal roots of 70 maxillary first molars were prepared, divided into four experimental groups of 0.5% CNPs, 10% CA, 17% EDTA, and distilled

water for 3 min and one control group The study concluded that CNPs remove smear layer with same efficiency as other irrigants utilized in this study at coronal and middle levels and more efficiently at the apical levels. IrriFlex was more effective than ProRinse in removing smear layer when used with EDTA and CA, while there was no difference when used with CNPs.[32]

14. Mathew et. al. investigated to evaluate the efficacy of smear layer removal, nanostructural and chemical changes caused by Chitosan and ethylenediaminetetraacetic acid (EDTA) on tooth surface using atomic force microscopic analysis and energy-dispersive X-ray (EDX) analysis. Forty single-rooted premolars were prepared and subjected to final rinse with 17% EDTA solution, 0.2% and 0.5% Chitosan solution for 1 min. The study concluded that AFM images showed no difference in elimination of smear layer and it also showed EDTA group had significantly higher surface alteration than Chitosan. EDX analysis showed that Ca/P ratio of root dentine in EDTA group is significantly lower than Chitosan group. Chitosan is an effective chelating agent with less alteration in radicular dentine.[33]

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.

- Chitosan was prepared at the KLE University's College of Pharmacy, KAHER, Belagavi, Karnataka.

- Confocal Laser Scanning Microscopy was carried out at the "Birla Institute Of Technology And Science, Pilani, K.K Birla Goa campus".

SAMPLE SIZE ESTIMATION:

The sample size was estimated using the formula:

$$n = \frac{2S^2 (Z_\alpha + Z_\beta)^2}{d^2}$$

Here, n= Sample size

Z = Critical value of the normal distribution at the required confidence

Level d = Mean difference

S1 = 1.22

S2 =1.01 ; d=0.9889

Z_α=1.96 at 5% α error

Z_β =0.84 at 20% β error

$S = \frac{S1+S2}{2} = 1.115$

$n = \frac{2S^2 (Z_\alpha + Z_\beta)^2}{d^2}$ = 20 in each group

There are four main groups, so the total sample size n= 20 x 4 = 80

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

INCLUSION CRITERIA

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

EXCLUSION CRITERIA:

- Teeth with calcified canals.
- Teeth with fracture/crack or a restoration.
- Teeth with internal and external root resorption.
- Teeth with presence of anatomic variations.

MATERIALS USED FOR STUDY

- Eighty Human mandibular premolar teeth
- 0.1% Thymol solution (S D FINE-CHEMICALS LIMITED, MUMBAI)
- 3% Sodium hypochlorite (VISHAL DENTOCARE, AHEMDABAD)
- 17% Ethylenediaminetetracetic Acid solution (EDTA) [GLIDE]
- Chitosan (SIGMA-ALDRICH, Bangalore)
- Paper points (Diadent Group International, Korea)
- Rhodamine B isothiocynatedye (Sigma Aldrich, Bangalore)
- Distilled water (NICE LIFE CARE, NEW DELHI)
- 1% Acetic acid (S.M. CORPORATION, Gujarat)
- Normal saline solution (Amanta Healthcare, Ahmedabad, Gujrat)
- AH Plus sealer (Dentsply, Germany)
- Gutta-percha points (Diadent Group International, Korea)
- Cavit (3M, ESPE, USA)

ARMAMENTARIUM USED FOR STUDY:

- Airotor (NSK, Japan)
- Micromotor (NSK, Japan)
- Gates Glidden Drills (MANI, Japan)
- K files (15-40) (MANI, Japan)
- ProTaper Universal nickel-titanium files (DentsplyMaillefer, Switzerland)
- Endomotor (X Smart, Dentsply)
- 5 ml syringe and 27 gauge needles (Dispovan, India)
- Ball burnisher (GDC, India)
- Spirit lamp (GDC, India)
- Agate spatula (GC Corporation, Tokyo, Japan)
- Plugger (GDC, India)
- Diamond disc (KWALITY DIAMOND TOOLS, Mumbai)
- Incubator (BIO TECHNICS, India)
- Ultrasonic system handpiece and Tips (Satelec,France)
- PUI adapter (E1, Guilin Woodpecker Medical Instrument Co., Limited, WOODPECKER, CHINA)
- Confocal laser scanning microscope (Olympus fluoview FV3000)
- Image J software (Fiji)

Preparation of Study materials:

Preparation of 0.2% Chitosan irrigant:

Chitosan solution was prepared by mixing 0.2g of Chitosan in 100 ml of 1% acetic acid, and stirred continuously using a magnetic stirrer for 2hrs.

METHODOLOGY: -

Eighty freshly extracted human mandibular premolar teeth were used and handled according to OSHA guidelines. Calculus and soft tissue debris was removed with an ultrasonic sealer and immersed in 0.1% Thymol solution till use.

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 15 mm. WL was established 1mm short of the length where #10 K- file exits the apical foramen. Cleaning and shaping was done using Protaper Universal (DENTSPLY) NiTi rotary instrument till F4 (size 40, 0.06 taper).

Samples were randomly divided in to four groups (n=20) based on the final irrigant (5ml) and irrigating technique used.

Group 1: 17% EDTA + Conventional Irrigation for 2 min.

Group 2: 0.2% Chitosan + Conventional Irrigation for 2 min.

Group 3: 17% EDTA + Passive Ultrasonic Irrigation for 1 min.

Group 4: 0.2% Chitosan + Passive Ultrasonic Irrigation for 1 min.

The canals were rinsed with 3ml of Saline solution and were dried with paper points. The root canals were obturated with Guttapercha points and AH Plus sealer labelled with fluorescent dye (Rhodamine B isothiocynate), to promote fluorescence under CLSM. Dye was added to the sealer during manipulation at an approximate ratio of 0.1% (weight). The root canal walls were coated by sealer with the help of master cone after which the master cone was placed in the canal up to working length(WL).

The root canal was obturated using accessory cones and excess gutta-percha seared off at orifice level and lightly condensed with a plugger. Teeth was sealed with Cavit (3M, ESPE) and incubated at 37°C and 100% humidity for a week to simulate clinical conditions.

Preparation of samples: Specimens were prepared by sectioning teeth 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 slices will be of 1mm thickness each and were categorized as A, B and C respectively. All sections were polished with silicon carbide abrasive paper. All specimens were mounted onto glass slides and the specimens were examined by using Confocal Laser Scanning Microscopy (CLSM).

Calculation of 'dentinal tubule penetration' (in µm): Images analysis was done using the Fiji Image J software and longest depth of sealer penetration for each specimen was measured. 'The depth of penetration was measured from the canal wall to the point of maximum sealer penetration using the measuring tool in the Image J software'. Analysis was done by a single operator and each measurement was repeated twice in order to ensure reproducibility and consistency.

STATISTICAL ANALYSIS:

The data was statistically analysed by-.

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

Study design

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

↓

All the teeth were radiographed and selected according to the inclusion and exclusion criteria.

↓

The teeth were decoronated using a diamond disc under copious water spray to acquire a standardized root length of 15 mm

↓

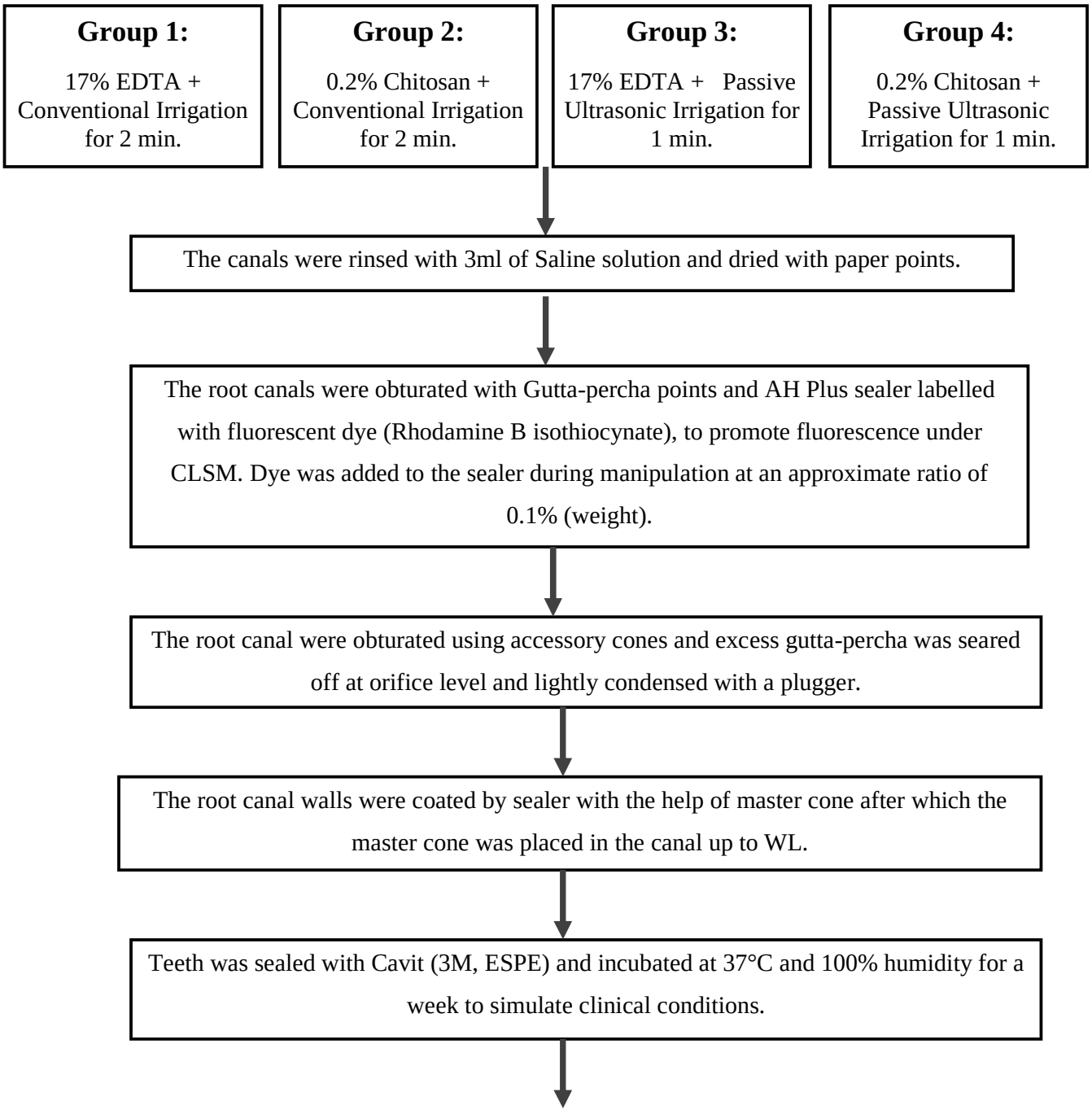
WL was established 1mm short of the length where # 10 K- file exits the apical foramen.

↓

Cleaning and shaping was done using Protaper Universal (DENTSPLY) NiTi rotary instrument till F4(size 40, 0.06 taper).

↓

Samples were randomly divided in to four groups (n=20) based on the final irrigant(5ml) and irrigating technique used.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The root canals were obturated with Gutta-percha points and AH Plus sealer labelled with fluorescent dye (Rhodamine B isothiocynate), to promote fluorescence under CLSM. Dye was added to the sealer during manipulation at an approximate ratio of 0.1% (weight).
The root canal were obturated using accessory cones and excess gutta-percha was seared off at orifice level and lightly condensed with a plugger.
The root canal walls were coated by sealer with the help of master cone after which the master cone was placed in the canal up to WL.
Teeth was 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) 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.

↓

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

↓

The results were statistically analysed using Two-way ANOVA and Tukey's Multiple Post hoc test

MATERIALS USED FOR THE STUDY:

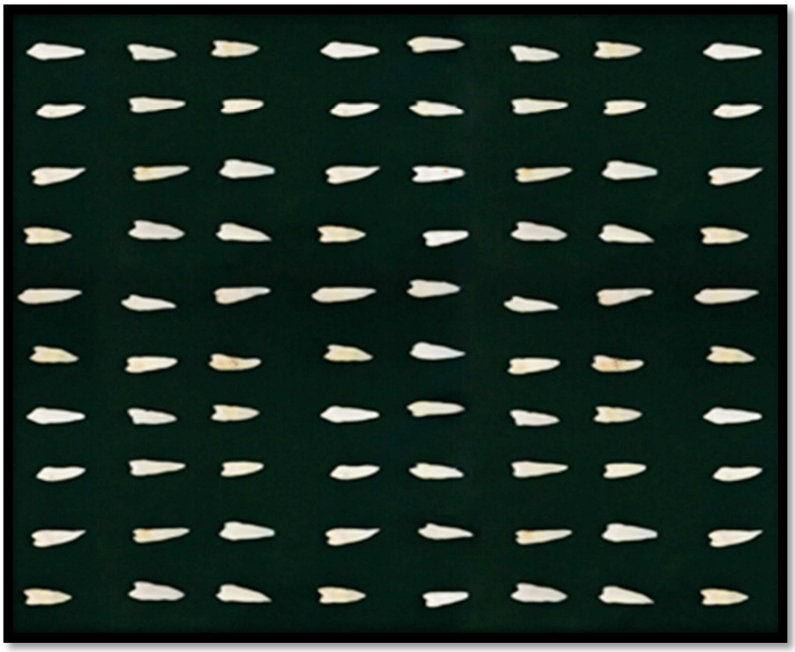


Fig 1: Total Sample Size (n=80)

Fig 2: Debris Removal

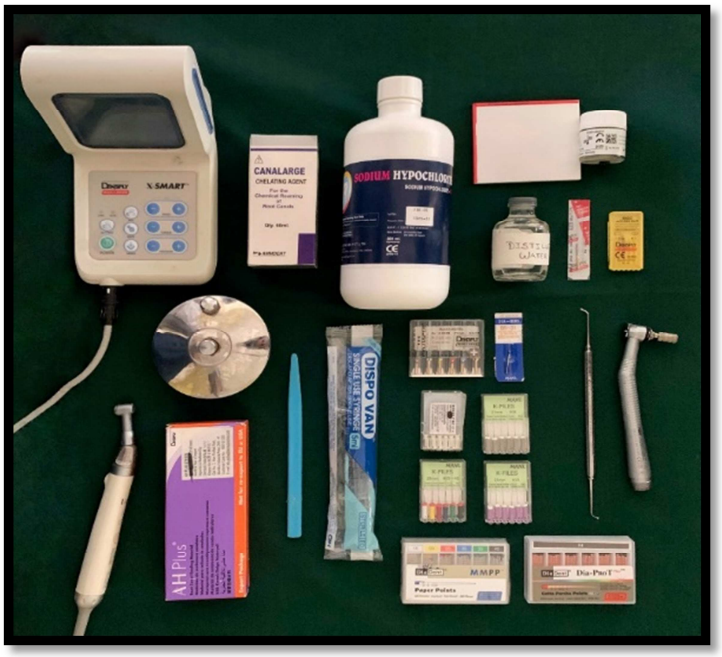


Fig 3: Materials and Armamentarium for access opening, biomechanical preparation and obturation of the tooth samples.

Fig 4: Materials

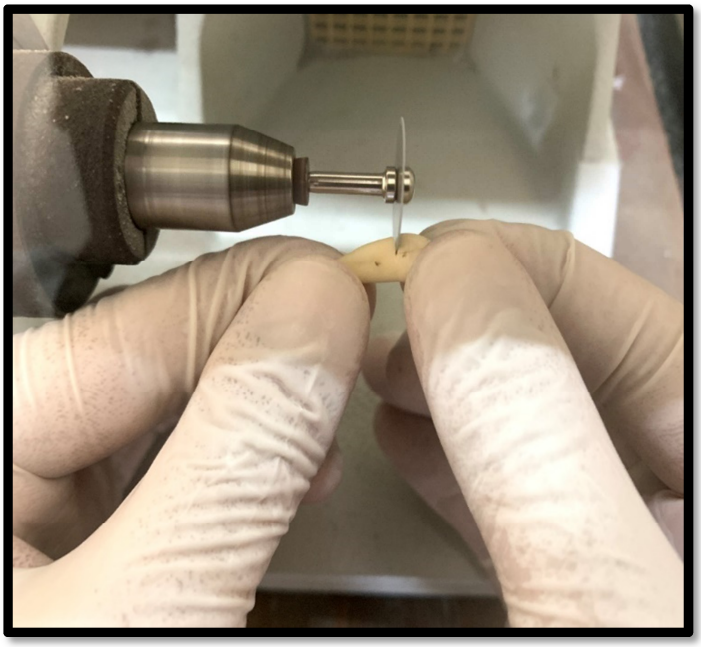


Fig 5: Sample decoronated at the level of CEJ using Diamond disc

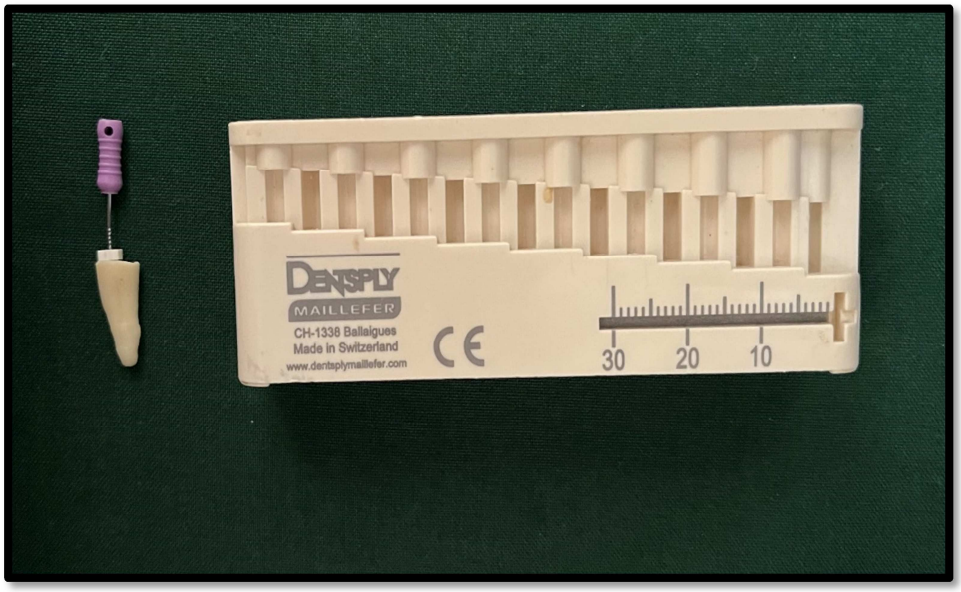


Fig 6: Working Length Determination

Fig 7: Bio-Mechanical Preparation

Fig 8: Sodium hypochlorite Irrigation in between instrumentation

Fig 9: Passive Ultrasonic Irrigation

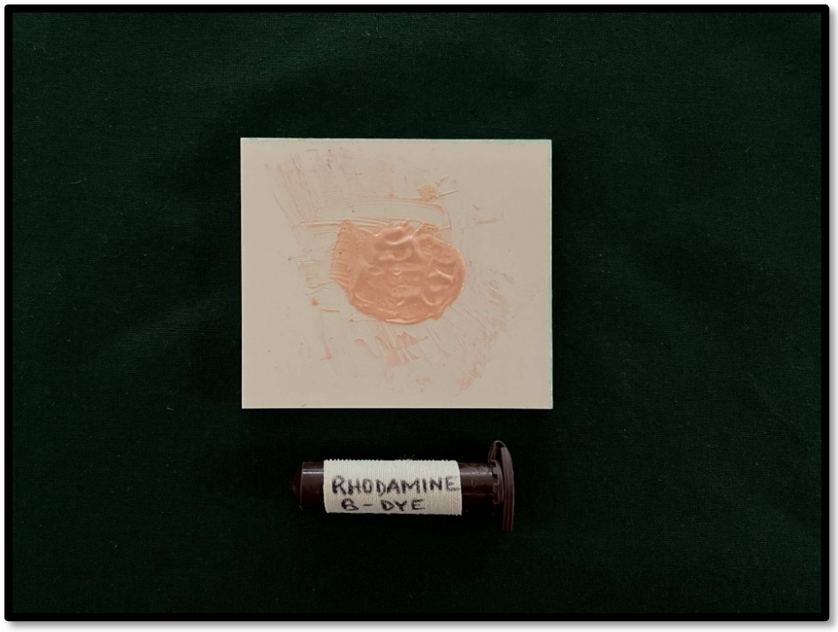


Fig 10: Dye Incorporation in Sealer

Fig 11: Drying Canals with paper points

Fig 12: Obturation of sample

Fig 13: Incubator

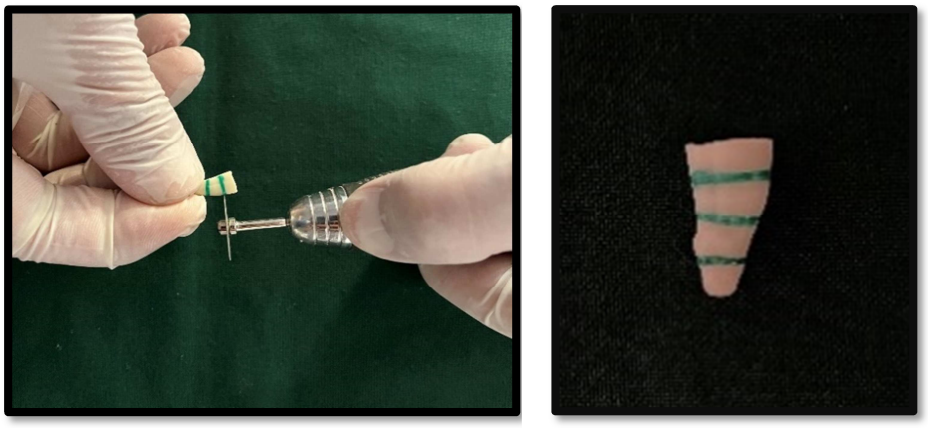


Fig 14: Sectioning of sample at Coronal, Middle and Apical third

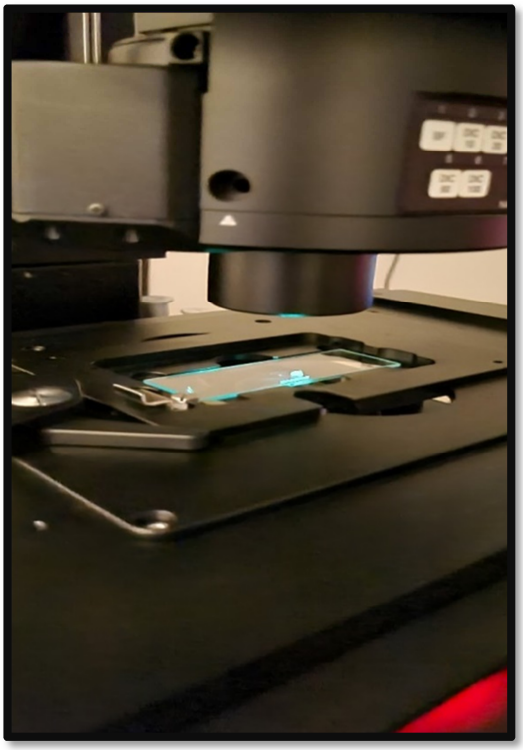


Fig 15: Confocal Laser Scanning Microscopy

RESULTS

Table 1: Summary of depth of sealer penetration in four groups and three regions

Factors	Level of factors	N	Mean	SD	SE	95% C.I for mean	
						Lower	Upper
Group	EDTA+CI	60	538.74	239.79	30.96	476.79	600.68
	Chitosan+CI	60	686.85	328.99	42.47	601.86	771.83
	EDTA + PUI	60	627.16	217.80	28.12	570.89	683.42
	Chitosan+PUI	60	716.44	308.39	39.81	636.78	796.11
Region	Coronal region	80	757.69	308.98	34.54	688.93	826.45
	Middle region	80	700.27	230.08	25.72	649.07	751.47
	Apical region	80	468.93	220.24	24.62	419.92	517.94
Interactions	EDTA+CI with Coronal	20	613.33	181.15	40.51	528.55	698.11
	EDTA+CI with Middle	20	657.16	241.15	53.92	544.30	770.02
	EDTA+CI with Apical	20	345.72	166.57	37.25	267.76	423.67
	Chitosan+CI with Coronal	20	895.56	371.14	82.99	721.86	1069.26
	Chitosan+CI with Middle	20	687.71	190.12	42.51	598.73	776.69
	Chitosan+CI with Apical	20	477.27	264.81	59.21	353.33	601.21
	EDTA + PUI with Coronal	20	606.86	227.47	50.86	500.40	713.31
	EDTA + PUI with Middle	20	708.33	185.96	41.58	621.29	795.36
	EDTA + PUI with Apical	20	486.44	116.54	37.24	408.50	564.38
	Chitosan+PUI with Coronal	20	915.01	287.58	64.30	780.42	1049.60
	Chitosan+PUI with Middle	20	747.87	294.37	65.82	610.11	885.64
	Chitosan+PUI with Apical	20	566.28	222.98	49.86	461.93	670.64

The mean depth of penetration of AH Plus sealer when irrigated with Chitosan with PUI was highest with a mean value of 716.44±39.8, followed by Chitosan with conventional irrigation (686.85±42.47). However, when EDTA was used as a final rinse, all the irrigation technique showed the lowest depth of sealer penetration, PUI with mean value of 627.16±28.12 followed by CI (538.74±30.96).

According to the sections of the tooth, highest depth of sealer penetration was observed at Coronal third (757.69±34.54) followed by middle third (700.27±25.72) and least was at the apical third (468.93±24.62).

However, when the interactions between the various irrigants, activation technique and various sections of the tooth was analysed, the mean depth of sealer penetration was observed to be the highest for Chitosan at the Coronal third with PUI (915.01±64.30) and the least was for EDTA at the apical third with CI (345.72±37.25).

Table 2: Comparisons of four groups and three regions with mean depth of sealer penetration by two way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	1106177.67	3	368725.89	6.3586	0.0004*
Regions	3738652.01	2	1869326.01	32.2364	0.0001*
2-way interaction effects					
Groups*Regions	1228425.63	6	204737.61	3.5307	0.0023*
Error	13221284.60	228	57988.09		
Total	19294539.91	239			

*p<0.05 indicates significant

Table 2 reports a statistical difference between both the main groups of irrigants with p value (0.0004) and regions of the tooth with p value (0.0001).

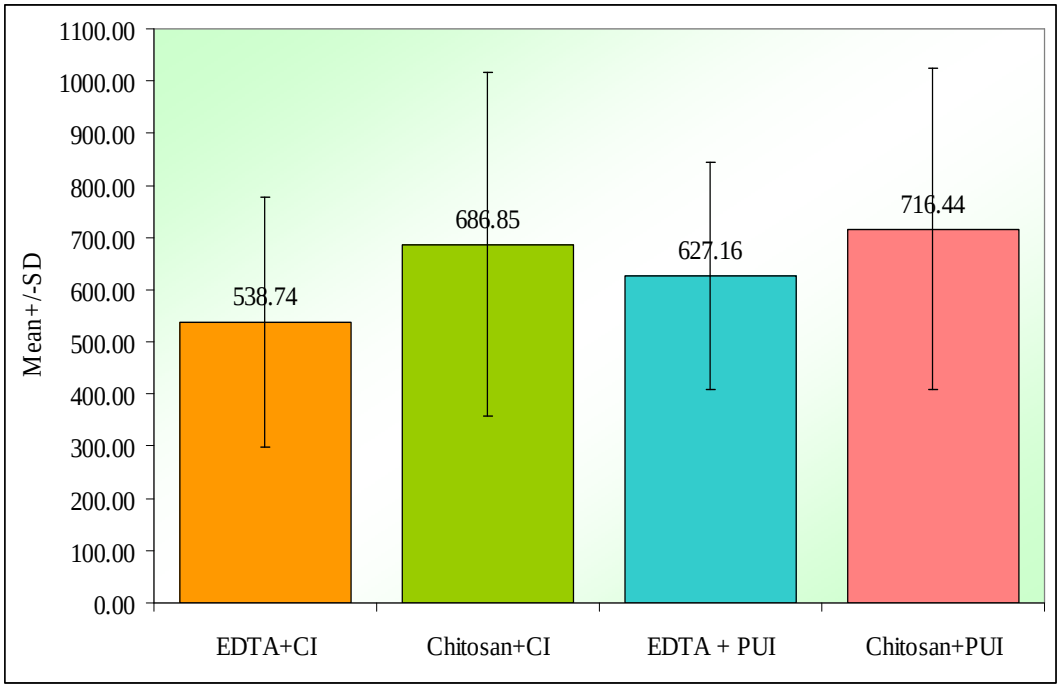
The 2-way interaction between the irrigants and regions of the tooth also showed a significant difference with p value (0.0023).

Table 3: Pair wise comparisons of four groups with mean depth of sealer penetration by Tukeys multiple posthoc procedures

Groups	EDTA+CI	Chitosan+CI	EDTA + PUI	Chitosan+PUI
Mean	538.74	686.85	627.16	716.44
SD	239.79	328.99	217.80	308.39
EDTA+CI	-			
Chitosan+CI	P=0.0042*	-		
EDTA + PUI	P=0.1838	P=0.5261	-	
Chitosan+PUI	P=0.0003*	P=0.9073	P=0.1766	-

*p<0.05 indicates significant

Graph 1: Comparisons of four groups with mean depth of sealer penetration



The table 3 and graph 1 shows inter-pairwise comparison of Chitosan and EDTA with CI and PUI using Tukey's post hoc test, which revealed statistically significant depth of sealer penetration values between Group 1&2; Group 1&4 ($p<0.05$).

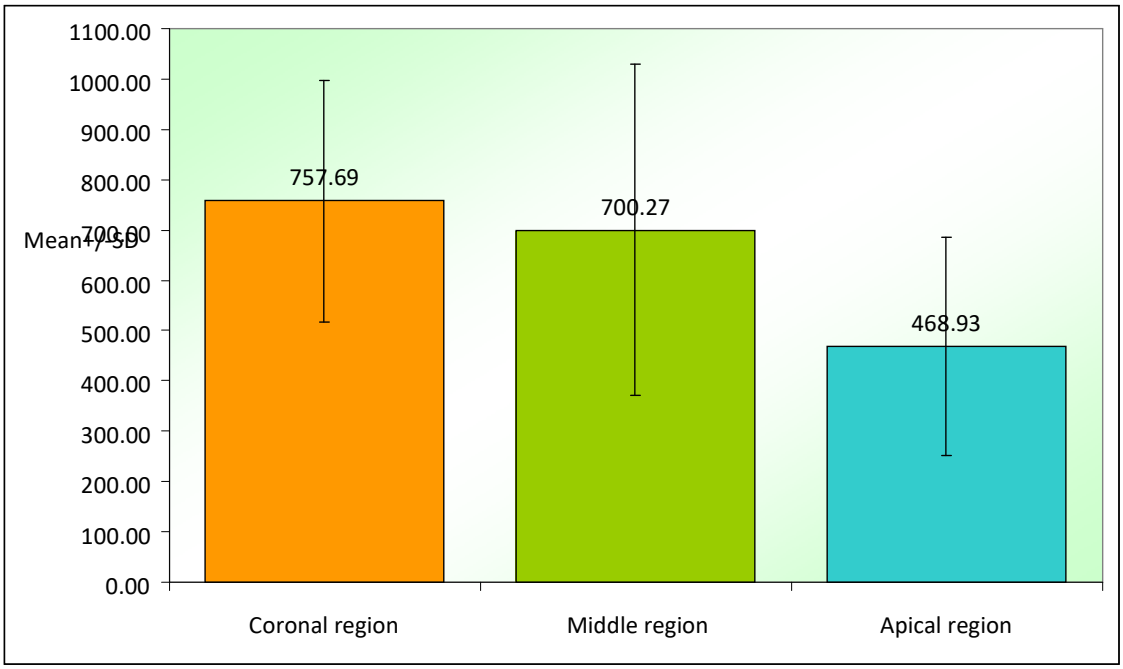
However, the comparison between all other Groups (Group 1&3; 2&3; 2&4) showed no significant difference ($p>0.05$).

Table 4: Pair wise comparisons of three regions with mean depth of sealer penetration by Tukeys multiple posthoc procedures

Groups	Coronal region	Middle region	Apical region
Mean	757.69	700.27	468.93
SD	308.98	230.08	220.24
Coronal region	-		
Middle region	P=0.2870		
Apical region	P=0.0001*	P=0.0001*	-

*p<0.05 indicates significant

Graph 2: Comparisons of three regions with mean depth of sealer penetration



The table 4 shows inter-pairwise comparison of mean depth of sealer penetration of various sections of the tooth using Tukey's post hoc test, which reported statistically significant difference between Coronal and Apical; Middle and Apical third (p=0.0001).

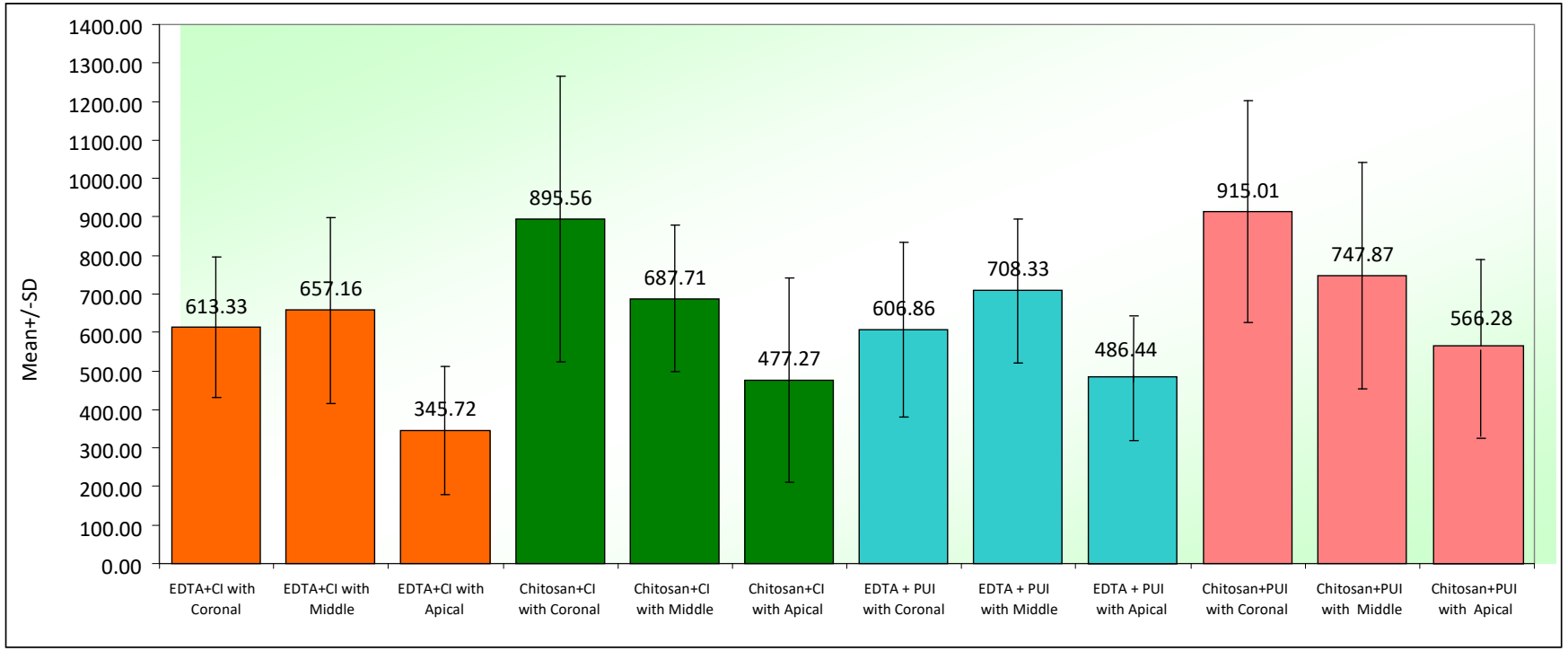
However, the comparison between Coronal and Middle third showed no significant difference.

Table 5: Pair wise comparisons of four groups and three regions with mean depth of penetration by Tukeys multiple posthoc procedures

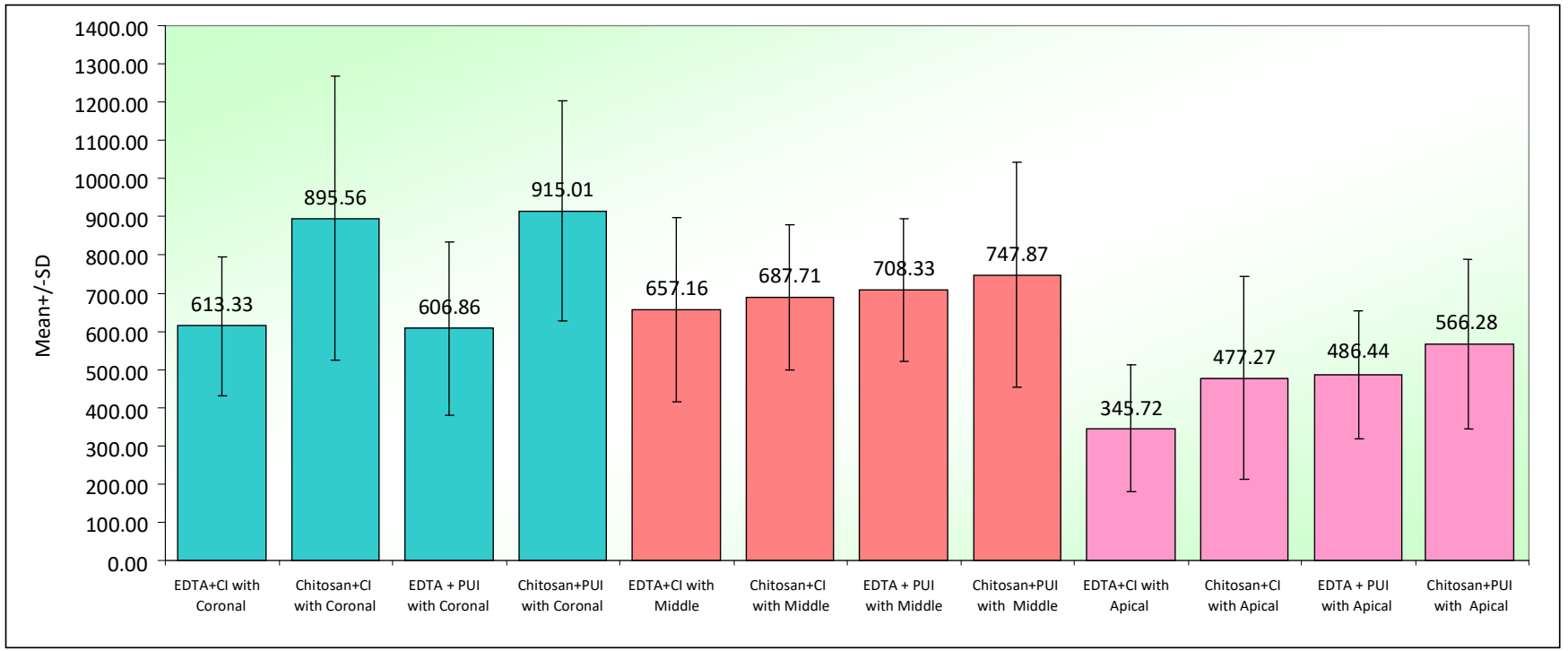
Interactions	EDTA+CI with Coronal	EDTA+CI with Middle	EDTA+CI with Apical	Chitosan+ CI with Coronal	Chitosan+ CI with Middle	Chitosan+CI with Apical	EDTA + PUI with Coronal	EDTA + PUI with Middle	EDTA + PUI with Apical	Chitosan+P UI with Coronal	Chitosan+P UI with Middle	Chitosan+ PUI with Apical
Mean	613.33	657.16	345.72	895.56	687.71	477.27	606.86	708.33	566.28	915.01	747.87	486.44
SD	181.15	241.15	166.57	371.14	190.12	264.81	227.47	185.96	222.98	287.58	294.37	166.54
EDTA+CI with Coronal	-											
EDTA+CI with Middle	**p=1.0000**	-										
EDTA+CI with Apical	**p=0.0224 ***	**p=0.0026***	-									
Chitosan+CI with Coronal	p=0.0114 *	p=0.0753	p=0.0001*	-								
Chitosan+CI with Middle	p=0.9982	p=0.0484*	p=0.0004*	**p=0.2116**	-							
Chitosan+CI with Apical	p=0.8256	p=0.4320	p=0.0495*	**p=0.0001***	**p=0.1958**	-						
EDTA + PUI with Coronal	p=1.0000	p=1.0000	p=0.0299*	p=0.0083*	p=0.9961	p=0.8679	-					
EDTA + PUI with Middle	p=0.9851	p=0.9999	p=0.0001*	p=0.3663	p=1.0000	p=0.0988	**p=0.9750**	-				
EDTA + PUI with Apical	p=1.0000	p=0.9896	p=0.1419	p=0.0009*	p=0.9115	p=0.9912	**p=1.0000**	**p=0.7809**	-			
Chitosan+PUI with Coronal	p=0.0043 *	p=0.0344*	p=0.0001*	p=1.0000	p=0.1128	p=0.0001*	p=0.0030*	p=0.2190	p=0.0003*	-		
Chitosan+PUI with Middle	p=0.8362	p=0.9897	p=0.0001*	p=0.7343	p=0.9998	p=0.0196*	p=0.7889	p=0.0498 *	p=0.4164	**p=0.5534**	-	
Chitosan+PUI with Apical	p=0.8835	p=0.5188	p=0.7912	p=0.0001*	p=0.2555	p=1.0000	p=0.9162	p=0.1358	p=0.0492*	**p=0.0001***	**p=0.0295**	-

*p<0.05 indicates significant

Graph 3: Comparisons of four groups and three regions with mean depth of sealer penetration



Graph 4: Comparisons of mean depth of penetration with four groups and three regions



The table 5 shows inter-pair wise comparisons of four groups and three regions with mean a depth of penetration of sealer by Tukeys multiple posthoc procedure. The mean difference between all the main groups and regions is statistically significant ($p<0.05$). Similarly, the difference between 1A & 3A, 1B & 3B, 1C & 3C, 2A & 4A, 2B & 4B, 2C & 4C are also significant ($p<0.05$).

CLSM Images depicting penetration of AH Plus sealer after final irrigation with 17% EDTA using CI technique at coronal, middle and apical sections (Group 1):

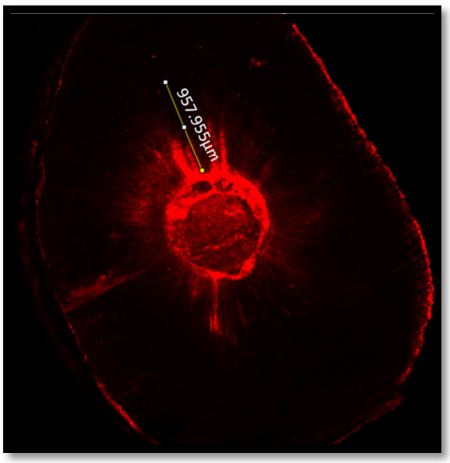


Fig 16 a: CORONAL [Group 1A]

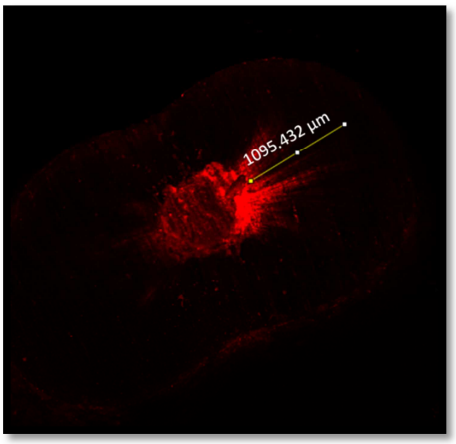


Fig 16 b: MIDDLE [Group 1B]

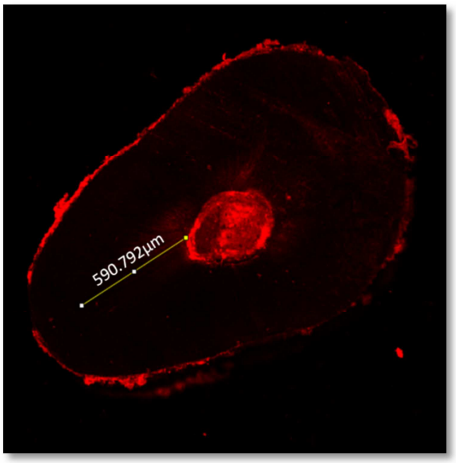


Fig 16 c: APICAL [Group

CLSM Images depicting penetration of AH Plus sealer after final irrigation with 17% EDTA using PUI technique at coronal, middle and apical sections (Group 2):

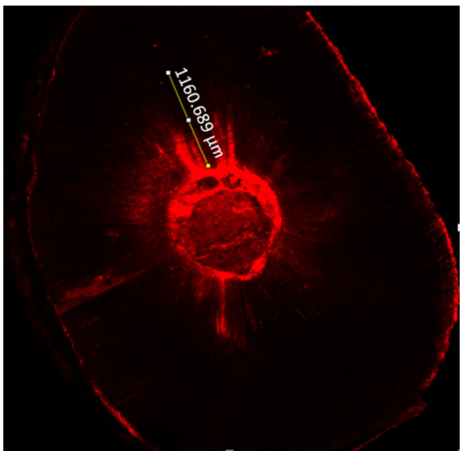


Fig 17 a: CORONAL [Group 2A]

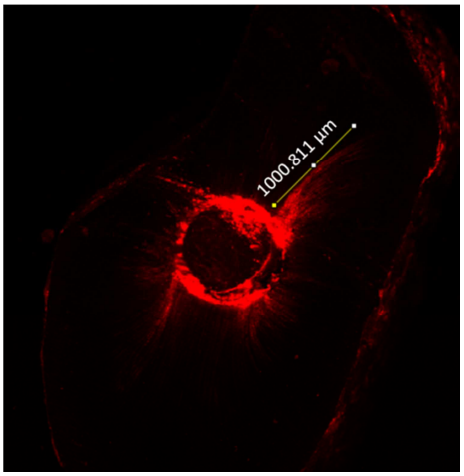


Fig 17 b: MIDDLE [Group 2B]

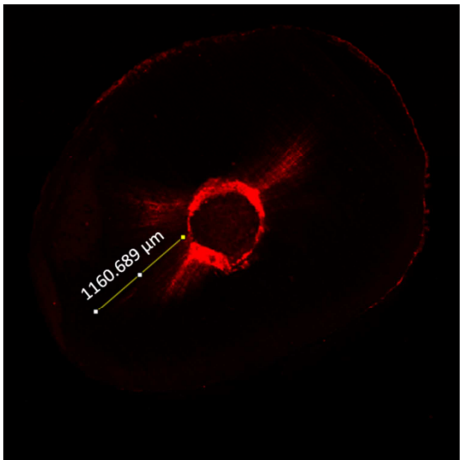


Fig 17 c: APICAL [Group 2C]

CLSM Images depicting penetration of AH Plus sealer after final irrigation with 0.2% CHITOSAN using CI technique at coronal, middle and apical sections (Group 3):

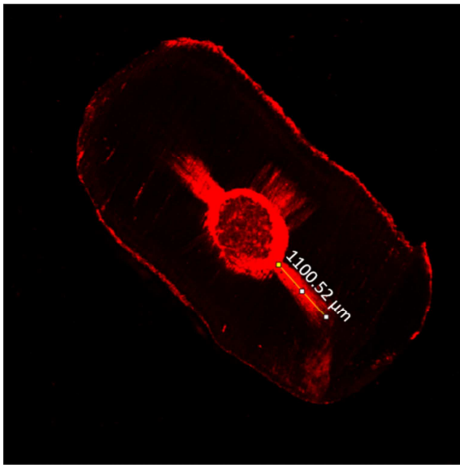


Fig 18 a: CORONAL [Group 3A]

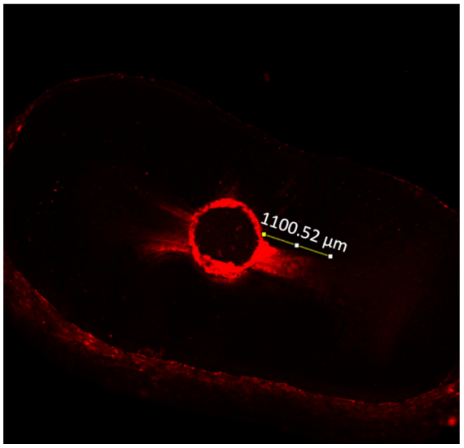


Fig 18 b: MIDDLE [Group 3B]

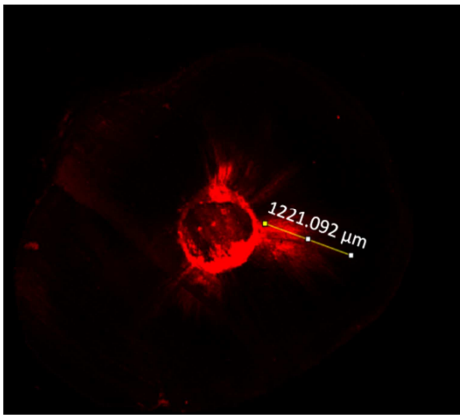


Fig 18 c: APICAL [Group 3C]

CLSM Images depicting penetration of AH Plus sealer after final irrigation with 0.2% CHITOSAN using PUI technique at coronal, middle and apical sections (group 4):

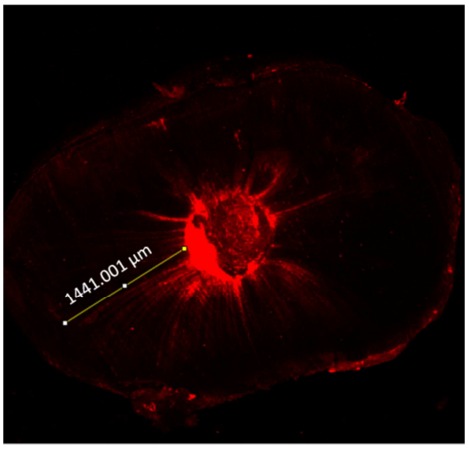


Fig 19 a: CORONAL [Group 4A]

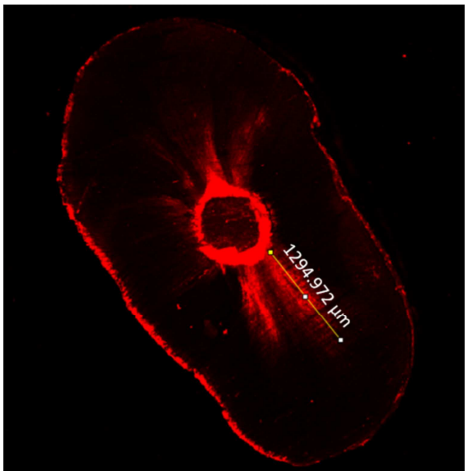


Fig 19 b: MIDDLE [Group 4B]

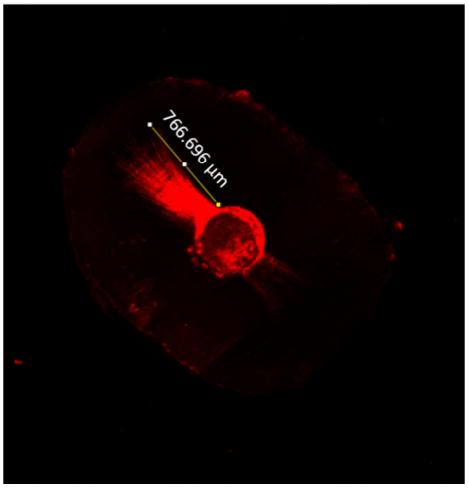


Fig 19 c: APICAL [Group 4C]

DISCUSSION

The quality of root canal disinfection and filling is a significant predictor of a successful outcome endodontic treatment.[22] One of the main objectives of endodontic treatment is adequate biomechanical preparation and three-dimensional obturation of root canal system.[33]

The pulp tissue, bacteria, dentin debris, and smear layer should be removed from the root canal system during root canal treatment[22]. The smear layer is an amorphous structure, about 1–2 μm thick that is created during biomechanical preparation.[4] Removal of the smear layer is a prerequisite in order to allow for opening of dentinal tubules to allow ingress of sealer.[35]

The main irrigating solution used in the endodontic treatment is sodium hypochlorite (NaOCl) due to its antimicrobial action and solvent capacity on organic tissues, but it does not affect the inorganic content. To remove debris and smear layer and improve the permeability of the root canal system, final irrigation with ethylenediaminetetraacetic acid (EDTA) is recommended as a prerequisite for a satisfactory sealing of the dentinal canaliculi.[22]

Chelating agents are most commonly used irrigant in endodontics in order to remove debris and smear layer and improve the permeability of the root canal sealers. These agents combine with calcium ions of tooth and decalcify the dentin.[36] EDTA in concentration of 15-17% eliminates calcium from dentine without any lethal damage to periapical tissues. The dual irrigation regime of NaOCl and EDTA has been used for removing debris and smear layer, resulting in successful debridement and disinfection of root canal.[26] Calt et al. and Torabinejad stated that longer contact

period of EDTA (> 1 min) could cause excessive peritubular and intertubular erosion and destruction of root dentin.[26,37] EDTA therefore imparts a negative influence on the hydration properties of cement because of its acidic nature, thereby inhibiting adhesion to materials.[38] Hence, search for newer alternatives was needed.

Chitosan, naturally obtained from "deacetylation of Chitin" is a biodegradable polymer. It contains copolymers of glucosamine and N-acetyl glucosamine and has been found to have antimicrobial and antifungal properties in the biomedical field.[39] An important property of Chitosan as an excipient is the ability to hydrate and form gels in a highly acidic aqueous environment and is thus used to prepare slow release drug delivery systems. It has been tested as a drug carrier in hydrocolloids and gels in vitro studies. It has also been proposed that Chitosan improves the bioavailability of drugs and can be useful for drug distribution to particular regions.[40]

Chitosan has shown to have the properties of a chelating solution and the highest effect was seen when it was solubilized in Acetic acid, similar to the 0.2% Chitosan used in this study.[26,37-41] It has chelation properties similar to EDTA and Citric acid with very less detrimental effects. Chelating solutions have the ability to eliminate smear layer, and expose large number of dentinal tubules[25] which encourages adhesion of sealer due to an increased contact area that would warrant better adaptation amid the sealer and root canal dentin.[33]Considering these factors, 0.2% Chitosan was used as an Experimental irrigant.

Along with the antimicrobial and chelating actions of endodontic irrigants, which play a critical role in disinfecting and cleaning the root canal system, many different devices and irrigant activation techniques have been developed to improve the efficiency and distribution of solutions during final irrigation.[42] Several techniques

used for root canal irrigation, include as conventional needle irrigation, manual dynamic activation (MDA), sonic irrigation (SI), and passive ultrasonic irrigation (PUI).

Passive ultrasonic irrigation (PUI) activates the irrigant by acoustic microstreaming transmitted from a smooth wire or an oscillating file at an ultrasonic frequency of 30 kHz.[22] Irrisafe tips (Satelec Acteon Group, Merignac, France) were used for passive ultrasonic irrigation (PUI) in our study. It also improves the cleaning and disinfection of the root canal system due to acoustic streaming and microcavitation and it allows the delivery of irrigants up to the working length of the root canal[13]. Its efficacy has been studied by Paragliola et. al. who concluded that the use of an ultrasonic agitation exhibited significantly more penetration of irrigants in tubules than sonic agitation.[43]

Accordingly, the present study was directed to evaluate and compare the depth of dentinal tubular penetration of an Epoxy resin based sealer following a final rinse of 17% EDTA and 0.2% Chitosan, with or without Passive Ultrasonic activation.

Following sufficient chemo-mechanical preparation, a hermetic sealing with a biocompatible material is another important objective of root canal treatment. Root canal sealers play a critical role in the success of endodontic therapy by eliminating the space between the root canal wall and core filling material.[44] Sealers should seal the canal laterally and apically to have a good adaptation to the root canal dentin. The penetration of sealer into dentinal tubules is essential as it enhances sealing ability and retention of the sealer.[25] Through its antibacterial effect, the penetration ability of sealers may also enable avoidance of the colonization of residual bacteria and reinfection of the root canal.[45]

In this study, a resin-based root canal sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany) and lateral condensation technique were used together. The pseudoplastic behaviour of AH Plus is a beneficial property which allows increased flow by reduction of viscosity during obturation and thus enhancing its penetration in tubules.[46] The covalent bonding of an epoxy group to the organic part of dentin (the collagen amine group released in dentin) may be the reason for the chemical bonding of AH Plus to the dentin molecule. According to Neto et al, previous studies have emphasized that the high bond strength of AH Plus could be due to the low polymerization stress of the sealer and its long-term dimensional stability.[47]

AH Plus shows other desirable properties of low rates of solubility, optimal adhesiveness and great radiopacity to the root dentin than others endodontic sealers and currently serve as gold standards for comparison.[25]

Lateral condensation technique of obturation is preferred as increased hydraulic forces occur throughout the process. In this technique, sealer can be pushed into the dentinal tubules. An easily detectable fluorescent dye, Rhodamine B, was used in low concentration (0.1%) to avoid impairing the physicochemical properties of the root canal sealer.[18]

CLSM was used in current study. CLSM uses high contrast points to identify sealer distribution within dentinal tubules. It works even with thick section where it collects serial optical sections by making possible control over depth and reducing background information. Unlike scanning electron microscopy, confocal microscopy does not require vacuum or metallization, which is responsible for sample dehydration and the occurrence of technical artifacts and has been widely used to observe and evaluate sealer penetration within the dentinal tubules.[48] In comparison to

conventional SEM, CLSM utilizes non-decalcified or hard tissue samples that do not require a sputter coating.[47] The disadvantage of Light microscopy is its ineffectiveness to differentiate between sealer and radicular dentin.[48]

The present study focused on the depth of sealer penetration following a final rinse with 17% EDTA and 0.2% Chitosan with or without passive ultrasonic activation. 0.2% Chitosan solution (Group 2=686.85±42.47, Group 4=716±39.81) showed higher depth of sealer penetration when compared with 17% EDTA (Group 1=538.74±30.96, Group 3=627.16±28.12) as shown in table 1 and graph 1; irrespective of the irrigation technique used.

Chitosan is hydrophilic in nature which favours intimate contact with dentin and removes smear layer. The decision of using a 0.2% Chitosan solution compared with 15% EDTA was based on the study by Silva, et al. (2012), which evaluated the effect of different concentrations of Chitosan on the dentin surface and smear layer removal. The authors verified that 0.1% Chitosan used for 3 minutes removes the smear layer but not the smear plug. Chitosan at 0.2% used for the same amount of time showed visible dentin, open tubules, and slight erosion of peritubular dentin. Chitosan at 0.37% cleaned the dentin walls similarly to 0.2% Chitosan, but with a much greater erosive effect.[10] Mathew et al, also concluded that Chitosan is an effective chelating agent and can be considered as a less invasive alternative to 17% EDTA.[49] Abraham et al, concluded that Diode laser and Endoactivator with 0.2% Chitosan proved better smear layer removal as compared to passive ultrasonic irrigation.[21]

The action of Chitosan can be explained by 2 mechanisms. First one being the bridge model in which two or more amino groups of Chitosan bind to the same

metal ion and in the second one is pendant model in which one amino group is utilized for binding, and the metal ion gets attached to amino group forming a pendant.[50] The above mentioned study concluded that 0.2% Chitosan removes the smear layer and unblocks dentinal tubules in a manner similar to EDTA.

Furthermore, Silva et. al. indicated that a 0.2% Chitosan solution was as effective as EDTA and CA with higher concentrations (15% EDTA and 10% CA) at removing the smear layer. Similar findings were observed with EDTA groups in the present study, where the sealer penetration was not as effective as seen with Chitosan groups which was found to be statistically significant (p=0.0042). EDTA when used as a final rinse, all the irrigation technique showed the lowest depth of sealer penetration, PUI with mean value of Group 3 =627.16±28.12 followed by CI (Group 1 = 538.74±30.96).

According to Filho et al compared effectiveness of 7 % maleic acid and 17 % EDTA in smear layer removal and concluded that effectiveness is improved with 7 % maleic acid in apical third of the tooth but the main disadvantage is that maleic acid causes more erosion of dentin and reduces the microhardness of the tooth.[51] Scelza et al, evaluated the time periods of EDTA, EDTA – T, Citric acid irrigation for effective opening of dentinal tubules and concluded that all three irrigants were effective at the 3 min. periods and after 3 min. there was saturation which leads to organic contents precipitation.[52]

Ultrasonic activation potentially improved the debris/smear layer removal by causing shear stress in the inorganic particles of the smear layer by acoustic streaming, facilitating its removal. However, the irrigant delivered by conventional needle only penetrates from 0 to 1.1 mm deeper than the tip of the needle, and gas

particles are produced and trapped in the apical portion, creating a vapor lock and hindering the efficacy of irrigant debridement. PUI allows the elimination of vapor lock effect, improving the efficiency of the irrigating solution. The safety of CI has also been questioned because the positive pressure used to deliver the solution into the canal may extrude it to the periapex, causing tissue damage and postoperative pain.[22]

These findings are consistent with other studies that observed a greater smear removal when supplementing the final irrigation with PUI (Group 2 =686.85±42.47, and Group 4 =716±39.81).The activity of EDTA though improved with the use of PUI, it was not able to match the activity of Chitosan with Conventional Irrigation.

Zhou et al, concluded that PUI improved effectiveness over XPF techniques for medicament removal and the fins and deltas, irrigation protocol, and intracanal medicaments time might determine the cleaning efficacy.[53] Plaza et al, stated that PUI with three cycles of 20 s (NaOCl-EDTA-NaOCl), two cycles of 60 s (EDTA-NaOCl), or one cycle of 60 s (NaOCl), were equally efficacious at removing dentinal debris and opening of dentinal tubules. This may be due to the flow generated by ultrasound with EDTA was more successful in removing the smear layer showing the importance of the physical way of enhancing the canal irrigation.[54]

These findings are consistent with other studies that observed a greater smear layer removal when supplementing the final irrigation with PUI. When the areas of root canals where considered overall penetration of endodontic sealer was greater in cervical and middle thirds irrespective of irrigant and technique used. There was a statistically significant difference observed between Chitosan and EDTA in terms of cleaning efficiency ($p=0.0004$). Chitosan proved better than EDTA in terms of cleaning efficiency, with both CI (686.85±42.47) and PUI (716.44±39.81).

Camilleri (2015) reported that AH Plus penetrated the dentinal tubules of the coronal and middle thirds of the root, whereas in the apical third, penetration was not always observed. This may have been due to two factors. First, the number and diameter of dentinal tubules decrease toward the apical third and, second, the apical third presents more sclerotic dentin and greater difficulty for irrigant delivery and smear layer removal, which has a direct effect on sealer penetration. This result agrees with previous studies that showed that irrigating solutions are less effective in the apical third[55] (Table 4, Graph 2).

However, in the apical third, the Chitosan + PUI (566.28±49.86) final irrigating protocol provided an improved debris/smear layer removal of the canal walls and a higher percentage of sealer penetration into the dentinal tubules. This finding is important because the apical third is considered the critical region of the root canal for presenting a greater amount of ramifications of the main root canal. These ramifications are inaccessible to the conventional chemo-mechanical preparation, which allows harboring remaining bacteria and their by-products and leads to the failure of the endodontic therapy.[22]

Within the limitation of the present study, we can conclude that 0.2% Chitosan was highly effective in terms of dentinal depth of sealer penetration, irrespective of the irrigation technique used. PUI further improved the effectiveness of both the irrigants. Cleaning the apical third of the root canal still remains an area of concern due to the reasons clearly discussed.

CONCLUSION

In the present study, 0.2% Chitosan as an irrigant showed higher quality and improved depth of penetration of sealer into the dentinal tubules when compared with 17% EDTA. This can be attributed to its chelating properties with less detrimental effects on the radicular dentin.

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

Higher depth of sealer penetration was seen in the coronal third of root canal and least in the apical third. The limited depth of sealer penetration in the apical third is due to the complex anatomy of the root canal in this region.

SUMMARY

The outcome of root canal therapy depends on the efficient administration of chemo-mechanical preparation and fluid tight seal of the root canal space. The root canal system has highly complex anatomy such as isthmus, canal fins and cul-de-sac. Large areas in the oval and flat canals may remain untouched despite careful instrumentation and thus have limited our ability to clean and disinfect it predictably. Therefore, much focus is given on endodontic irrigant as to impact those areas and obtain thorough decontamination.

Chelating agents are most commonly used irrigant in endodontics in order to remove debris and smear layer and improve the permeability of the RCS. Although, EDTA is one of the most widely used chelating agents, many studies revealed that EDTA was not effective in smear layer removal in the apical third of the root canals.

Chitosan, a natural polysaccharide, is prominently used in dentistry because it is biocompatible, biodegradable, bioadhesive, and non-toxic, with broad-spectrum antimicrobial properties and chelating activity[15,16]. It has the ability to remove the smear layer and unblock dentin tubules without promoting significant dentin erosion[17]. Hydrophilic nature of Chitosan increases the contact area to dentin, thereby enhancing the adhesion of sealer to root canal dentin which avoids the penetration of microbes in root canal and provides a good hermetic seal. Chitosan solution is used as a chelating agent which removes the smear layer exposing a greater number of dentinal tubules resulting in better adaptation and penetration of sealer due to increased contact area.

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.

There are very few studies have evaluated and compared the effect of EDTA and Chitosan on radicular dentin and the penetration of sealers into dentinal tubules. Keeping these rationales in mind, this study aims to explore the effects of final canal irrigation with Chitosan and EDTA, with and without passive ultrasonic agitation on sealer penetration.

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

Eighty freshly extracted human mandibular premolar teeth were cleaned of calculus and soft tissue debris immersed in 0.1% Thymol solution till use. Teeth were then radiographed and selected as per the inclusion and exclusion criteria. Decoronation of teeth up to the level of CEJ was done with the help of a diamond disc to produce a standardized root length of 15mm. The root canals were prepared using Protaper Universal (DENTSPLY) NiTi rotary instrument till F4(size 40, 0.06 taper).

Samples were randomly divided in to four groups (n=20) based on the final irrigant (5ml) and irrigating technique used.

Group 1: 17% EDTA + Conventional Irrigation for 2 min.

Group 2: 0.2% Chitosan + Conventional Irrigation for 2 min.

Group 3: 17% EDTA + Passive Ultrasonic Irrigation for 1 min.

Group 4: 0.2% Chitosan + Passive Ultrasonic Irrigation for 1 min.

The canals were irrigated with 3ml of Saline solution, dried with paper points and then obturated with Guttapercha points and AH Plus sealer labelled with fluorescent dye(Rhodamine B isothiocynate). Dye was added to the sealer during manipulation at an approximate ratio of 0.1% (weight). The root canal walls were then coated by sealer with the help of master cone and obturated using lateral condensation technique. Teeth were then sealed with Cavit (3M, ESPE) and incubated at 37°C and 100% humidity for a week.

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 slices were of 1mm thickness each and were mounted onto glass slides. Specimens were examined by using Confocal Laser Scanning Microscopy (CLSM). Calculation of depth of penetration would be done by using Image J software.

Statistical analysis of the data obtained was done by Two-way ANOVA and Tukey's Post hoc test. The results indicated that there is significant difference between all the four groups (p value <0.01).

The mean depth of penetration of AH Plus sealer when irrigated with Chitosan with PUI was highest followed by Chitosan with conventional irrigation. However, when EDTA was used as a final rinse, both irrigation techniques showed low depth of sealer penetration.

According to the sections of the tooth, highest depth of sealer penetration was observed at Coronal third followed by middle third and least was at the apical third.

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

penetration was observed to be the highest for Chitosan at the Coronal third with PUI and the least was for EDTA at the apical third with CI.

Therefore, null hypothesis stating that there will be no difference in the depth of penetration of an Epoxy resin based sealer into the dentinal tubules after the use of 17% EDTA and 0.2% Chitosan as a final rinse with or without Ultrasonic activation was rejected.

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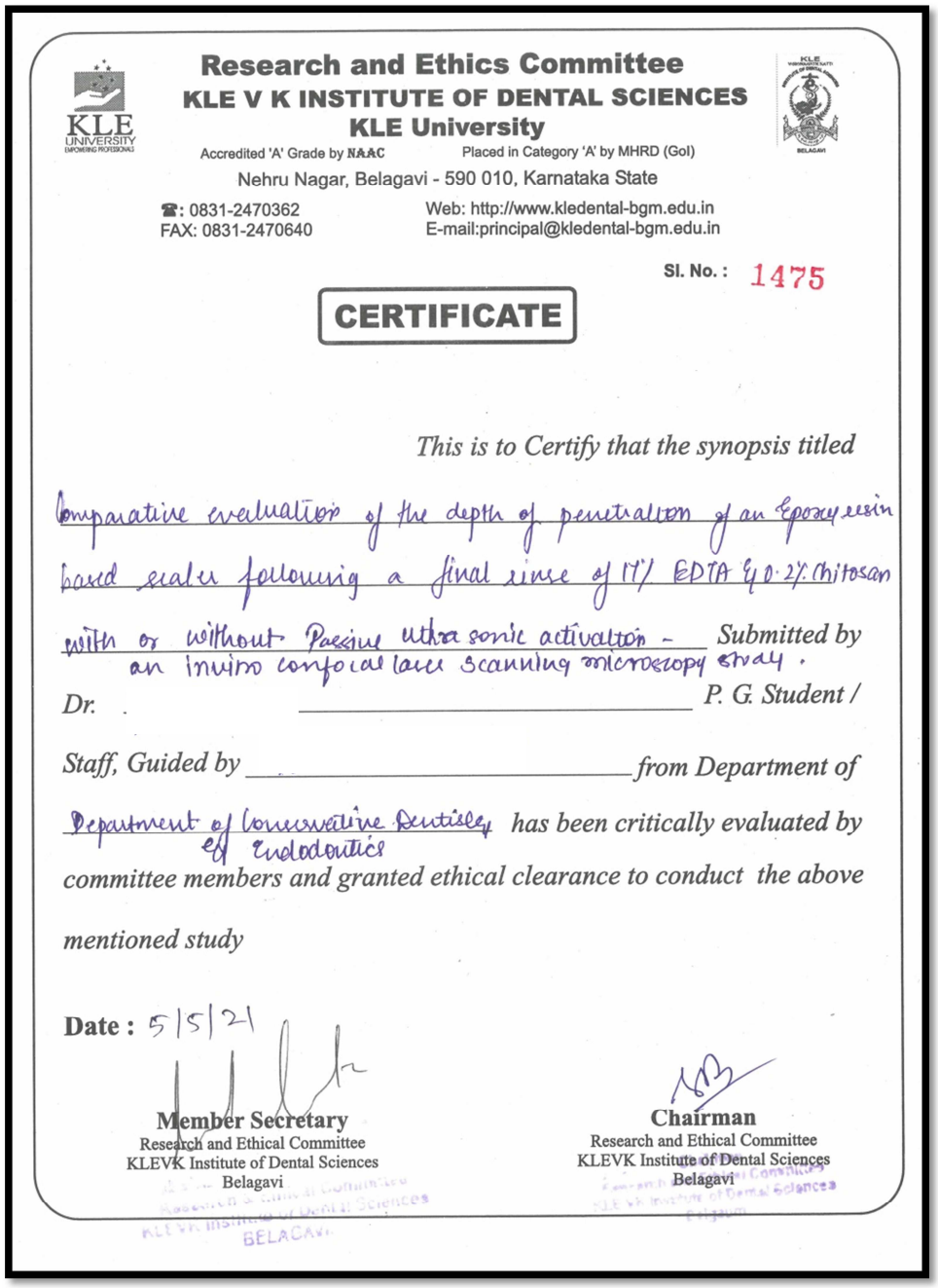
ANNEXURE – I

ETHICAL CLEARANCE



Research and Ethics Committee
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Sl. No. : 1475

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of the depth of penetration of an Epoxy resin based sealer following a final rinse of 17% EDTA & 0.2% Chitosan with or without Passive Ultra sonic activation - an invitro confocal laser scanning microscopy study.

Submitted by

Dr. _____ *P. G. Student /*

Staff, Guided by _____ *from Department of*

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

Date : 5/5/21

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE – II

BIOSTATISTIC CLEARANCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
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E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Post Graduate Student**, under the guidance of **Professor, Department of Conservative Dentistry and Endodontics**, entitled **"Comparitive evaluation of the depth of penetration of an Epoxy resin based sealer following a final rinse of 17% EDTA and 0.2% Chitosan, with or without passive ultrasonic activation: An in-vitro confocal laser scanning microscopy study"** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 14/11/2022

Name & Signature of Biostatistician
(Dr. S.B. Javali)
Sr. Asso. Prof. in Statistics
USM KLE Dmp. Belagavi

ANNEXURE – III

PLAGIARISM CHECK CERTIFICATE

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

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

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

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Date : 26/12/2022 Serial No. : 140

PLAGIARISM CHECK REPORT

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

The soft copy of Research Work / Manuscript by ..... entitled "Comparative evaluation of the depth of penetration of an epoxy resin based sealer following a final rinse of 17% EDTA and 0.2% chitosan, with or without PUI: An invitro CLSM study" under the guidance of ..... has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of 3%, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

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