"COMPARATIVE EVALUATION OF THE EFFECT OF 17% EDTA AND 0.2% CHITOSAN USED AS A FINAL RINSE ON THE PUSH OUT BOND STRENGTH OF THREE DIFFERENT ROOT CANAL SEALERS TO THE RADICULAR DENTIN-AN INVITRO STUDY"

By

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Dissertation

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Under the Guidance of

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DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS

KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES

BELAGAVI, KARNATAKA

2019-2022

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UNDERTAKING

I, **Dr.BHAVNA SHARMA**, hereby declare that the information and data mentioned in my thesis entitled **"COMPARATIVE EVALUATION OF EFFECT OF 17% EDTA AND 0.2% CHITOSAN USED AS A FINAL RINSE ON THE PUSH-OUT BOND STRENGTH OF THREE DIFFERENT ROOT CANAL SEALERS TO THE RADICULAR DENTIN-AN INVITRO STUDY"** belongs to me and is original.

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This dissertation is dedicated

To

Almighty god,

My parents, &

My elder sister

And brother

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Thank you, one and all.

Dr. BHAVNA SHARMA

LIST OF ABBREVIATIONS

SR. NO	ABBREVIATIONS	FULL FORM
1.	NaOCl	Sodium Hypochlortie
2.	EDTA	Ethylene Diaminetetracetic acid
3.	CEJ	Cemento Enamel Junction.
4.	CA	Citric acid
5.	POBS	Push out bond strength
6.	UTM	Universal testing machine
7.	SEM	Scanning electron microscope
8.	NMR	Nuclear Magnetic Resonance.
9.	° C	Degree Celsius
10.	MPa	Megapascals
11.	mm	Millimeter
12.	ANOVA	Analysis of Variance
13.	SD	Standard Deviation
14.	n	Number of specimens
15.	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
16.	>	Greater than
17.	<	Less than

ABSTRACT

Aim and Objectives: To evaluate the effect of 17% EDTA and 0.2% Chitosan as final flush on push-out bond strength of three different root canal sealers to radicular dentin.

Methodology: Sixty-six extracted human mandibular premolar teeth were selected, disinfected and decoronated to obtain a standardized root length of 15 mm. The teeth were sectioned below the CEJ to obtain 2 sections each of 4 mm thickness. The root canals were then prepared upto no.3 Gates Glidden drill and then divided into two groups depending on the irrigants used as final flush.

Group 1: 3% NaOCl + 17 % EDTA

Group 2: 3% NaOCl + 0.2% Chitosan

After following the respective irrigation protocols, the sections were again divided into three sub-groups depending on the sealer to be filled.

Sub-Group 1A and 2A: AH Plus sealer

Sub-Group 1B and 2B: Apexit Plus sealer

Sub- Group 1C and 2C: MTA Fillapex sealer.

The sealers were mixed according the manufacturer's instructions and then filled.

The sections were placed in an incubator at 37°C and 100% humidity for 7 days.

The push-out bond strength test was evaluated using Universal Testing Machine.

Results: 0.2% Chitosan when used as a final flush produced a positive impact on the push-out bond strength of epoxy resin based, calcium hydroxide based, and MTA resin based sealers when compared with EDTA. The highest push-out bond strength was seen in Group 2A (0.2% Chitosan + AH Plus sealer) and least was seen in Group 1C (17% EDTA + Apexit Plus sealer).MTA Fillapex sealer produced higher bond strength than Apexit Plus but less than AH Plus.

Conclusion: Chitosan when used as a final flush improves the push-out bond strength of all the three sealers among which epoxy resin based sealer has highest bond strength.($p<0.05$)

Key words: Chitosan, EDTA, Push-out bond strength, AH Plus, Apexit Plus, MTA Fillapex.

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INTRODUCTION

The endodontic triad for successful root canal treatment comprises of shaping and cleaning, disinfection and 3D obturation of the root canal system. Various studies have shown that even after using newer generation file systems, large areas of root canal wall (which is approximately 35%), remains untouched due to complex anatomy of root canal.[1]

During chemomechanical instrumentation there is production of 1-2 mm thick smear layer which blocks the dentinal tubules, hindering the penetration of medicaments, irrigants, resulting in inadequate disinfection of root canals and hampering the adhesion of the root filling material to dentin[2].

Apart from smear layer, the other factors which affect the adhesion of sealer to dentin include nature of root dentin in specific tooth, chemical composition of irrigants and sealer and their interaction with dentin[3]. Surface treatment of dentin with different irrigating solutions affect chemical and structural architecture of dentin changing the permeability and solubility of dentin, thereby affecting the adhesion of filling materials to dentin surfaces.[4]

Goldman et al has shown that the canals treated without irrigation have more amount of residual tissue than the canals in which irrigants were used.[5]

As a result, irrigation is considered an important aspect of root canal debridement since it enables for cleaning beyond what root canal instrumentation alone can accomplish. However, there is currently no one irrigant that satisfies all of

the criteria for an ideal irrigating solution. A successful treatment outcome is aided by the use of a combination of products in the proper irrigation sequence and method.[6]

Sodium hypochlorite is the most commonly used irrigating solution in endodontics to remove organic debris and necrotic tissues whereas chelating agents are used to remove the inorganic dentin and smear layer .Sequential use of EDTA and sodium hypochlorite shows a concentration and time dependant erosion of root canal dentin.[7] Many studies have addressed the aggressive effect of 17% EDTA on canal walls causing erosion and decalcification of dentin at 20-30 µm depth within 5 min. This erosive behaviour of EDTA causes alteration of its mechanical properties resulting in more difficulties in adaptation of filling material.

Lately, a newer irrigant Chitosan used in 0.2% concentration, has attracted attention of dental research due to its biocompatibility, biodegradability, bioadhesion and lack of toxicity.[8] Chitosan is a natural polysaccharide obtained by deacetylation of chitin, which is found in crab and shrimp shells and has shown excellent chelating properties and antibacterial against gram positive, gram negative organisms and fungi.[9]

Studies have shown that 0.2% Chitosan irrigation has been as effective as 15% EDTA and 10% Citric acid in smear layer removal with less of toxic effect.[10]

Aldo Del Carpio et al showed that when chitosan was used as final irrigant it gives dual benefit of smear layer removal and inhibits bacterial recolonization on root dentin.[11]

Obturation of the root canal involves placement of gutta percha core in combination with a root canal sealer. An ideal root canal sealer should adhere firmly to both dentin and core filing materials to form a hermetic seal.[12]

AH Plus is an epoxy resin based sealer that provides better long term sealing ability compared to conventional sealers due to its reported expansion over time. It is biocompatible, radiopaque, has short setting time, low solubility and good flow characteristics. Nonetheless, this sealer exhibits some toxicity when freshly mixed which reduces after setting.[13] To overcome the problem of toxicity, new Calcium silicate sealers have been developed.

Apexit Plus is a non-shrinking, radiopaque root canal sealer containing calcium hydroxide. Apexit Plus is the further development of proven Apexit sealer and the only difference is it is supplied in a more convenient delivery form (Automix syringe) and has a more hydrophilic formulation. It has excellent tissue tolerance; therefore, a biological balance is re-established around the tooth after root canal treatment.[14]

A MTA-based sealer (MTA Fillapex) has been proposed as an endodontic sealer, which consists of MTA, salicylate resin, natural resin, bismuth oxide and silica. This material has attracted the researchers' attention due to its excellent biocompatibility, bioactivity and osteoconductivity.[15] MTA simultaneously releases free calcium ions to accelerate the healing process by stimulating the regeneration of the adjacent tissues. It has 0.1% solubility which provides better marginal seal.[16]

A study showed that MTA Fillapex sealer has suitable physiochemical properties such as good radiopacity, flow and alkaline pH.[17] Oliveira et al concluded

that MTA-based sealers reported the maximum leakage into the root canal walls over time when compared with AH Plus sealer[18]. Esin Ozlek has reported in his study that the canals irrigated with chitosan improves the dislocation resistance of MTA Fillapex when compared to EDTA and normal saline.[19] A recent study by Jesline Maria Jose has compared the effect of EDTA and Chitosan on MTA-Fillapex and bioRoot RCS and concluded that Chitosan along with NaOCl has a better debridement effect on root canal wall and bioRoot RCS showed higher bond strength values.[20]

Bond strength testing is a popular method for determining the effectiveness of adhesion between endodontic materials and tooth structure.[21] The push-out test measures the interfacial shear strength formed between different surfaces, thereby providing additional information about the evaluation of adhesion properties.[22]

The purpose of this test is to assess the extent to which the sealer and core material are bound together as a solid mass, as well as its bond strength to the root canal wall.

No study till date has been done to compare the push-out bond strength of AH sealer, Apexit Plus and MTA Fillapex after different irrigation regimens. Keeping these concepts in mind, this study aims to evaluate the effect of EDTA and chitosan on the push out bond strength of different sealers to radicular dentin.

AIM OF THE STUDY:

To evaluate and compare the effect of 17% EDTA and 0.2% Chitosan when used as a final rinse on the push out bond strength of three different root canal sealers to the radicular dentin.

OBJECTIVES:

1. To evaluate the effect of 17% EDTA and 0.2% Chitosan when used as final rinse on the push out bond strength of Epoxy resin based sealer, calcium hydroxide based sealer, MTA resin based sealer to radicular dentin using Universal Testing Machine.
2. To compare the effect of 17% EDTA and 0.2% Chitosan when used as final rinse on the push out bond strength of Epoxy resin based sealer, calcium hydroxide based sealer, MTA resin based sealer to radicular dentin using Universal testing machine.

HYPOTHESIS

Null Hypothesis

There is no difference of 17% EDTA and 0.2% Chitosan used as final flush on the push out bond strength of three different root canal sealers to radicular dentin.

Alternate hypothesis

There is a difference of 17% EDTA and 0.2% Chitosan used as final flush on the push out bond strength of three different root canal sealers to radicular dentin

REVIEW OF LITERATURE

1. An invitro study was done to investigate 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 decoronated, standardized to length of 15 mm and prepared with Protaper upto F3 rotary files. The samples were irrigated with sodium hypochlorite as initial rinse and divided into 4 experimental groups and 2 control groups (1-17% EDTA, 2- normal saline) depending on the type of final rinse solution used, that is, 2% Chitosan, 4% C-citrate, 10% CA, and 1% Acetic acid. The samples were then dehydrated, split buccolingually, and examined under field emission scanning electron microscope. The study concluded that group 4,5,3 i.e. 4% Chitosan citrate, 10% citric acid and 2% Chitosan as final rinse showed least amount of smear layer, debris and erosion at the apical, middle, and coronal one-third of root canal.

   M. Praveen, G. Aarthi,P.KMeenapriya Journal of pharmacy and bioallied sciences-2017

2. An invitro study was done to investigate the pushout bond strength of three different sealers such as MTA Fillapex, AH Plus and Apexit Plus. Forty-five freshly extracted maxillary central incisors were sectioned at CEJ to obtain a root length of 12 mm and then instrumented with ProTaper rotary instruments. The samples were irrigated with 5.25% NaOCl between instrumentation followed by 17% EDTA and final flush by saline. The samples were divided into three groups for obturation with respective sealers and then roots were sectioned horizontally into 3 sections – coronal, middle and apical of 3mm

thickness and subjected to push-out bond strength testing using UTM. The study concluded that AH Plus sealer showed significantly higher bond strength values than MTA Fillapex and Apexit plus sealer. There was no significant difference between MTAFillapex and Apexit plus however (p>0.05).

Deepak Kurup, Ajay kumarNagpal, Journal ofBioinformation- January 31 2021.

3. An invitro study was done to investigate the interaction between EDTA and Sodium hypochlorite: A nuclear magnetic Resonance analysis. This study has demonstrated that the use of EDTA as a final flush resulted into erosion of dentinal walls. The objective of this study was to verify through nuclear magnetic resonance (NMR) analysis if the oxidizing property of sodium hypochlorite inactivates EDTA. The study confirmed that the reaction between the sodium hypochlorite and EDTA lead to a very slow but progressive degradation of this compound.

   Nicola Maria Grande, GianlucaPlotino, JOE, MAY 2006.

4. An invitro study was done to investigate the smear layer removal after root canal final irrigation with 17% Ethylenediaminetetraacetic acid (EDTA), 10% citric acid (CA), Biopure MTAD, and 0.2% chitosan solutions. Fifty extracted maxillary central incisors were decoronated to a root length of 16 mm. They were instrumented using ProTaper system up to size F4 and 2.5% sodium hypochlorite (NaOCl) irrigation throughout instrumentation. The specimens were divided into 5 equal groups according to the final irrigation solution; Group I: 17% EDTA, Group II: 10% CA, Group III: MTAD, Group IV: 0.2% chitosan, Group V (control): 2.5% NaOCl. Samples were split longitudinally and examined under Scanning electron microscope for smear layer presence at

coronal, middle and apical root canal levels. 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 efficient in smear layer removal suggesting of supreme irrigant.

A M Daran, Tanta Dental Journal, 2014

5. A study was done to evaluate the effects on the adhesion of various root canal sealers after Er: YAG Laser and irrigants are used on the dentin surface. The teeth were sectioned and divided into 3 main groups by sealer (AH Plus, Endosequence and Real seal) and then divided into 5 subgroups by dentin treatment (distilled water, calcium hydroxide, sodium hypochlorite, EDTA, and Er.YAG laser). The specimens were then incubated and push-out bond strength was applied and 3 specimens from each group were examined under SEM. The result showed that the resin based root canal sealers had higher push-out bond strength than the bio-ceramic sealer.

   Ismail Ozkocak, DDS,PhD, and Bade Sonat, DDS,PhD,JOE-Volume 41, Number 8, August 2015

6. An invitro study was done to investigate the time dependant effects of EDTA on dentin surfaces. Six extracted single-rooted teeth were instrumented to #60. Apical and coronal thirds of each root were removed, leaving a 5 mm middle third that was then cut longitudinally into two equal segments. Using 10 ml of 17% EDTA solution, halves belonging to the same root were irrigated 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.

Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. Journal of endodontics. 2002 Jan 1; 28(1):17-9.

7. An invitro study was done to investigate the dentinal tubule penetration and push-out bond strength of AH 26, BioRoot RCS and MTA Plus root canal sealers in presence or absence of smear layer. The root canals of 90 extracted mandibular premolars were divided into 2 groups: smear layer preserved and smear layer removed and then the roots were further divided into 3 subgroups according to the sealer used i.e. AH Plus, BioRoot RCS and MTA plus. The specimens were then obturated with gutta percha and the sealer was mixed with 0.1% Rhodamine B. The specimens were sectioned from the middle third in 1mm slices and the push out test was done and the remaining slice was used to calculate the dentinal tubule penetration. The study concluded that the retention of MTA Plus and BioRoot RCS was higher than AH 26 when smear layer preserved and BioRoot RCS showed lowest penetration when smear layer was removed.

   SevincAktemurTurker, EmelUzunoglu

   Journal of dental research vol 12, 4th December 2018

8. An invitro study was done to investigate the effect of different chelating agents 17% EDTA and 0.2% Chitosan on the push out bond strength of MTA Fillapex and bioRoot RCS . Sixty premolars were divided into three groups and each group was then subdivided into A and B. Group 1A: 17% EDTA + 5.25% NaOCl with MF sealer (n = 10); Group 1B: 17% EDTA + 5.25% NaOCl with BRCS sealer (n = 10); Group 2A: 0.2% Chitosan + 5.25% NaOCl

with MF sealer (*n* = 10); Group 2B: 0.2% Chitosan + 5.25%NaOCl with BRCS sealer (*n* = 10); Group 3A: 5.25% NaOCl + MF sealer (*n* = 10); and Group 3B: 5.25% NaOCl + BRCS sealer (*n* = 10). After obturation, they were sectioned horizontally into 1.5 mm thick and then POBS was calculated using a universal testing machine. The study reported that the bond strength of BRCS was higher when the root canal was irrigated with 0.2% Chitosan + 5.25% NaOCl whereas MTA Fillapex has shown lesser bond strength values.

Jose JM, Krishna EM, Journal of Conservative Dentistry: JCD. 2021 Mar; 24(2):195.

9. An invitro study was done to investigate the push out bond strength of three different sealers: Mineral trioxide aggregate (MTA)-based sealer (MTA Fillapex), An epoxy resin-based sealer (AH Plus), and a zinc oxide eugenol-based sealer (EndoFill) Thirty extracted single-root human teeth were prepared using #3 and #2 Gates Glidden drills in the cervical portion of the canal and K3(r) rotary instruments to a size #25/0.06 to working length. The root canals were irrigated with 0.5 mL 2% chlorhexidine gel before and 1 mL saline after each instrument. The smear layer was removed with 3 mL 17% EDTA for 3 min and then the samples were sectioned horizontally into eight sections of 1±0.1 mm-thick serial slices. The push-out test was carried out using UTM and it was concluded that AH Plus sealer showed significantly higher bond strength while MTA Fillapex showed the least.

   Gurgel-Filho ED, Leite FM. Brazilian Journal of Oral Sciences. 2014 Apr; 13:114-7.

10. A systematic review was done to investigate the influence of use of chelating agents as final irrigant on the push-out bond strength of epoxy resin-based root canal sealers. The study included irrigants 17% EDTA,Peracetic acid (0.5% and 1%), 0.2% Chitosan, Etidronic acid in concentration of 0.9 and 18%, QMix, Smearclear,7% Maleic acid and their effect on push out bond strength of an epoxy resin based sealer. The study revealed that most of the final irrigation protocols with chelating agents had a positive impact and promoted an improvement in the dislodgment resistance of the epoxy resin-based sealers to the root dentin.

    Carla M. Augusto,Australian Endodontic Journal, 2021

11. An invitro study was done to evaluate the push-out bond strength of polydimethylsiloxane endodontic sealers to dentin. Thirty root slices of 1±0.1 mm thick were prepared from the middle third of 10 mandibular teeth. Three holes of 0.8 mm were prepared on each root slice. Standardized irrigation was done followed by drying the canals with paper point. The three holes in each root slice were filled with three root canal sealers: AH Plus, GuttaFlow 2 and GuttaFlow Bioseal. The push out strength was evaluated for each root canal sealer on a root slice and it as concluded that AH Plus has significantly superior bond strength than GuttaFlow 2 and GuttaFlow Bioseal.

Dem K, Wu Y, Kaminga AC, Dai Z, Cao X, Zhu B. The push out bond strength of polydimethylsiloxane endodontic sealers to dentin. BMC oral health. 2019 Dec; 19(1):1-6.

12. A Nuclear magnetic resonance analysis was done to investigate the interaction between EDTA and sodium Hypochlorite. Erosion of dentinal walls following the use of EDTA as a final flush has been reported by recent studies. The objective of this study was to verify through NMR if the oxidizing property of sodium hypochlorite inactivates EDTA and it was reported that the reaction between these NaOCl and EDTA lead to a very slow but progressive degradation of EDTA.

Grande NM, Plotino G, Falanga A, Pomponi M, Somma F. Interaction between EDTA and sodium hypochlorite: a nuclear magnetic resonance analysis. Journal of endodontics. 2006 May 1;32(5):460-4.

13. A contempory overview of endodontic irrigants – a review

This article reviews the potential irrigants with their advantages and limitations with their future in endodontic irrigation. A clinical irrigating regimen is proposed based on the actions and interactions of currently available solutions. Furthermore, some technical aspects of irrigating the root canal system are discussed, and recent trends are critically inspected.

Agrawal Vineet S, Rajesh M, Sonali K, Mukesh P. A contemporary overview of endodontic irrigants–A review. J Dent App. 2014 Oct; 1(6):105-5.

14. An invitro study was done to investigate the effect of final irrigation protocols on the push-out bond strength of an epoxy resin based sealer to dentin. Eighty single-rooted anterior teeth were used. The root canals were partially prepared using a rotary system and the final diameter was standardised using a #5 Gates-Glidden drill prior to the push-out bond test. The teeth were irrigated with 5.25% NaOCl or 2% CHX gel. For smear layer removal, 17% ethylenediaminetetraacetic acid (EDTA) or QMix 2 in 1 was applied for 3

min. As final irrigant, 1 mL of NaOCl, CHX solution or distilled water was used. It was observed that the group which were irrigated with NaOCl/EDTA/NaOCl showed significantly higher bond strength than other groups. The study concluded that 5.25% NaOCl proved to be the best solution for the final irrigation when combined with EDTA.

Leal F, Simão RA, Fidel SR, Fidel RA, do Prado M. Effect of final irrigation protocols on push-out bond strength of an epoxy resin root canal sealer to dentin. Australian Endodontic Journal. 2015 Dec;41(3):135-9.

15. An invitro study was done to compare the effect of smear layer on the penetration depth and push-out bond strength of AH 26, BioRoot RCS, and MTA Plus root canal sealers. Ninety extracted human mandibular premolars were divided into 2 groups: smear layer preserved and smear layer removed and the roots were further divided into 3 subgroups according to the sealer: AH 26, BioRoot RCS and MTA Plus. The 1mm thick slices were obtained from the middle third of each root and evaluated for push-out bond strength. The study concluded that bond strength was significantly affected by the sealer type and smear layer removal/ preservation. BioRoot RCS showed higher bond strength compared to MTA Plus and AH 26 when smear layer was removed.

    Türker SA, Uzunoğlu E, Purali N. Evaluation of dentinal tubule penetration depth and push-out bond strength of AH 26, BioRoot RCS, and MTA Plus root canal sealers in presence or absence of smear layer. Journal of dental research, dental clinics, dental prospects. 2018;12(4):294.

16. An invitro study was done to investigate the shear bond strength of two sealers and assess the failure after displacement. Forty mandibular premolars were selected and instrumented using rotary and then divided into two groups according to the sealer- Group 1 MTA Fillapex, Group 2- AH Plus sealer. The roots were filled with sealer without gutta-percha and sectioned into 1 mm thick slices inorder to evaluate the push-out bond strength. The results concluded that AH Plus sealer had higher bond strength than MTA Fillapex with less resin tag formation.

    Baechtold MS, Mazaro AF, Crozeta BM, Leonardi DP, Tomazinho FS, Baratto-Filho F, Haragushiku GA. Adhesion and formation of tags from MTA Fillapex compared with AH Plus® cement. RSBO RevistaSul-Brasileira de Odontologia. 2014; 11(1):71-6.

MATERIALS AND METHODS

SOURCE OF DATA

- The study was carried out in the Department of Conservative Dentistry and Endodontics, V.K Institute of Dental sciences, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka.
- Chitosan (0.2%) irrigant was prepared at KLE University's College of Pharmacy (KAHER), Belagavi, Karnataka.
- Push out bond strength testing was carried out at K.L.E Society's Dr. M.S. Sheshgiri College of Engineering and Technology, Belagavi, Karnataka.

SAMPLE SIZE ESTIMATION:

The following formula helps in calculating the minimum sample size which is required to know the adequate proportion of sample along with confidence level and the margin error.

Here, n= Sample size

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

d = Mean difference

S1 = 1.28

S2 =1.15 ; d=0.97

Z_α=1.96 at 5% α error

Z_β= 1.282 at 90% power

$S = \frac{S1+S2}{2} = 1.215$

$n = \frac{2S^2 (Z_\alpha + Z_\beta)^2}{d^2}$ = 33in each group

There are two main groups, so the total sample size N=66.

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

INCLUSION CRITERIA

- Extracted human single canal single rooted mandibular premolar teeth with straight patent canals.

EXCLUSION CRITERIA:

- Carious teeth.
- 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

- 66 single root mandibular premolar teeth
- 0.1% Thymol solution (S D FINE-CHEMICALS LIMITED, MUMBAI)
- 3% Sodium hypochlorite (Thermo Fischer SCIENTIFIC)
- 17% Ethylenediaminetetracetic Acid (EDTA) [GLIDE]
- AH Plus sealer (DENTSPLY, Germany)
- Apexit Plus sealer(Ivoclar Vivadent)
- MTA Fillapex sealer (Angelus dental)
- 1% Acetic acid (S.M. CORPORATION, Gujarat)

- Chitosan (SIGMA-ALDRICH, BANGALORE)
- Paper points (DIADENT GROUP INTERNATIONAL, KOREA)
- 5ml 27-gauge syringe (DISPOVAN, HINDUSTAN SYRINGES LTD FARIDABAD)

ARMAMENTARIUM USED IN THE STUDY.

- Micromotor(NSK, JAPAN)
- Gates Glidden Drills (DENSTPLY SIRONA,USA)
- Carborundum disc(KWALITY DIAMOND TOOLS, Mumbai)
- Cold cure acrylic resin(DENTAL PRODUCTS OF INDIA)
- Mixing pad
- Agate spatula
- 5ml 27-gauge syringe (DISPOVAN, HINDUSTAN SYRINGES LTD FARIDABAD)
- Incubator (BIO TECHNICS, India)
- Universal testing machine (ENKAY ENTERPRISES, New Delhi)

Preparation of Study materials:

Preparation 0f 0.2% chitosan irrigant

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

METHODOLOGY: -

Sixty six freshly extracted human mandibular premolar teeth were selected based on the selection criteria and handled according to OSHA guidelines. Calculus and soft tissue debris was removed with an ultrasonic scaler and immersed in 0.1% thymol solution till use.

The teeth were decoronated and then transversely sectioned 4mm and 8mm below the cementoenamel junction to obtain 2 sections each with the help of a diamond disc under copious water spray to obtain 132 specimens. The root canal of each specimen was prepared using no.3 Gates Glidden drill and then the specimens were divided into two groups depending on the irrigant used as final flush.

Group 1: 3% Sodium hypochlorite + 17% EDTA (n=66)

Initial irrigation was done with 5ml of 3% sodium hypochlorite for 1 minute and final irrigation was done with 5 ml of 17% EDTA for 2 minutes

Group 2: 3% Sodium hypochlorite + 0.2% Chitosan. (n=66)

Initial irrigation was done with 5ml of 3% sodium hypochlorite for 1 minute and final irrigation was done with 5 ml of 0.2% Chitosan for 2 minutes.
The canals were then dried with sterile absorbent paper points.

Specimens in Group 1 and Group 2 were further subdivided into three sub-groups according to the sealer to be placed.

Subgroup A: Epoxy resin-based sealer- AH Plus. (n=22)

Subgroup B: Calcium Hydroxide based sealer- Apexit Plus. (n=22)

Subgroup C: MTA resin-based sealer- MTA Fillapex. (n=22)

The sealers were mixed according to the manufacturer's instructions and filled into the root canals. The sealer was allowed to set by placing the specimens in an incubator at 37º C and 100% humidity for a week to simulate clinical conditions.

Push out bond strength assessment:-

The 4 mm thick root specimens were centered inside the aluminium rings and embedded in the acrylic resin. A push out force was applied with a cylindrical piston measuring 0.8mm in diameter at a cross head speed of 1mm/min. perpendicular to the sample surface using the universal testing machine. The maximum failure load was recorded in Newton's and then converted into MPa by using the formula given below

Calculation of push-out strength:

$$\textbf{Push –out bond strength (MPa)} = \frac{\text{MAXIMUM LOAD FOR DISLODGEMENT}}{\text{SURFACE AREA}}$$

STATISTICAL ANALYSIS:

The data was statistically analysed by-.

- Two-way ANOVA test for intra-group pairwise comparison.
- Tukeys post-hoc test for inter-group pairwise comparison.

STUDY DESIGN

Sixty-six extracted human mandibular premolar teeth were collected 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

The teeth were sectioned transversely at the level of 4mm and 8mm below the cementoenamel junction to obtain 132 specimens.

Root canal of each specimen were prepared using no. 3 Gates Glidden drill and divided into two groups based on the irrigants for final flush as follows:

Group 1:

3% NaOCl + 17% EDTA.

(n=66)

Group 2:

3% NaOCl+ 0.2% Chitosan

(n=66)

In both the groups, 5 ml of 3% NaOCl will be used as an initial rinse for 1 min followed by 5ml of irrigant (EDTA/Chitosan) as a final rinse for 2 min. The canals will then be dried with paper points.

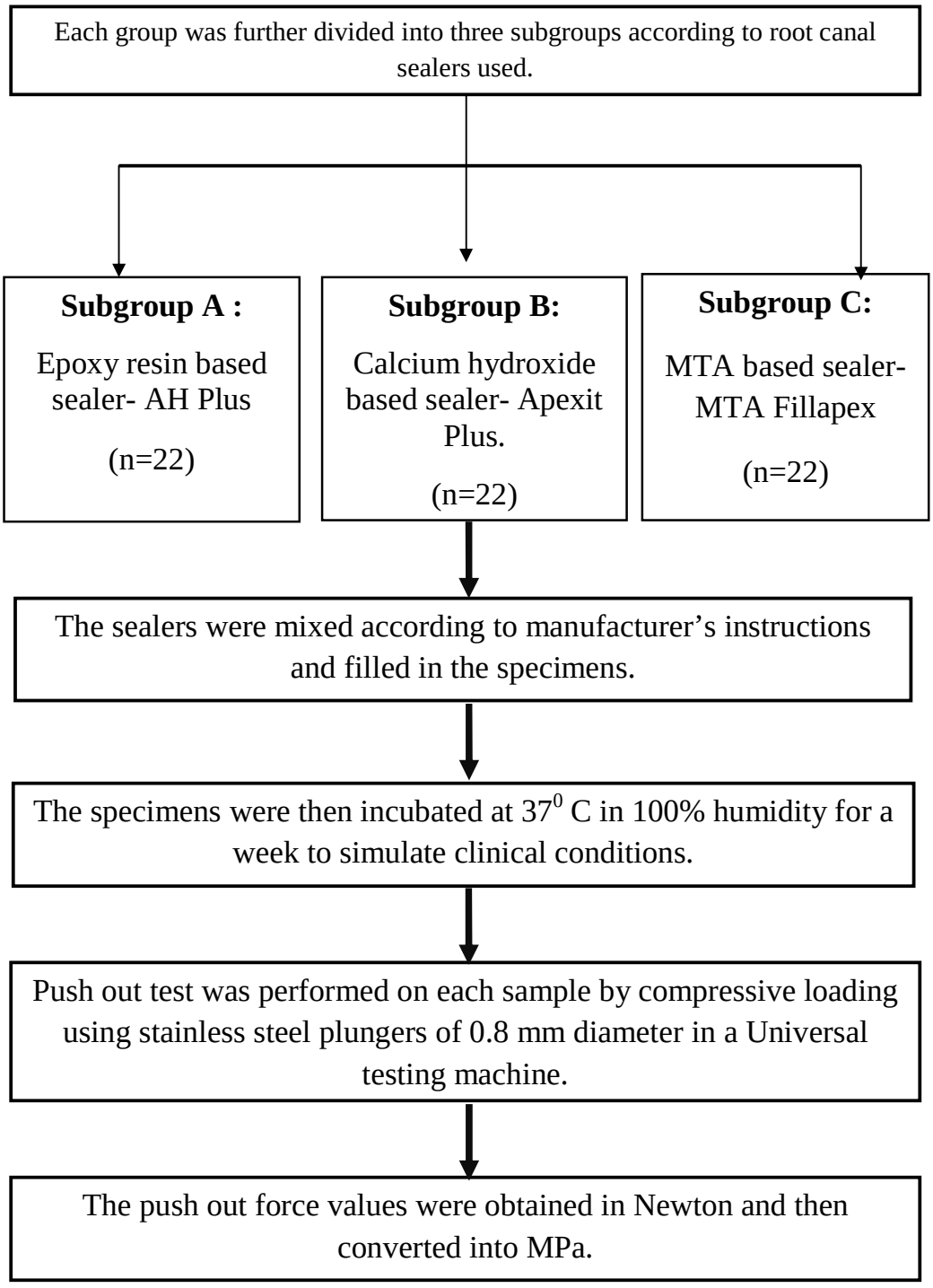

Each group was further divided into three subgroups according to root canal sealers used.
Subgroup A :
Epoxy resin based sealer- AH Plus
(n=22)
Subgroup B:
Calcium hydroxide based sealer- Apexit Plus.
(n=22)
Subgroup C:
MTA based sealer- MTA Fillapex
(n=22)
The sealers were mixed according to manufacturer's instructions and filled in the specimens.
The specimens were then incubated at 37^0 C in 100% humidity for a week to simulate clinical conditions.
Push out test was performed on each sample by compressive loading using stainless steel plungers of 0.8 mm diameter in a Universal testing machine.
The push out force values were obtained in Newton and then converted into MPa.

Fig 1: Total Sample Size (n=66)

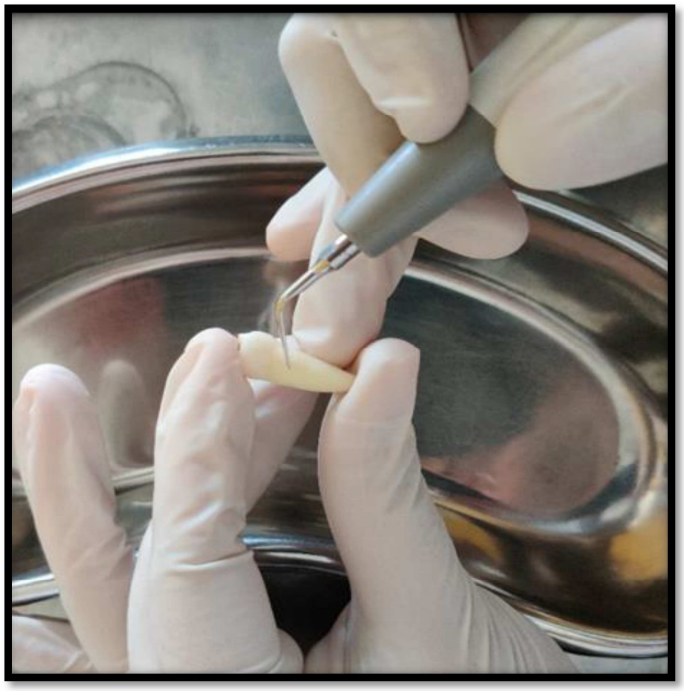


Fig 2: Ultrasonic scaling to remove debris and calculus

MATERIALS AND ARMAMENTARIUM

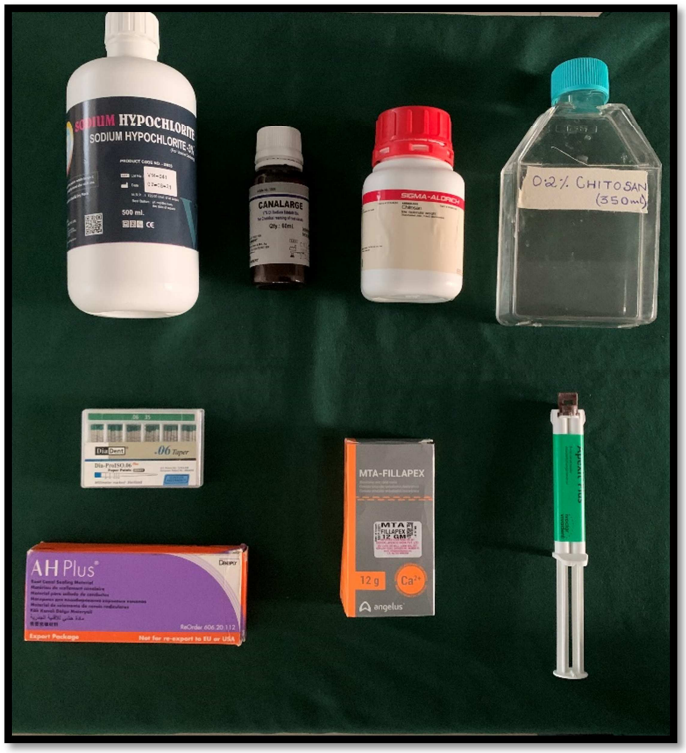


Fig 3: Materials used for the study.

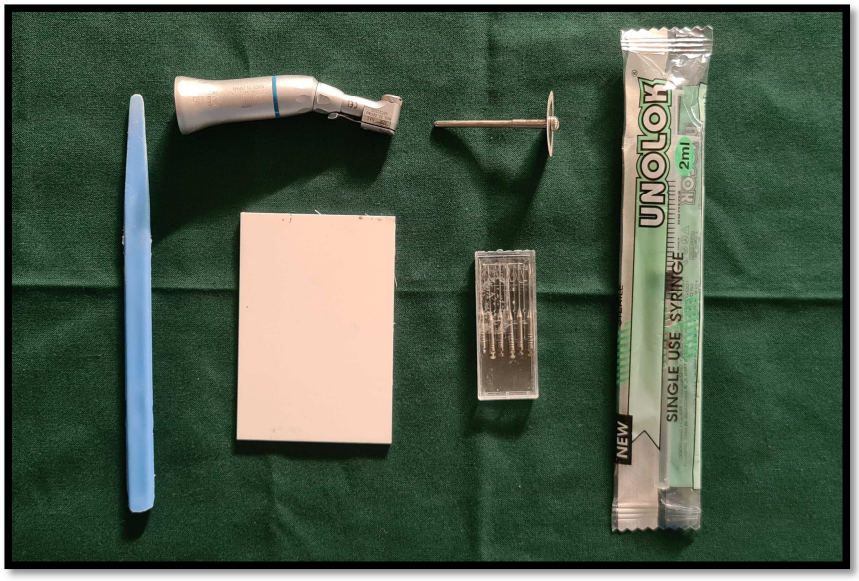


Fig 4: Instruments used for preparation of the sections.

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

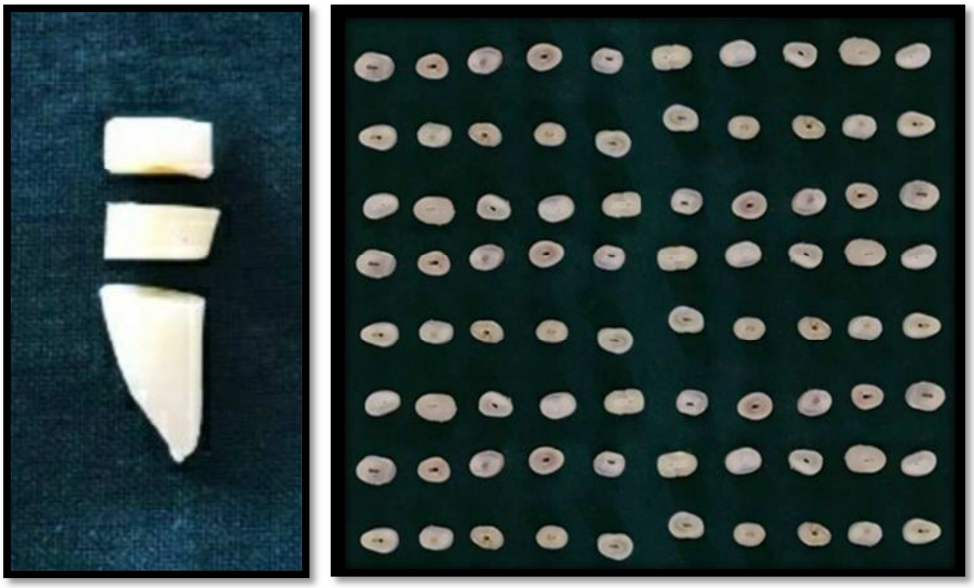


FIG 6:A} Samples sectioned 4mm and 8mm below the CEJ to obtain 4 mm thick dentin disc B} Dentin discs .

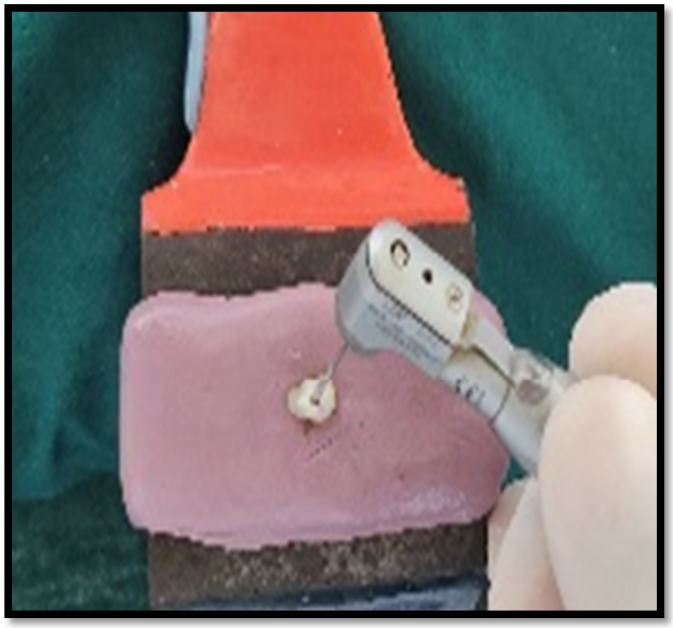


FIG 7: Preparation of canals using Gates Glidden drills

PREPARATION OF 2% CHITOSAN SOLUTION

Fig 8 :0.2 g of Chitosan powder weighed by placing it on top of filter paper

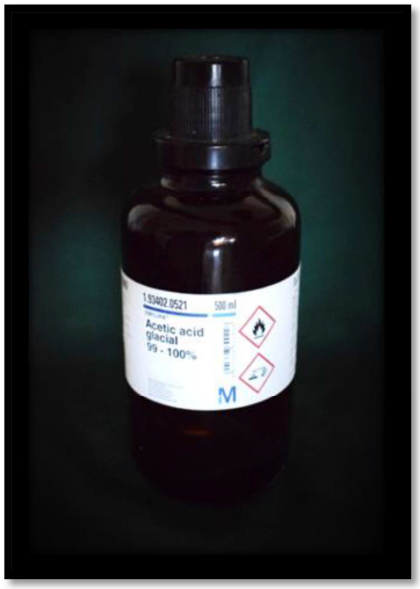


Fig 9: Acetic Acid used to dissolve Chitosan powder

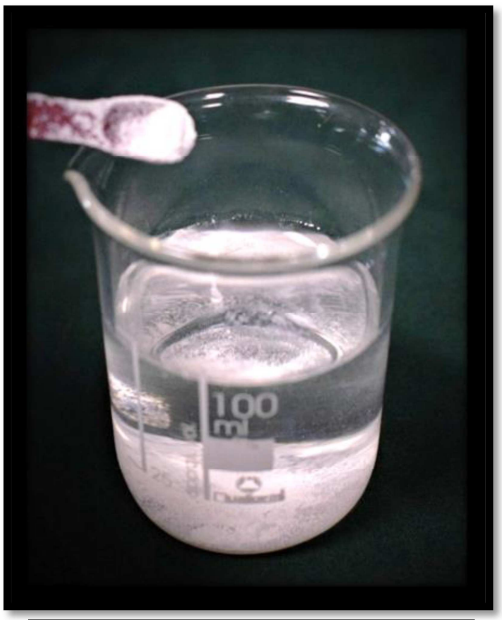


Fig 10: 0.2 g Chitosan Powder dispensed into 100 ml Acetic acid

Fig 11:Magnetic Stirrer used to get a 0.2% Chitosan solution.

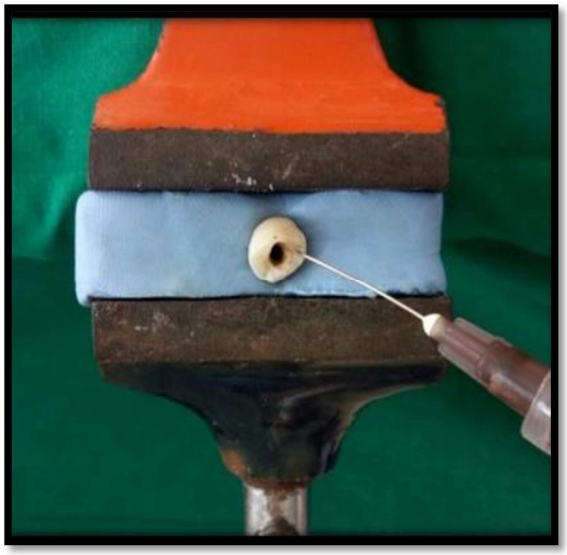


Fig 12. : Irrigation of sections using 17% EDTA /0.2% Chitosan in respective groups.

Fig 13: Manipulation of sealer as per Manufacturer's Instructions

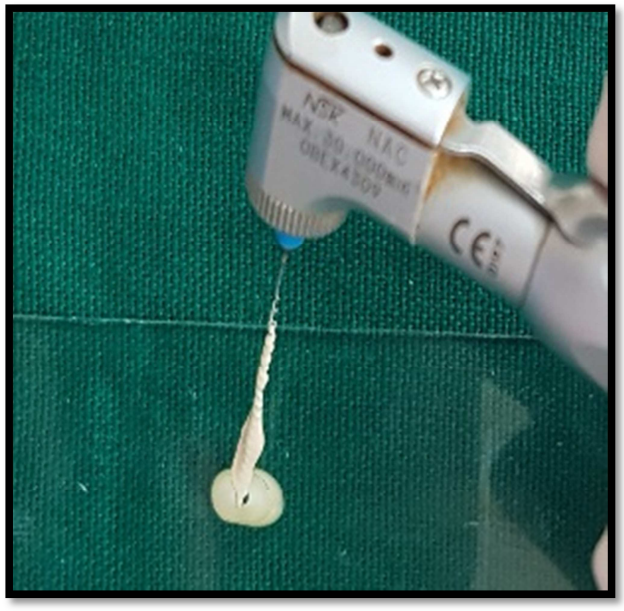


Fig 14: Placement of sealer in sections using lentulospiral.

Fig 15: Storage of dentin discs in incubator at 100% humidity and 37^0C.

Fig 16: Pushout bond strength testing using a Universal testing machine at a cross head speed of 1mm/min

RESULTS

Table 1: Summary of Mean push out bond strength in two irrigants (EDTA and Chitosan) and three sealers (AH plus, Apexit plus and MTA Fillapex)

Irrigants	Sealers	n	Mean	SD	SE	CV
EDTA	AH Plus	22	3.08	0.26	0.05	8.36
	Apexit Plus	22	2.38	0.38	0.08	15.94
	MTA Fillapex	22	2.73	0.30	0.06	10.89
Chitosan	AH Plus	22	3.13	0.37	0.08	11.70
	Apexit Plus	22	2.71	0.25	0.05	9.39
	MTA Fillapex	22	2.96	0.27	0.06	8.98

The push-out bond strength of AH Plus sealer when the root canals irrigated with Chitosan showed highest push-out bond strength with mean value of 3.13±0.37 followed by MTA Fillapex (2.96±0.27) and least with Apexit Plus sealer (2.71±0.05). However, when EDTA used as final flush, all the sealers showed lower bond strength values, AH Plus sealer with mean value (3.08± 0.26) followed by MTA Fillapex (2.73 ±0.30) and least was seen with Apexit Plus (2.38±0.38).

Table 2: Comparison of two irrigants (EDTA and Chitosan) and three sealers (AH plus, Apexit plus and MTA Fillapex) with mean push out bond strength by two way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Main effects					
Irrigants	1	1.3774	1.3774	14.5662	0.0002*
Sealers	2	6.8400	3.4200	36.1667	0.0001*
2-way interaction effects					
Irrigants x Sealers	2	0.4273	0.2136	2.2591	0.1087
Error	126	11.9148	0.0946		
Total	131	20.5595			

*$p<0.05$

Table 2 reports a statistical difference between both the main groups of irrigants with p value (0.0002) and all the three sealers with p value (0.0001).

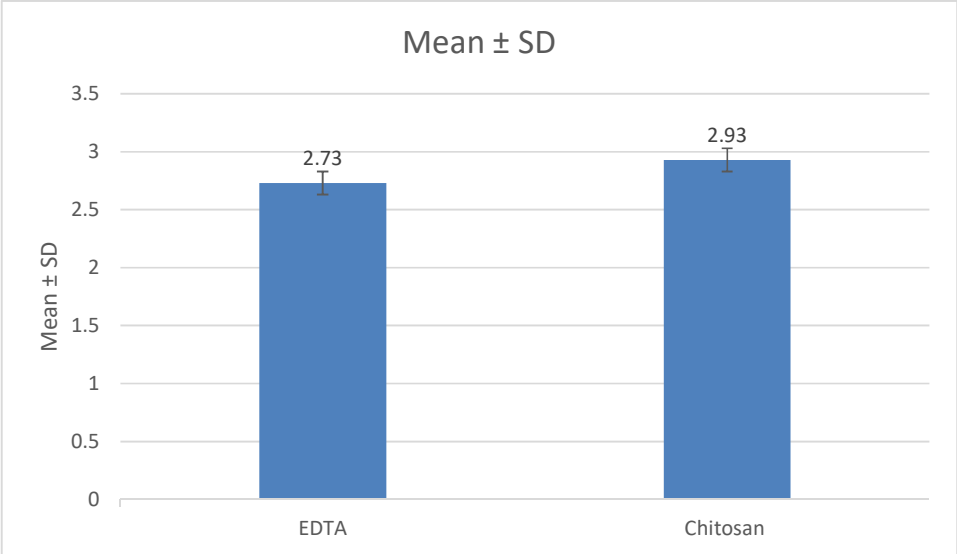
However, the 2-way interaction between the irrigants and sealers showed no significant difference.

Table 3: Comparison of two irrigants (EDTA and Chitosan) with mean push out bond strength by Tukeys multiple posthoc procedures

Irrigants	EDTA	Chitosan
Mean	2.73	2.93
SD	0.42	0.34
Chitosan	P=0.0001*	-
EDTA	-	P=0.0001*

*p<0.05

Figure 1: Graph Comparing the mean push out bond strength of the two irrigants (Chitosan and EDTA) when used as a final flush



The table 3 and figure 1 shows inter-pairwise comparison of Chitosan and EDTA using Tukeys post hoc test, which revealed statistically significant high bond strength values with Chitosan as compared to EDTA.

Table 4: Comparison of three sealers (AH plus, Apexit plus and MTA Fillapex) with mean push out bond strength by Tukeys multiple posthoc procedures.

Sealers	AH plus	Apexit plus	MTA Fillapex
Mean	3.10	2.55	2.84
SD	0.31	0.36	0.30
AH plus	-		
Apexit plus	P=0.0001*	-	P=0.0001*
MTA Fillapex	P=0.0002*		-

Figure 2- Shows the Comparison of three sealers (AH plus, MTA Fillapex and Apexit plus) with mean push out bond strength and indicates that AH Plus shows statistically significant highest bond strength followed by MTA Fillapex and least with Apexit Plus.

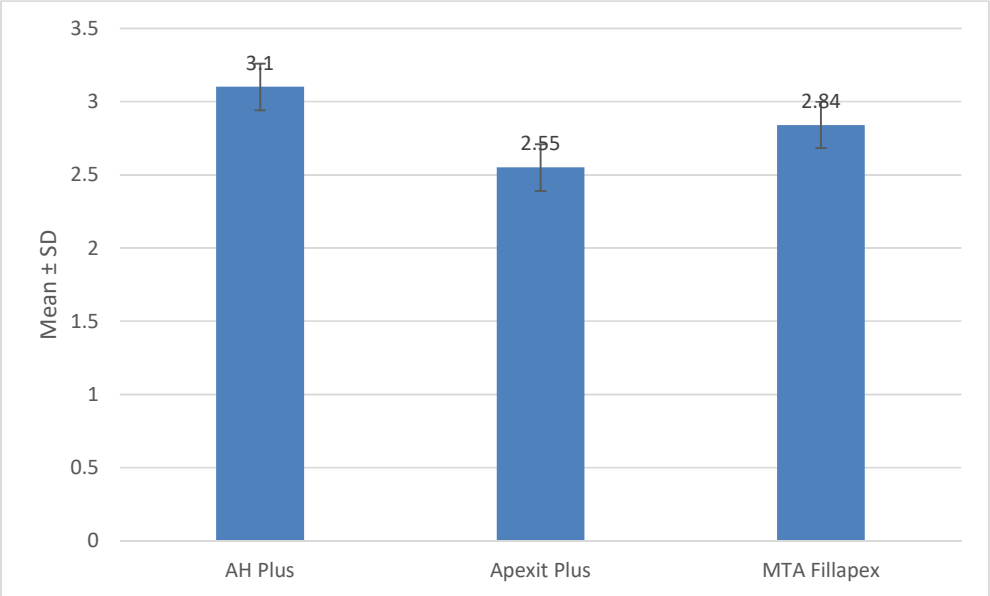


Table 5: Pair wise comparison of two irrigants (Chitosan and EDTA) and three sealers (AH plus, MTA Fillapex and Apexit plus) with mean push out bond strength by Tukeys multiple posthoc procedures.

Groups with side	EDTA and AH plus	EDTA and Apexit Plus	EDTA And MTA Fillapex	Chitosan and AH plus	Chitosan and Apexit Plus	Chitosan and MTA Fillapex
Mean	3.08	2.38	2.73	3.13	2.71	2.96
SD	0.26	0.38	0.30	0.37	0.25	0.27
EDTA and AH plus	-			p=0.9921	p=0.0012*	p=0.7916
EDTA and apexit plus	p=0.0001*	-	p=0.0026*	p=0.0001*	p=0.0051*	p=0.0001*
Edta And MTA Fillapex	p=0.0024*		-	p=0.0002*	P=1.0000	p=0.1325
Chitosan and AH plus				-		
Chitosan and Apexit plus				p=0.0001*	-	p=0.0851
Chitosan and MTA Fillapex				p=0.4191		-

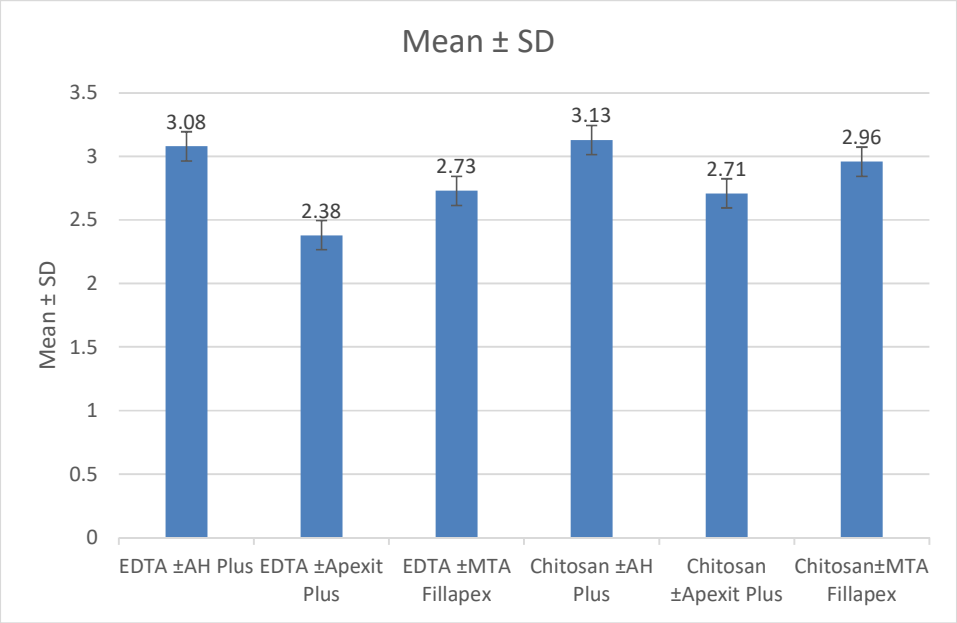
*p<0.05

The mean difference between all the sealers is statistically significant (p<0.05)

Difference between subgroup 1A and 1B; 1B and 1 C is significant.

Similarly the difference between 2A and 2B ; 2B and 2C is also significant.

Figure 3: Pair wise comparison of two irrigants (Chitosan and EDTA) and three sealers (AH plus, MTA Fillapex and Apexit plus) with mean push out bond strength.



The following figure shows comparison of three sealers (AH plus, MTA Fillapex and Apexit plus) in each of the two irrigants (Chitosan and EDTA) with mean push out bond strength.

A significant difference was seen in subgroups A, B and C shown in the table 4 and figure 2. The root canals when irrigated with 0.2% Chitosan irrigant as final flush showed higher bond strength values in all the sealers among which AH Plus sealer showed highest bond strength, followed by MTA Fillapex. The root canals when irrigated with 17% EDTA showed lower bond strength values in all the sealers than Chitosan and Apexit Plus showed the least bond strength values.

DISCUSSION

A sturdy and long lasting link between the root canal wall and the root canal core filling material is established by the endodontic sealer. This is considered as one of the important aspect in the prevention of root canal infection caused either by regrowth of microorganism or new infection due to coronal or apical leakage[23]. A bacteria tight seal of the root canal established by the endodontic sealer is therefore a major aspect when evaluating the properties of different sealers.[24]

The factors which affect the adhesion of sealer to dentin include the smear layer produced during chemomechnical preparation of the root canals, nature of root dentin in specific tooth, chemical composition of irrigants and sealer; and their interaction with dentin.[25] Surface treatment of dentin with different irrigating solutions affect the chemical and structural architecture of dentin, changing the permeability and solubility of dentin, thereby affecting the adhesion of filling materials to dentin surfaces.[26]

A study by Sevinc Türker et al suggested that removal of smear layer significantly affects the adhesion of filling material as the layer prevents intimate contact between the sealer and dentin walls.[27] There are various methods to remove the smear layer which includes chemical, ultrasonic, and laser, none of which is very effective or has received universal acceptance.[28,29]

Chelating agents are most commonly used irrigant in endodontics in order to remove smear layer. These agents combine with calcium ions of tooth and decalcify the dentin[31].

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.[3] Studies have shown that efficiency of chelating agent depends on several factors such as application time, pH, concentration and volume of the solution.[29] 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.[29,32] EDTA therefore imparts a negative influence on the hydration properties of cement because of its acidic nature, thereby inhibiting adhesion to materials.[33]

Because of this recognized limitation of this irrigant, newer irrigant such as Chitosan remains an area of great interest.

The present study focused on the effect of use of two chelating agents 17% EDTA and 0.2% Chitosan as final flush on the bond strength of sealers. 0.2 % Chitosan solution (Group 2=2.93±0.34) when used as final flush showed significantly higher bond strength of all sealers when compared with 17% EDTA (Group 1=2.73±0.42) as shown in Table 3 and Figure 1. Chitosan is hydrophilic in nature which favours intimate contact with dentin and removes smear layer by the formation of complexes with metal ions due to adsorption, ionic exchange, and chelation and is responsible for the elimination of dentin calcium ions.[34] The higher values observed with Chitosan could be attributed to these properties of Chitosan.

Mathew et al also concluded that Chitosan is an effective chelating agent and can be considered as a less invasive alternative to 17% EDTA.[35]

Push-out bond strength, also known as dislodgement resistance is regarded as a relevant factor to evaluate the bonding of sealer to canal wall and filling material. This test is usually preferred over conventional tests such as micro tensile or shear bond strength because the fracture occurs parallel at dentin-bonding interface. Also this method is less sensitive to small variations of stress distribution during load application. The most crucial step of this test is the ratio of pin diameter and the specimens' diameter[36,37].

Chen et al suggested that the plunger tip of universal testing machine for bond strength evaluation should be 0.85 times smaller than the size of filling material.[34] A ratio of less than 0.6 was reported to influence the push-out bond strength. In the present study the pin diameter used was 0.8 mm while the specimen was of 0.9 mm in diameter.[36] Both the diameters were designed to be within this range. All the specimens were prepared to no. 3 Gates Glidden drill in order to obtain the same root canal diameter and standardization. The sealers were mixed according to manufacturer's instructions and placed in the specimens. The sealer was vibrated gently with lentulospiral so as to avoid bubble formation and then filled with hand pluggers.

AH Plus is an epoxy resin based sealer that provides better long term sealing ability compared to conventional sealers due to its reported expansion over time. It is biocompatible, radiopaque, has short setting time, low solubility and good flow characteristics.[36] Nonetheless this sealer exhibit some toxicity when freshly mixed which reduces after setting. To overcome the problem of toxicity, new Calcium silicate sealers have been developed.[37]

Apexit Plus is a non-shrinking, radiopaque root canal sealer containing calcium hydroxide.[38] Apexit Plus is the further development of proven Apexit sealer and the only difference is it is supplied in a more convenient delivery form (Automix syringe) and has a more hydrophilic formulation. It has excellent tissue tolerance; therefore, a biological balance is re-established around the tooth after root canal treatment.[39]

A MTA-based sealer (MTA Fillapex) has been proposed as an endodontic sealer, which consists of MTA, salicylate resin, natural resin, bismuth oxide and silica. This material has attracted the researchers' attention due to its excellent biocompatibility, bioactivity and osteo-conductivity.[40]MTA simultaneously releases free calcium ions to accelerate the healing process by stimulating the regeneration of the adjacent tissues.[41] It has 0.1% solubility which provides better marginal seal.

In this present study, AH Plus sealer (3.10±0.31) exhibited statistically significant better results than MTA Fillapex (2.84± 0.30) and Apexit Plus (2.55±0.36) shown in Table 5 and Figure 2. Several studies compared the bond strength of epoxy resin based sealer with newer endodontic sealer and concluded that AH Plus sealer had highest bond strength similar to the observations of the present study.[13, 26,42,45,47]

The highest bond strength values obtained by AH Plus sealer in both the groups could be related to its capacity of creating a covalent bonding with an opened epoxy ring to any amine group exposed in collagen , giving long-term dimensional stability and low polymerization tension. The chemical composition of MTA- based cement could also influence on its bonding capacity[45]. A recent study discovered that the rationale behind the low bond strength of MTA Fillapex is its low bonding

capacity to dentinal tubules because of the formation of apatite by MTA, over its own surface, thus creating a similar structure that is different from the tag which prevents its leakage[45]. Sagsen et al stated that AH Plus exhibited longer and uniform tags, showing its higher mechanical imbrication and resulting in greater bonding capacity while MTA Fillapex displayed little or none formation of tags.[43]

Apexit Plus sealer which is a calcium hydroxide based sealer provides excellent tissue tolerance and convenient application because of the automix syringe. U Salz et al reported the sealing ability of Apexit Plus sealer is better than AH Plus because of the low solubility (0.03) of AH Plus than Apexit Plus (0.05%) which resulted in less bacterial leakage.[44]

However calcium hydroxide based sealer might tear down leaving behind obturation voids which results in leakage. Also these sealers have poor cohesive strength which might be the reason why these sealers have shown poor bond strength values. A study by Deepak Kurup et al also evaluated push out bond strength of dentin sealer interface for AH Plus sealer, MTA Fillapex and Apexit Plus and it was reported that epoxy resin based AH Plus sealer showed highest bond strength followed by MTA Fillapex and Apexit Plus which is similar to our study[47]. However the difference between the MTA Fillapex and Apexit Plus was not statistically significant.

A study showed that MTA Fillapex sealer has suitable physiochemical properties such as good radiopacity, flow and alkaline pH. However Oliveira et al concluded that MTA-based sealers showed increase in the leakage into the root canal walls over time when compared with AH Plus.[18] Esin Ozlek has reported in his study that the canals irrigated with Chitosan improves the dislocation resistance of MTA

Fillapex when compared to EDTA and normal saline.[49] A recent study by Jesline Maria Jose has compared the effect of EDTA and Chitosan on MTA-fillapex and bioRoot RCS and concluded that Chitosan along with NaOCl has a better debridement effect on root wall and bioRoot RCS showed higher bond strength values[50].

Chitosan has shown a positive effect on the push-out bond strength of all the three sealers, among which AH Plus (3.13±0.37) sealer has highest bond strength whereas Apexit Plus (2.38±0.38) showed lowest bond strength when flushed with 17% EDTA shown in Table 1 and figure 3.

Therefore the null hypothesis that there is no effect of 17% EDTA and 0.2% Chitosan on the push-out bond strength of three different sealers to radicular dentin is rejected.

CONCLUSION

Under the limitations of study, it can be concluded that:

1. Chitosan performed better than EDTA when used as a final irrigant in terms of push-out bond strength of all the three sealers to radicular dentin.
2. AH Plus sealer showed highest bond strength when irrigated with 0.2% chitosan as final flush.
3. MTA Fillapex produced better push-out bond strength values as compared to Apexit Plus sealer but lower than AH Plus sealer, irrespective of the irrigant used as final flush.

SUMMARY

Successful endodontic treatment requires adequate shaping and cleaning and fluid tight seal of the root canal space. The root canal system has highly complex and variable anatomy which have limited our ability to clean and disinfect it predictably. Difficulties occur to remove the organic tissue and disinfect the canal properly in areas such as isthmus, canal fins and cul-de-sac, large areas in the oval and flat canals which may remain untouched despite careful instrumentation. Therefore much focus is given on endodontic irrigant as to impact those areas and obtain thorough decontamination, as it is rightly said, *"What You Take Out Of Root Canal May Be More Important Than What You Put In It"*

Sodium hypochlorite is the most commomly used irrigant in concentration of 0.5%- 6% in order to remove the organic tissue. During biomechanical preparation smear layer is produced which blocks the dentinal tubules thereby inhibiting the proper adhesion of sealer to dentin walls. To remove this smear layer, chelating agent such EDTA is most frequently used which opens up the dentinal tubules. The sequential use of NaOCl and EDTA has an erosive effect which alters the mechanical properties of dentin.

Chitosan as an irrigant has been extensively studied because of its antimicrobial, high bioactivity, biodegradability, antimicrobial activity and chelating properties. Chitosan is hydrophilic in nature, increases the contact area to dentin, thereby enhancing the adhesion of sealer to root canal dentin.

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.

Adhesion of sealers to radicular dentin avoids the penetration of microbes in root canal and provides a good hermetic seal. Studies have reported that chemical composition of sealers directly influences physical-chemical behaviour of dentin.

There are few studies which have compared the push-out bond strength of epoxy resin based sealer, MTA Fillapex and Apexit Plus sealer irrigated with 5.25% NaOCl followed by 17% EDTA and final flush with saline. However, no study was done to compare the effect of 17 % EDTA and 0.2% Chitosan on the push-out bond strength of these sealers on radicular dentin.

Keeping these rationale in mind this study aims to evaluate the effect of 17% EDTA and 0.2% chitosan as final flush on the push out bond strength of three different sealers to radicular dentin.

Sixty six extracted human mandibular premolar teeth selected as per the inclusion and exclusion criteria were cleaned of calculus and soft tissue were stored in 0.1% Thymol. All teeth were then decoronated up to the level of CEJ with the help of a diamond disc under copious water spray to produce a standardized root length of 15mm. After decoronation, each tooth was sectioned in 4 mm thick disc from coronal and middle third to obtain 132 specimens. The root canal of each specimen was prepared till no. 3 Gates Glidden drill and then divided into two groups based on the irrigant used as final flush.

Group 1(n=66):3% NaOCl + 17% EDTA.

Irrigation was done with 5 ml of 3% NaOCl for 1 min followed by 5ml of 17% EDTA for 2min

Group 2(n=66):3% NaOCl+ 0.2% Chitosan

Irrigation was done with 5 ml of 3% NaOCl for 1 min followed by 5ml of 0.2% Chitosan for 2min

The sections after irrigation were dried using paper points and further subdivided into three sub-groups according to the sealer to be placed.

Subgroup A: Epoxy resin-based sealer- AH Plus.

Subgroup B: Calcium Hydroxide based sealer- Apexit Plus.

Subgroup C: MTA resin-based sealer- MTA Fillapex.

The sealers were mixed as per manufacturer's instructions and filled into the root canals. All the sections were placed in the incubator for a week in order to simulate the natural conditions. The 4 mm thick sections were embedded in the acrylic resin and push-out bond strength test was done using Universal testing machine. The failure load was recorded in Newton and then calculated in MPa.

Statistically analysis of the data was done by using two way ANOVA for intra pair wise comparison and Tukeys post hoc test for inter-pairwise comparison. The test results indicated that there is significant difference between both the Groups 1 and 2 and all the three subgroups. (p value <0.05).

0.2 % Chitosan group showed highest bond strength when compared to EDTA.

Among all the three subgroups, subgroup 2A- 0.2% Chitosan+ AH Plus sealer (3.13 ± 0.37) showed the highest bond strength which can be attributed to its resin tag formation in dentin.

Chitosan solution is used as a chelating agent and shows its highest effect when it is mixed with 1% acetic acid. The solution removes the smear layer exposing more number of dentinal tubules resulting in better adaptation of sealer due to increased contact area. The results showed that Chitosan has a positive impact on bond strength of sealer. Therefore null hypothesis that there is no difference in the effect of 17% EDTA and 0.2% Chitosan as final flush on the push out bond strength of three different root canal sealers to radicular dentin was rejected.

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ANNEXURE – I

ETHICAL CLEARANCE CERTIFICATE

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CERTIFICATE

This is to Certify that the synopsis titled

COMPARATIVE EVALUATION OF THE EFFECT OF 17% EDTA AND 0.2% CHITOSAN USED AS A FINAL RINSE ON THE PUSH-OUT BOND STRENGTH OF ~~ER~~ THREE DIFFERENT ROOT CANAL SEALERS : IN-VITRO STUDY *Submitted by*

Dr. BHAVNA SHARMA *P. G. Student / Staff, Guided by* DR. ANAND PATIL *from Department of* CONSERVATIVE DENTISTRY & ENDODONTICS *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

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ANNEXURE – II

BIOSTATISTIC CLEARANCE CERTIFICATE

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Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Dr. Bhavna Sharma, Post Graduate Student**, under the guidance of **Dr. Anand C. Patil**$_{M.D.S}$, **Professor, Department of Conservative dentistry and Endodontics**, entitled **"Comparative evaluation of the effect of 17% EDTA and 0.2% Chitosan used as final flush on the push-out bond strength of three different root canal sealers to the radicular dentin- An Invitro study"** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 23/12/2021

Name & Signature of Biostatistician
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ANNEXURE – III

PLAGIARISM CHECK CERTIFICATE

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PLAGIARISM CHECK REPORT

Name of the Applicant : DR. BHAVNA SHARMA
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The soft copy of Research Work / Manuscript by DR. BHAVNA SHARMA entitled "COMPARATIVE EVALUATION OF THE EFFECT OF 17% EDTA AND 0.2% CHITOSAN USED AS FINAL RINSE ON THE PUSH OUT BOND STRENGTH OF THREE DIFFERENT ROOT CANAL SEALERS TO RADICULAR DENTIN - AN INVITRO STUDY"

under the guidance of DR. ANAND C. PATIL 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.

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