# AN IN-VITRO EVALUATION OF PUSH OUT BOND STRENGTH OF TWO ENDODONTIC SEALERS TO DENTIN USING DIFFERENT CHELATING AGENTS AS FINAL IRRIGANTS

*Dissertation submitted to*

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# CERTIFICATE

This is to certify that this dissertation titled **"An in-vitro Evaluation of Push out Bond Strength of two Endodontic sealers to Dentin using different Chelating agents as final Irrigants"** is a bonafide record of work done by **Dr. S. MOHAN KUMAR** under my guidance and to my satisfaction during his postgraduate study period, 2013 – 2016. This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfilment for the award of the degree of Master of Dental Surgery in Conservative Dentistry and Endodontics, Branch IV. It has not been submitted (partially or fully) for the award of any other degree or diploma.

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**Dr. S. MOHAN KUMAR**

# CONTENTS

# INTRODUCTION

The present century has seen much newer advancement in the practice of endodontics – material science, techniques, equipment and instrument design. However, the goals of nonsurgical endodontic therapy still remain the same: "Root canal systems must be cleaned , shaped and disinfected : cleaned of their organic remnants and shaped to receive a three-dimensional hermetic (fluid-tight seal) filling of the entire root canal space."[1]

Disinfection is one of the major steps which decreases microbial load and stimulates healing of periapical lesion.[2]This is achieved by shaping the canal, disinfection with an irrigant and an intracanal medicament; and fluid tight obturation of the canal.[3]During canal preparation, smear layer containing debris, vital and necrotic pulp tissue, bacteria, blood cells and odontoblastic processes is formed which blocks the dentinal tubules preventing the sealer penetration.[4] Till date many irrigants have been tested to attempt complete removal of this smear layer. EDTA is the most commonly used chelating agent along with sodium hypochlorite for this purpose.[5] However many studies have reported on the relative ineffectiveness of these agents in the removal of smear layer from the apical third of root canals.[6] EDTA solution is used at the end of a procedure to remove the smear layer but does not prevent future bacterial penetration between root canal fillings and canal walls. Hence in the current scenario developing new and better irrigating solutions which meet these challenges remains an area of great interest.

Chitosan is a natural, cationic aminopolysaccharide copolymer of glucosamine and N-acetylglucosamine obtained by the alkaline, partial deacetylation of chitin which is obtained from shells of crustaceans and shrimps. Chitosan possesses high chelating capacity for various metal ions including Zinc, Cobalt, Iron, Magnesium, and Copper ions in acidic conditions.[7,8] **A.M. Darrag etal** stated that final irrigation with 0.2% chitosan solution was more efficient in smear layer removal when compared to 10% CA and MTAD.

The Toronto landmark study by **Farzaneh et al** stated that the success or failure of endodontic therapy is dependent on obtaining hermetic seal to the full length of the canal system.[9] Root canal sealers have evolved over the years and research is still going on in search of the sealer that fulfils all the ideal criteria outlined by Grossman, while also being non-mutagenic or carcinogenic. Good adhesion to tooth material within the root canal is one of the ideal properties of a sealer cement, which potentially influences both leakage and root strength. Adhesion of the root canal filling to the dentinal walls is advantageous for two main purposes. In a static situation, it should eliminate any space that may allow percolation of fluids between the filling and the canal wall. In a dynamic situation, it is needed to resist dislodgement of the filling during subsequent manipulation.[10,11]

The epoxy resin-based sealer, ***AH Plus (Dentsply)***, is cytocompatible. In addition, no genotoxic or mutagenic effects have been found with AH PlusThe bond strength of an epoxy-resin based sealer may be attributed to its ability to react with any exposed amino groups in collagen to form covalent bonds between the resin and collagen when the epoxide ring opens, although other mechanisms may also contribute. ***ApexitPlus (IvoclarVivadent)***is a radiopaque, non-shrinking root canal sealer paste that is based on calcium hydroxide.**U.Salz et al** evaluated bacterial leakage of Apexit Plus, in comparison with AH Plus and reported that Apexit Plus had a better sealing ability than AH Plus.[13]

There are various methods for evaluating the adhesion of dental material to dentin. These are tensile, shear, and push-out strength tests. The push-out test is based on shear stresses, which occur in clinical conditions and can be imitated by this test method. As the push-out test generates parallel fractures in the interfacial area of the dentin-bonding, it presents a better method to evaluate bond strength for root canal sealers than any other conventional tests.

The ability of irrigant to remove smear layer directly influences the adhesion of the sealer to the root canal dentin. **Lester &Boyde** in the year 1977 stated that smear layer can act as a barrier between filling materials and the canal wall and therefore compromise the formation of a satisfactory seal. There is limited literature comparing the efficiency of smear layer removal of chitosan with the other commonly used chelating agents and there have been no studies on the effect of Chitosan when employed as a final rinse to determine the adhesion of the filling to the root canal wall, which is crucial for a fluid tight seal. Hence, the objective of this study was to compare the effectiveness of 0.2% Chitosan and Smear clear on the adhesion of Apexit plus and AH plus root canal sealers to root canal dentin using push out bond strength test.

# AIM & OBJECTIVE

The purpose of this study was :

- To assess and compare the push out bond strength of AH plus and Apexitplus endodontic sealers to dentin using 0.2 % Chitosan and 17% EDTA as final irrigants.

- To evaluate surface changes of the root canal dentin after rinsing with two different chelating agents by using scanning electron microscopy.

# REVIEW OF LITERATURE

**Hayashi et al** (2007)[14] designed a study to confirm whether chewing chitosan-containing gum will more mechanically and effectively suppressed the growth of oral bacteria than a mouth rinse using chitosan solution, and to also demonstrate whether the salivary secretion increased by chewing chitosan-containing gum in comparison to chewing control gum. They found that Chitosan-containing gum chewing had a greater antibacterial effect and it also increases salivary secretion through its little astringent and/or bitter taste. This suggested that the application chitosan was useful for oral health.

**Arnaud et al** (2010)[15] evaluated the in vitro effect of chitosan (concentration and time of action) treatment on enamel de-remineralization behaviour upon a pH cycling assay. They found that Chitosan interfered with the process of demineralization of the tooth enamel inhibiting the release of phosphorus in this laboratory study. Demineralization was influenced by the concentration and exposure time of the biopolymer to the enamel. They stated that Microhardness measurements may be used as an indication of mineral loss from tooth enamel. Additionally their OCT images supported the idea that chitosan may act as a barrier against acid penetration, contributing to its demineralization inhibition.

**Uysal et al** (2011)[16] evaluated the in vivo effects of a chitosan-containing dentifrice in reducing enamel demineralization around orthodontic brackets and to compare the chitosan-containing dentifrice with the conventional non fluoridated dentifrice. To date, the use of chitosan-containing chewing gum and mouth rinse has been found to be an effective method for preventing demineralization of enamel. The highly deacetylated and lower molecular chitosan also showed bactericidal activity.

Authors suggested that the supplementation of chitosan to dentifrice is an effective method to control the demineralization of enamel around brackets.

**Keegan et al** (2012)[17] aimed to manufacture and characterise chitosan microparticles containing NaF using a spray drying route previously reported for the matrix microencapsulation of metoclopramide, a highly water soluble anti-emetic.Authors concluded that Bioadhesive chitosan/fluoride microparticles manufactured using a spray-drying protocol have been extensively characterised and further opportunity for optimisation identified. These microparticles may provide a means of increasing fluoride uptake from oral care products to provide increased protection against caries.

**Mahapoka et al** (2012)[18] evaluated the antimicrobial effect of nano-sized chitosan whiskers impregnated into resin sealant against *Streptococcus mutans*. In addition, the physical properties of thischitosan-resin sealant were evaluated for the depth ofcure, hardness and degree of double bond conversion. In this study, the chitosan whiskers in resin sealant exhibited antibacterial property against *S. Mutans* (UA159). The morphological data of incorporated chitosan whiskers indicated a mostly scattered distribution of small-size fibrous figures. The gross appearance of the sealant is clear and transparent. The two percent by weight of distributed chitosan whiskers showed acceptable physical properties compared with control, including greater depth of cure and double bond conversion degree. While the incorporation of fibrous whiskers moderately reduced the hardness value, the high aspect ratio in absorbing and spreading out the biting force could prove beneficial. Authors concluded that, the chitosan whiskers from shrimp shells can be used in dimethacrylate-based sealant as an alternative antimicrobial pit and fissure sealant.

**Lee et al** (2012)[19] examined the use of a polymer coating to deter the effects of acid erosion of Hydroxyapatite. Of particular interest is the ability of polymers to modify the native properties of the host surface by changing the wettability, surface topography, or chemical reactivity.They concluded that both the cross-linked chitosan/artificial saliva surface and the physically adsorbed chitosan/artificial saliva surface served as a novel method to prevent acid erosion of the model dental hydroxyapatite surface.

**Pimenta et al** (2012)[20] evaluated the action of 0.2% chitosan, 15% EDTA and 10% citric acid on root dentin microhardness. The use of EDTA, either alone or combined with sodium hypochlorite, reduces the microhardness of root dentin significantly The authors found that there was no significant differences among 0.2% chitosan, 15% EDTA and 10% citric acid solutions in the reduction of root dentin microhardness. Distilled water, which was used as a control, did not alter the microhardness.

**Silva et al** *(*2012)[21] evaluated the efficacy of smear layer removal of chitosan compared with different chelating agents using scanning electron microscopy. They found that 15% EDTA, 0.2% chitosan and 10% citric acid effectively removed smear layer from the middle and apical thirds of root canals. In addition, 15% EDTA and 0.2% chitosan were associated with the greatest effect on the root dentine demineralization.

**Darrag** (2014)[22] evaluated the smear layer removal ability of 17% EDTA, 10% CA, MTAD, and 0.2% chitosan solutions using scanning electron microscope (SEM) on the coronal, middle and apical thirds of instrumented root canals. He found that the tested chelating agents could effectively, but not completely, remove the smear layer. They were more effective in the coronal two thirds than in the apical third of the root canal. He stated that studies were needed to investigate in details the physical, chemical and biological properties of 0.2% chitosan solution to verify the benefits of their use as root canal chelating agents.

**Suzuki et al** (2014)[23]focused on citric acid as a solution to dissolve chitosan and determined its antibacterial properties against *Enterococcus faecalis*and its efficacy of removing the smear layer from root canal dentin. They concluded that, chitosan-citrate solution showed antibacterial activity and enabled removal of smear layer. As this ability depended on chitosan, it was considered that the action was enhanced by chitosan. They stated that the Chitosan citrate solution could be indicated as a possible root canal irrigant.

**Delivanis et al (**1983)[24] in their landmark study proved that a fluid tight obturation is essential for prevention of survival of micro-organisms in the root canals by using the F43 strain of Streptococcus sanguis. Their results confirmed Grossman's hypothesis that if a canal is completely filled laterally and apically any micro-organism remaining inside the canal will not be capable of surviving for a long time.

**Drummond et al** (1996)[25]evaluated the effect of the following variables on shear dentin-bonding test results: mode of testing (cyclic fatigue versus static loading), surface treatments (32% phosphoric acid, 10% phosphoric acid, and no treatment (unetched), and type of shear test (traditional planar versus push-out). Authors suggested that the push-out test provides a better evaluation of bonding strength than the conventional shear test because using the push-out test, fracture occurs parallel to the dentine–bonding interface, which makes it a true shear test for parallel-sided samples.

**Ureyen Kaya et al** (2007)[26] compared the interfacial strength and failure mode of root fillings consisting of different technique-material combinations. The results showed push-out test provides a better evaluation of the bonding strength than the conventional shear test because with the push-out test fracture occurs parallel to the dentine-bonding interface, which makes it a true shear test for parallel-sided samples. It has the benefit of more closely simulating the clinical condition. Interfacial strength and dislocation resistance between the root filling material and intraradicular dentine were evaluated using thin-slice push-out tests.

**Jainaen et al** (2007)[27] evaluated the push-out bond strength of the dentine–sealer interface with and without main cone for three resin sealers AH Plus, EndoREZ or Resilon. This study found a difference in the mode of failure between thin film and bulk sealer of the three resin sealers. With a thin film, failure was cohesive within the sealer itself, whereas bulk sealer showed adhesive failure between dentine and sealer, leaving partially pulled out resin tags in the dentinal tubules. Authors concluded that the epoxy resin-based sealer had the highest push-out bond strength when compared to UDMA-based sealers when used with a main cone and sealer. The bond strengths after filling with sealer alone were higher than those with main cone and sealer, and may reflect different patterns of behaviour when the sealer is present as a thin layer.

**Alfredo et al** (2008)[28] evaluated the bond strength of AH Plus and Epiphany sealers to human root canal dentine irradiated with a 980 nm diode laser at different power and frequency parameters, using the push-out test. The specimens were assigned to five groups (n = 12):one control (no laser) and four experimental groups that were submitted to 980 nm diode laser irradiation at different power (1.5 and 3.0 W) and frequency (continuous wave and 100 Hz) parameters. The push-out test was performed. The specimens irradiated with the diode laser and filled with AH Plus had significantly higher bond strength values (8.69 ± 2.44) than those irradiated and filled with Epiphany (3.28 ± 1.58) and the non- irradiated controls (3.86 ± 0.60). There was a predominance of adhesive failures at Epiphany–dentine inter- face (77%) and mixed failures at AH Plus–dentine interface (67%).

**Teixeira et al** (2009)[29] compared the Shear bond strength test and push-out test in their ability to measure accurately the bond strength of a resin-based endodontic sealer (AH Plus) to dentin and gutta-percha. The secondary goal of this study was to assess the failure modes on the debonded surfaces by scanning electron microscopy (SEM). SEM analysis showed a predominance of adhesive and mixed failures of AH Plus sealer. The comparison of the employed methodologies showed that the Shear bond strength test produced significantly lower bond strength values than the push-out test.

**Amara et al** (2011)[30]compared the bond strength of Epoxy resin based and UDMA based sealers and to assess the relative bond strengths between Dentin-Sealer and Sealer-main cone, by testing canals filled with and without a main cone. Sealers failed in cohesive mode within the thin film, leaving a layer of sealer on the canal surface. Epoxy resin based sealer had the highest push-out bond strength compared with UDMA based sealers when used with main cone.The bond strengths after filling with sealer alone were higher than those with main cone and sealer and may reflect different patterns of behavior when the sealer is present in thin layer.

**Kandaswamy et al** (2011)[32] evaluated the effect of various final irrigants (EDTA, MTAD and HEBP) on shear bond strength of AH plus sealer. EDTA showed higher bond strength followed by HEBP, the possible reason might be due to the removal of smear layer. Smear layer removal procedures allow the sealer penetration into the dentinal tubules and thus could increase the dentin bond strength of resin based sealer as well as an enhanced seal. Even though MTAD had better smear layer removal efficacy and demineralized dentin zone (8 to 12µm) compared to EDTA and HEBP, shear bond strength showed less. Reason for that might be the degradation product, which might interfere in the sealing ability of the sealers.

**Barbizam et al** (2011)[32] evaluated the bond strength of different sealers to dentin, in presence or absence of smear layer, using push-out tests. It was considered the hypotheses that bond strength of resin-based sealers is similar among them, but higher than that of ZOE-based sealer, and that bond strength of all sealers is higher in the absence of smear layer. The bond strength values decreased when smear layer was present, independently of the sealers composition. Authors concluded that epiphany sealer presented higher bond strength values to dentin in both irrigating protocols, and the use of 2.5% NaOCl and 17% EDTA increased the bond strength values for all sealers.

**Sagsen et al** (2011)[33] assessed the push-out bond strength of two new calcium silicate-based endodontic sealers in the root canals of extracted teeth. In this study the push-out bond strengths in the middle and apical specimens were significantly higher than those of the coronal specimens. There were no significant differences between the push-out bond strengths in the middle and apical specimens. The higher bond strengths in the middle and apical specimens could be related to deeper sealer penetrations because of higher lateral condensation forces or as a result of the dentine structure in these parts of the roots. MTA Fillapex root canal sealer had low adhesion strength to root dentine. Authors suggested that the reason for the low bond strength of MTA Fillapex is due to the low adhesion capacity of tag-like structures formed by controlled mineral nucleation on dentine.

**Assmann et al** (2012)[34] aimed to evaluate the bond strength to root dentin of 2 mineral trioxide aggregate (MTA)-based sealers (Endo-CPM sealer and MTA Fillapex) and of 1 epoxy resin-based sealer (AH Plus sealer). Irrigation with 2.5% NaOCl and a final rinse with 17% ethylenediaminetetraacetic acid and distilled water were performed. Canals were filled by using Endo- CPM sealer, MTA Fillapex, or AH Plus sealer by means of the gutta-percha lateral condensation technique. The authors quote that accordingly, AH Plus can be considered the gold standard material for testing endodontic sealers' resistance to dislodgment, because previous studies have pointed out that it presents advantages in comparison with other materials usually used.

**Candeiro et al** (2012)[35] evaluated the physicochemical properties of a bioceramic root canal sealer, Endosequence BC Sealer. Radiopacity, pH, release of calcium ions (Ca2+), and flow were analyzed, and the results were compared with AH Plus cement. The release of Ca2+ and pH were measured at periods of 3, 24, 72, 168, and 240 hours with spectrophotometer and pH meter, respectively. The bio- ceramic endodontic cement showed radiopacity (3.84 mm Al) significantly lower than that of AH Plus (6.90 mm Al). The pH analysis showed that Endosequence BC Sealer showed pH and release of Ca2+ greater than those of AH Plus during the experimental periods. The flow test revealed that BC Sealer and AH Plus presented flow of 26.96mmand 21.17 mm, respectively.

**Patil et al** (2013)[36]attempt to evaluate and compare the push-out bond strength of gutta-percha/AH Plus, Resilon/Epiphany SE and gutta-percha/EndoREZ to dentin. The results study challenge the claim of "monoblock" formation by the new resin-based sealers. The adhesiveness quality to root dentin promoted by both resin-based sealers is compromised even when teeth with simple anatomic features were obturated under well-monitored laboratory conditions. The higher push-out bond strength found in the gutta-percha/AH Plus root fillings reiterate the fact that the era of conventional nonbonding root filling has not yet come to an end.

**Guneser et al** (2013)[37] evaluated the effect of various endodontic irrigants on the push-out bond strength of Biodentine (Septodont, Saint MaurdesFoss_es, France) in comparison with contemporary root perforation repair materials. The push-out bond strength of Dyract AP, amalgam, IRM, and Biodentine was not significantly different when immersed in NaOCl, CHX, and saline solutions, whereas MTA lost strength when exposed to CHX. Authors find that the Biodentine showed considerable performance as a perforation repair material even after being exposed to various endodontic irrigants, whereas MTA had the lowest push-out bond strength to root dentin.

**Mozayeni et al** (2013)[38] aimed to evaluate the push-out bond strength of AH26 sealer to rootcanal dentin with a micro-push-out technique subsequent to a final irrigation of MTAD compared with combination of NaOCl/EDTA. Combined use of NaOCl and EDTA was reported the most effective approach for smear layer removal. In this study, NaOCl/EDTA final irrigation protocol significantly increased push-out bond strength compared with saline. Authors found that that smear layer removal improved bond strength of AH26 sealer to root canal walls and this improvement is statistically greater with application of MTAD as a final rinse to that of combination of NaOCl and EDTA final rinse.

**Topcuoglu et al (**2013)[39] evaluated the fracture resistance of teeth filled with 3 different endodontic sealers, namely EndoSequence BC sealer, MTA-based sealer and AH Plus jet sealer. All root specimens were stored for 2 weeks at 100% humidity to allow the complete setting of the sealers. Each specimen was then subjected to fracture testing by using a universal testing machine at a crosshead speed of 1.0 mm/min until the root fractured. It was seen that in contrast to MTA based sealer, Endosequence BC and AH Plus Jet sealer increased the force to fracture in root-filled single-rooted premolar teeth.

**Zhou et al** (2013)[40] evaluated the pH change, viscosity and other physical properties of 2 novel root canal sealers (MTA Fillapex and Endosequence BC) in comparison with 2 epoxy resin-based sealers (AH Plus and ThermaSeal), a silicone-based sealer (Gutta- Flow), and a zinc oxide-eugenol–based sealer (Pulp Canal Sealer) . The flow, dimensional change, solubility, and film thickness of all the tested sealers were in agreement with ISO 6876/2001 recommendations. The MTA Fillapex sealer exhibited a higher flow than the Endosequence BC sealer. The MTA Fillapex and Endosequence BC sealers showed the highest film thicknesses among the tested samples. The Endosequence BC sealer exhibited the highest value of solubility, which was in accordance with 3% mass fraction recommended by the ISO 6876/2001, and showed an acceptable dimensional change. The MTA Fillapex and Endosequence BC sealers presented an alkaline pH at all times. The pH of fresh samples of the AH Plus and ThermaSeal sealers was alkaline at first but decreased significantly after 24 hours. The viscosity of the tested sealers increased with the decreased injection rates.

**Shokouhinejad et al**, (2013)[41] assessed the effect of different irrigation protocols for smear layer removal on the bond strength of EndoSequence BC Sealer, a new bioceramic sealer, to root canal dentin. The middle third of forty-four extracted human teeth were sectioned horizontally to obtain 128 dentin disks. After dentin treatment, two specimens of each group were prepared for investigation with scanning electron microscopy (SEM). Surface of root canal wall was assessed in each specimen. Then the canal spaces were filled with EndoSequence BC Sealer in the remaining specimens. Push-out bond-strength and failure modes were assessed. There was no significant difference between the bond strengths of test groups. The bond failure was mainly cohesive for all groups.

**Nagas et al** (2013)[42] tested the influence of dentin moisture conditions on the bond strength of 4 root canal sealers namely, AH Plus, iRoot SP, MTA Fillapex, Epiphany. Eighty root canals were prepared using rotary instrument and, thereafter, were assigned to 4 groups with respect to the moisture condition tested. Bond strengths of the test materials to root canal dentin were measured using a push-out test setup at a crosshead speed of 1 mm/min. Irrespective of the moisture conditions, iRoot SP displayed the highest bond strength to root dentin. Statistical ranking of bond strength values was as follows: iRoot SP > AH Plus > Epiphany ≥ MTA Fillapex. The sealers displayed their highest and lowest bond strengths under moist and wet conditions, respectively.

**Shokouhinejad et al** (2013)[43] conducted a study to compare the bond strength of a new bioceramic sealer (EndoSequence BC Sealer) and AH Plus in the presence or absence of smear layer. Extracted single-rooted human teeth were prepared and randomly divided into four groups. In groups 1 and 3, the root canals were finally irrigated with 5.25% NaOCl and smear layer was not removed, but in groups 2 and 4, the root canals were finally irrigated with 17% EDTA followed by 5.25% NaOCl in order to remove the smear layer. Push- out bond strength and failure modes were evaluated. The bond strength of gutta-percha/AH Plus and gutta-percha/EndoSequence BC Sealer was not significantly different. The presence or absence of smear layer did not significantly affect the bond strength of filling materials. The mode of bond failure was mainly cohesive for all groups. In conclusion, the bond strength of the new bioceramic sealer was equal to that of AH Plus with or without the smear layer.

**Vemisetty et al** (2014)[44]compared the push-out bond strengths of AH Plus, Pulp Canal Sealer EWT and Apexit Plus endodontic sealers with and without amoxicillin. To prevent new bacterial growth, obturation materials and sealers should have anti-microbial properties which upon contact with microbes and biofilms, will prevent re-infection of root canal system. Endodontic sealers with added amoxicillin showed inhibition of bacterial cell growth initially, but also demonstrated inhibition after 7 days of sealer set. Authors concluded that AH plus, Apexit plus, Pulp canal sealer EWT have showed no significant ($p > 0.05$) difference in push-out bond strength when mixed with amoxicillin. The also suggested that addition of amoxicillin to the endodontic sealers has no effect on push out bond strength.

# MATERIALS AND METHOD

**SOURCE OF SAMPLES:**

Forty extracted mandibular premolars with single canal were collected from Department of Oral and Maxillofacial surgery, Sri Ramakrishna Dental College & Hospital, Coimbatore.

**Materials Used:**

- Normal saline ***(Claris Otsuka LTD, Ahmedabad, India)***
- 3% NaOCl irrigating solution ***(Vensons India, Bangalore, India)***
- Low molecular weight Chitosan ***(Sigma Aldrich,Missouri, United States).***
- 17% EDTA ***(smear clear, Sybron Endo).***
- ProTaper F4 gutta-percha points ***(DentsplyMaillefer, Ballaigues, Switzerland)***
- AH plus root canal sealer.***(Dentsply de Trey GmbH, Konstanz, Germany)***.
- Apexit Plus ***(IvoclarVivadent AG, Schaan, Liechtenstein).***

**Armamentarium:**

1. Diamond disk
2. Scale
3. Endoblock ***(DentsplyMaillefer, Ballaigues, Switzerland)***
4. Stainless steel ball burnisher
5. Stainless steel hand GP condenser ***(Dispodent, Chennai, India)***
6. Disposable 6ml Syringe ***(DISPO VAN, Hindustan Syringes and Medical Devices LTD, Faridabad, India)***
7. X-Smart Endomotor and Handpiece ***(DentsplyMaillefer, Ballaigues, Switzerland)***
8. ProTaper Rotary Endodontic Files ***(DentsplyMaillefer, Ballaigues, Switzerland)***
9. AirotorHandpiece***(NSK, Japan)***
10. No. 2 Round burs ***(Mani Inc., Japan)***
11. Spirit Lamp
12. Glass Slab
13. Stainless steel Cement spatula
14. Stainless steel plastic instrument

15. Humidity Chamber
16. K files - ISO 10 and 15 ***(Mani Inc., Japan)***
17. Nikon – Coolpix 4500 Digital Camera
18. Digital Image Analyzing Software ***(Image-Pro Express Version 6.0)***
19. Electronic weighing device.
20. Scanning electron microscope ***(carlzeiss)***
21. Autoclave ***(unique clave C-79, confident)***
22. Universal testing machine ***(Zwick Roell Z010)***
23. Saw Microtome ***(Leica SP 1600)***

| Sealer | Manufacturer | Classification | Composition |
|---|---|---|---|
| **AH Plus** | Dentsply De Trey Gmbh, Germany | Epoxy resin based | Epoxy paste: diepoxy, calcium tungstate, zirconium oxide, aerosol, and dye. |
| | | | Amine paste: 1-adamantane amine, N.N’dibenzyl-5 oxanonandiamine-1,9, TCD-diamine, calcium tungstate, zirconium oxide, aerosol, and silicon oil. |
| **Apexit plus** | IvoclarVivadent | Calcium hydroxide based | **Base** Calcium hydroxide / Calcium oxide,Hydrated collophonium,Fillers and other auxiliary materials (highly dispersedsilicon dioxide, phosphoric acid alkyl ester) |
| | | | **Activator** : Disalicylate, Bismuth hydroxide / Bismuth carbonate ,Fillers and other auxiliary materials (highly dispersedsilicon dioxide, phosphoric acid alkyl ester) |

## METHOD OF COLLECTION OF SAMPLES

Forty mandibular premolars with single canal (fig.1) were collected from the Department of Oral and Maxillofacial surgery, Sri Ramakrishna Dental College & Hospital, Coimbatore, which were indicated for extraction due to poor periodontal prognosis and orthodontic reasons.

**Infection Control protocol for the teeth collected for this study:**

Collection, storage, sterilization and handling of extracted teeth were followed according to the Occupational Safety and Health Administration (OSHA) and Centre for Disease Control and Prevention (CDC) recommendations and guidelines:[45,46]

1. Handling of teeth was always done using gloves, mask and protective eyewear.
2. Teeth were cleaned of any visible blood and gross debris.
3. Distilled water was used in wide mouth plastic jars for initial collection.
4. Teeth were immersed in 10% formalin for 7 days, following which the liquid was discarded and the teeth were transferred into separate jars containing distilled water.
5. The initial collection jars, lids and the gloves employed were discarded into biohazard waste receptacles.
6. As and when the teeth were required, they were removed from the jars with cotton pliers and rinsed in tap water.

**Inclusion Criteria:**

- Teeth with completely formed roots.
- Teeth with normal anatomical roots.
- Absence of caries and root canal fillings
- Patent single canal
- Root canal with apical diameter of size 15 K file

**Exclusion criteria:**

- Teeth with fractured roots.
- Multi-rooted teeth
- Teeth with open apices
- Calcified root canals
- Internal or external resorption
- Cracks on examination
- Attrition/abrasion

**PROCEDURE**

**Removal of external residual tissues:**

Teeth were placed in 2.5% sodium hypochlorite solution for ten minutes to remove the soft tissues. Calculus was mechanically removed from the root surfaces using hand scalers. Teeth were again stored in fresh saline solution until use.

**Preparation of 0.2% chitosan solution:**

0.2g of low molecular weight chitosan was measured using Electronic weighing device. The solution was prepared by dissolving 0.2 g of chitosan [47] in 100 mL of 1% acetic acid. The mixture was agitated using a magnetic agitator[48]for 2 h to obtain a homogenous clear solution.

**Root canal treatment:**

The samples were decoronated with a double faced diamond disk to leave 13mm root (Fig.2) measured with help of calliper. Access was prepared on each tooth using high speed diamond burs with copious water spray. A size 10 K file was placed in the canal until it was visible at the apical foramen. The working length was determined by subtracting 1 mm from this measurement.

Endodontic treatment was performed using ProTaper Universal NiTi rotary instruments (Fig.4). Canals were enlarged up to F4 at working length. During instrumentation all canals were irrigated between each instrument with 2.5mL of 3% NaOCl. Finally, the root canals were rinsed with 5 mL of distilled water and randomly divided into two groups (n = 20) according to the final irrigating solution used for smear layer removal. Preparation was deemed complete when the irrigating solution appeared clear of debris.

Group I: 1 mL of 0.2% chitosan (Sigma Aldrich) was used for 3 min.

Group II: 1 mL of 17% EDTA solution (Smear clear, Sybron endo)was used for 1 min.

The irrigating solutions were delivered via a sterile 30-gauge nickel titanium needle[49]which penetrated to 2 mm of the working length. The root canals were then flushed with 5 mL of distilled water,[50] dried with sterile paper pointsand sterilized cotton pellets were placed in the root canal orifices.

**OBTURATION:**

Each group was further subdivided into two subgroups (n = 10) according to the obturation system used.

GROUP I: Teeth specimens rinsed with 0.2% chitosan.

GROUP II: Tooth specimens rinsed with 17% EDTA (Smear clear, Sybron endo)

**Group IA& Group II A: AH plus**

AH Plus sealer is available as a two paste system (Fig.7). According to the manufacturer’s instruction, both the pastes were dispensed on to paper pads for equal amounts. The two pastes were mixed using a spatula until a uniform mix was obtained. TheLentulo spiral was introduced into the root canal to a location 2 to 3 mm short of the working length and then slowly withdrawn from the canal, with continuous rotation. For standardization lentulo spiral was used for ten seconds only in all the canals. A guttapercha cone of F4 size was used for the obturation of the canal and the excess was sheared off.

**Group IB & Group II B: Apexit plus**

Apexit Plus is a calcium hydroxide based two-component material (Fig.8), which sets by complex formation. Lentulo spiral was introduced into the root canal to a location 2 to 3 mm short of the working length and then slowly withdrawn from the canal, with continuous rotation. For standardization lentulo spiral was used for ten seconds only in all the canals. A guttapercha cone of F4 size was used for the obturation of the canal and the excess was sheared off.

The specimens were stored in an incubator (Fig.10) at 37 degree centigrade in 100% humidity for 14 days to allow the sealer to set completely.

**Preparation of specimens for Push out Bond Strength testing:**

Samples were embedded in self cure acrylic resin (Fig.11).The roots were sectioned to obtain specimens of 2 mm ± 0.1 mm thickness using water-cooled hard tissue microtome (Fig.12). One section each from the middle third of each root specimen to give a final sample size of 10 per group were analysed to test the bond strength. (n=10).

**Measurement of Push out Bond strength:**

The bond strength measurement was done in the apico- coronal direction using the Universal testing machine with a plunger of 0.50 mm diameter at a cross-head speed of 1mm/min with a force of 1N until bond failure occurred (Fig.14). The force was measured in Newtons. The bond strength measurement was converted to MPa by dividing the force in Newtons by the area of bonded surface. The area of bonded surface is given by the formula $2\pi r*h$. $\pi$ is a constant with an approximate value of 3.14 , r is the internal diameter of the root canal and h is the height of the specimen.

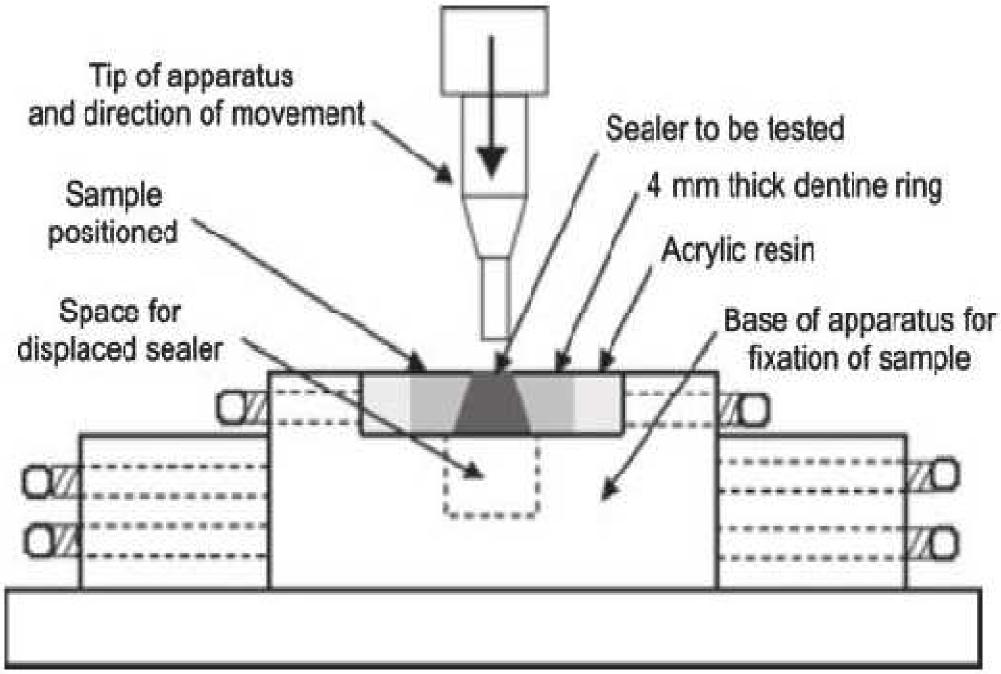



**Diagram depicting the experimental set up involving a Universal Testing Machine to measure the Push out Bond Strength**

**SEM ANALYSIS**:

Two Representative specimens of each group were separately prepared for scanning electron microscopy (SEM) to analyze the surface morphology of root canal dentin after irrigation with different irrigants. Longitudinal grooves were made on the buccolingual surfaces on each root by using a diamond disk[51]at low speed without penetrating the canal, the roots were then split in two halves with a chisel. The coded specimens were secured on metal stubs, desiccated, sputter coated with gold (Fig.15), and examined under Scanning Electron Microscope[52] (Sigma FE-SEM, Zeiss) at X1000 magnification (Fig.16).

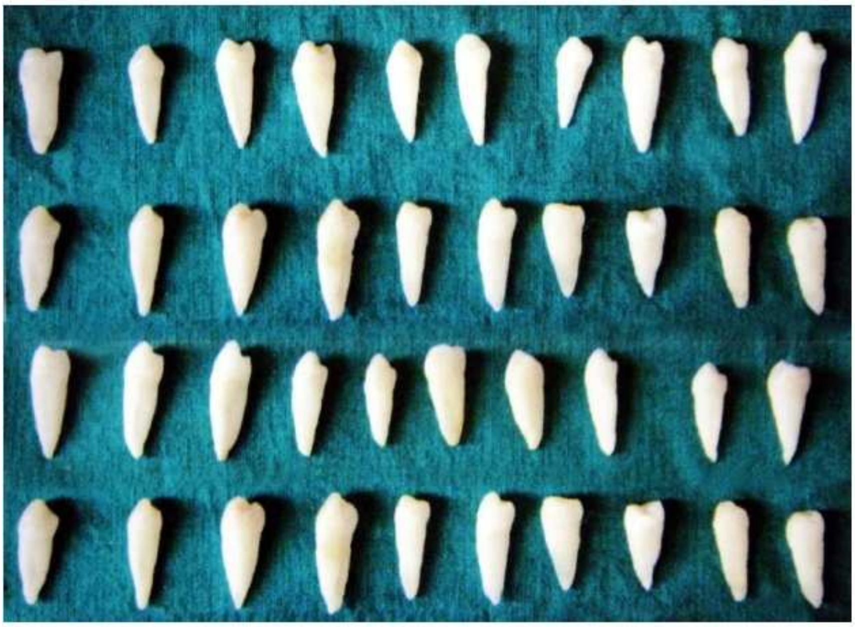


**FIG.1: HUMAN SINGLE ROOTED TEETH USED IN THE STUDY**

**FIG 2: DECORONATED TEETH SPECIMEN**


**FIG 3: X-SMART ENDOMOTOR AND HANDPIECE**

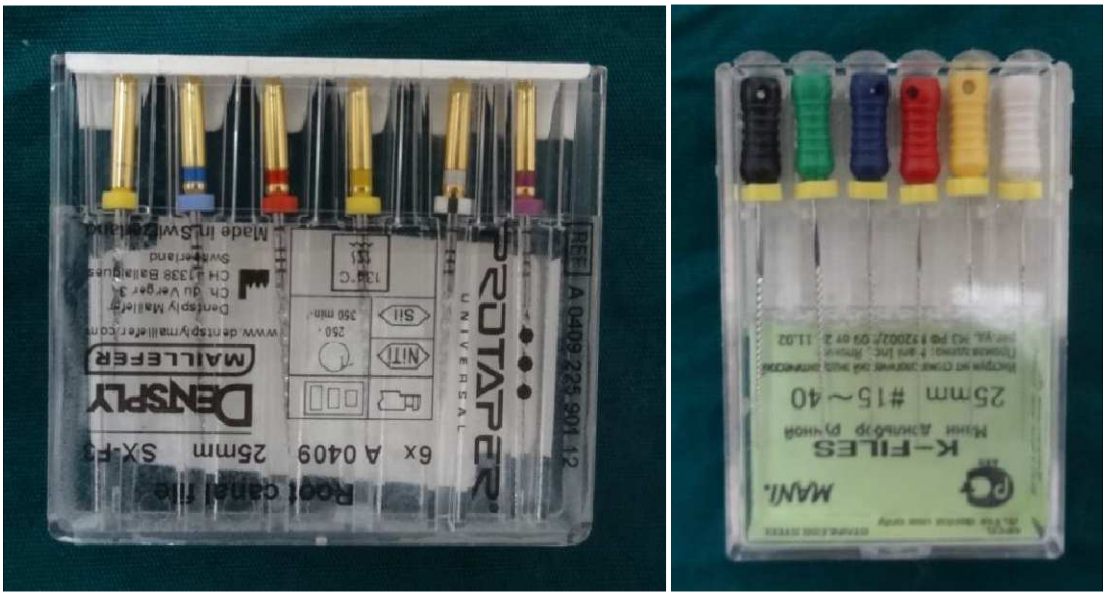


**FIG 4: PROTAPER ROTARY SYSTEM AND K - FILES**


**FIG 5: 17 % EDTA *(smear clear, Sybron Endo*)**

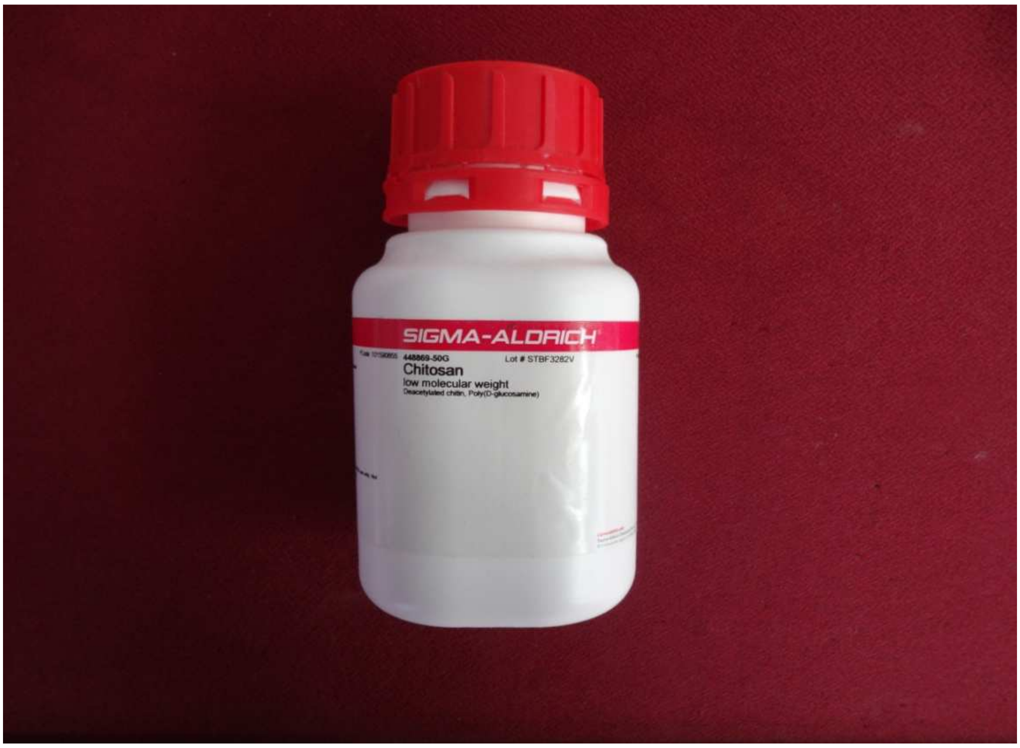



**FIG 6: LOW MOLECULAR WEIGHT CHITOSAN *(sigma Aldrich)***


**FIG 7: AH PLUS ROOT CANAL SEALER**

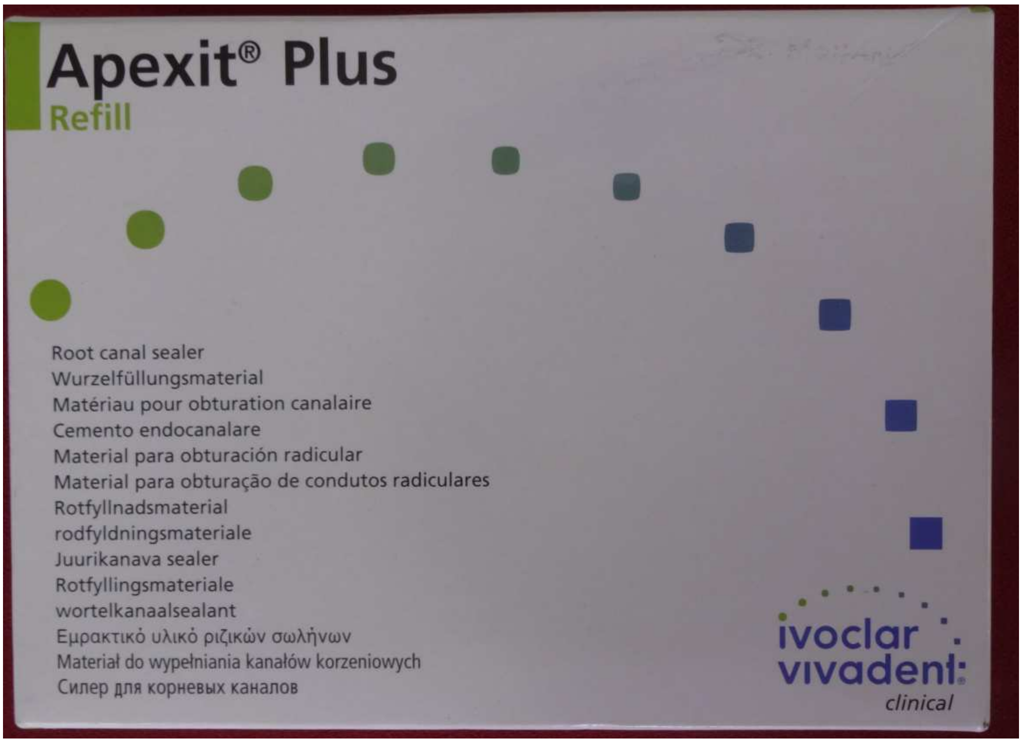



**FIG 8: APEXIT PLUS ROOTCANAL SEALER**

FIG 9: ENDODONTIC PREPARATION OF DECORONATED SAMPLES

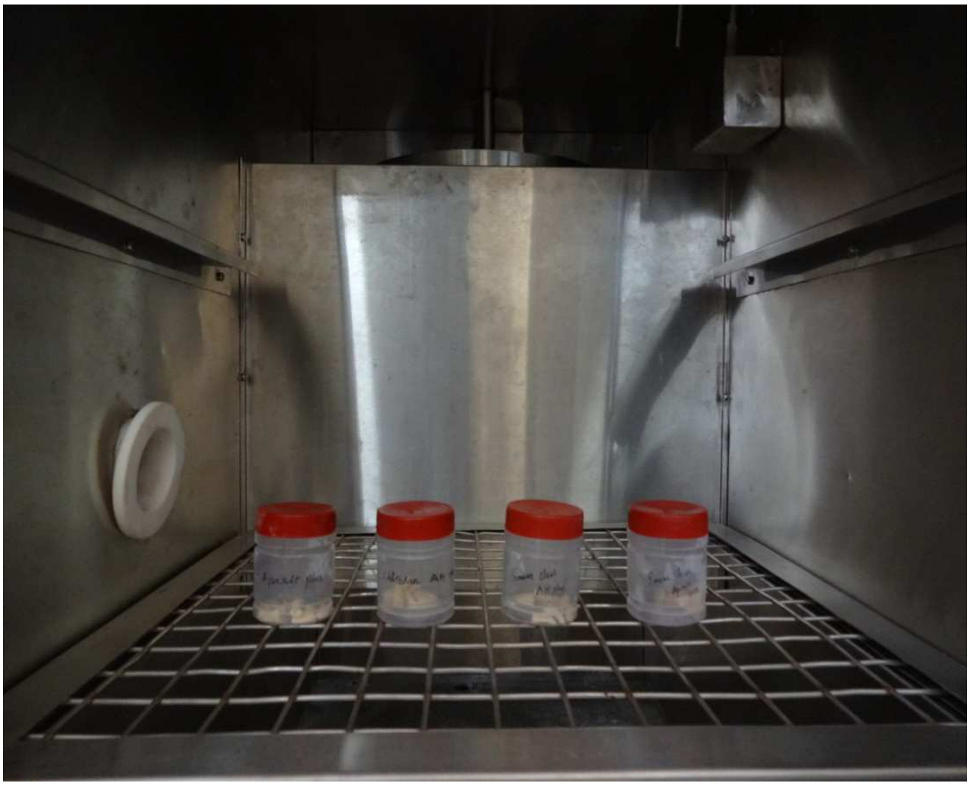


FIG 10: TEETH SPECIMENS INSIDE THE HUMIDITY CHAMBER

**FIG 11: SAMPLES EMBEDDED IN SELF CURE ACRYLIC**

**FIG 12: CROSS SECTION OF MIDDLE THIRD DONE WITH HARD TISSUE MICROTOME**

**FIG 13: CROSS SECTION AT THE MIDDLE THIRD OF THE SAMPLES**

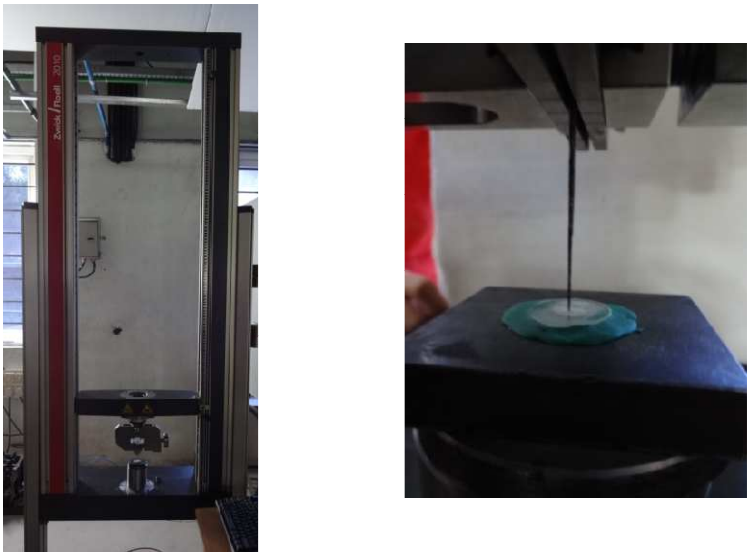


**FIG 14: EXPERIMENTAL SET UP WITH THE UNIVERSAL TESTING MACHINE AND THE SAMPLE IN POSITION**

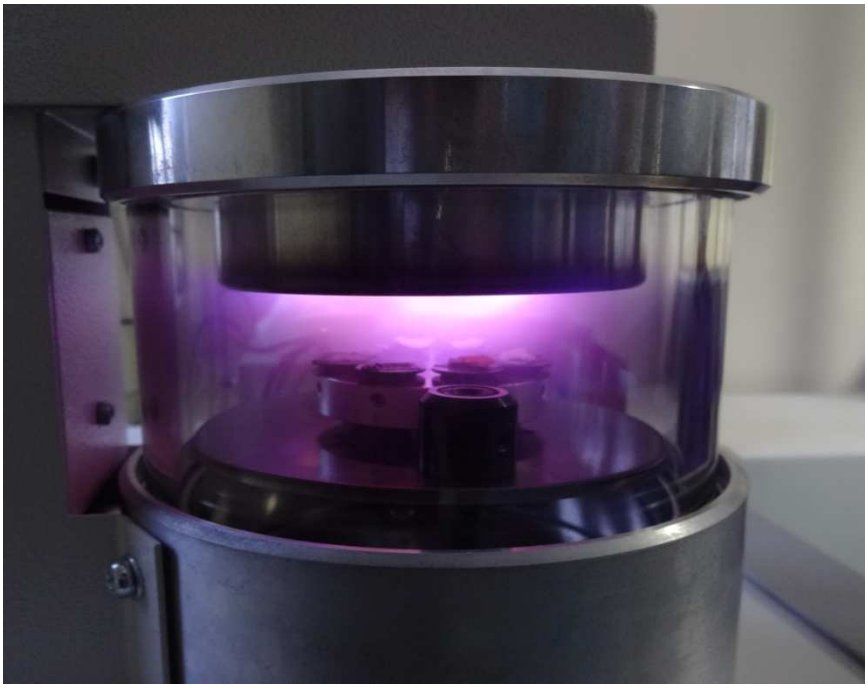


**FIG 15: GOLD SPUTTER COATING OF TEETH SPECIMENS**


**FIG 16: SCANNING ELECTRON MICROSCOPE**

**STATISTICAL ANAYSIS**

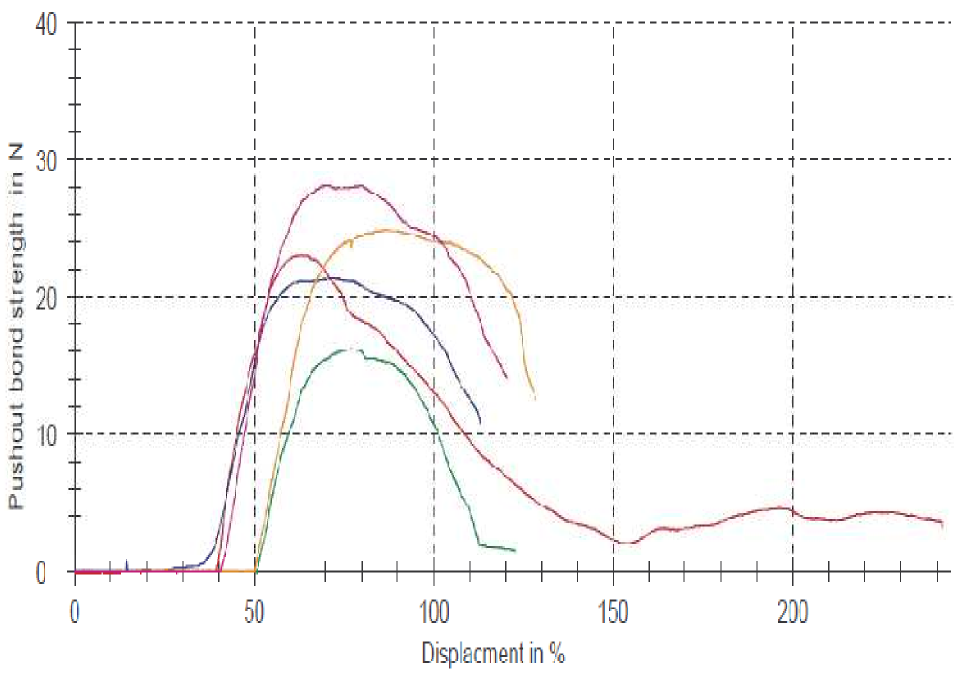
The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analysed using SPSS (Statistical Package for Social Science, Ver.10.0.5) package. The level of significance was set at a $P < 0.05$ and with a confidence interval level of 95%.The results were averaged (mean + standard deviation) for continuous data are presented in Table 1. Inter and intra group comparisons were done with one-way ANOVA followed by a pairwise comparison with Tukey's *post hoc* test for evaluating the push out bond strength of compared groups (Table.2).

# RESULTS

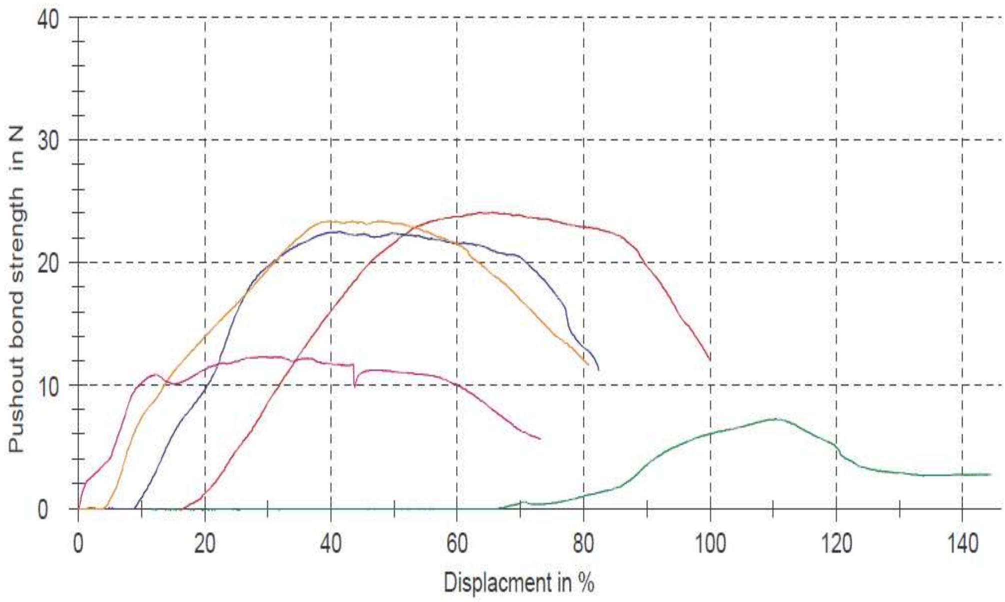
**PUSH OUT BOND TESTING:**

- The values at the time of dislodgement were recorded in Newtons for each specimen, captured electronically.
- The measurements of each sample of four different groups were plotted in the Stress – Strain Graph. Where, Y- axis denotes applied Load (or) Stress and X-axis denotes Displacement (or) strain

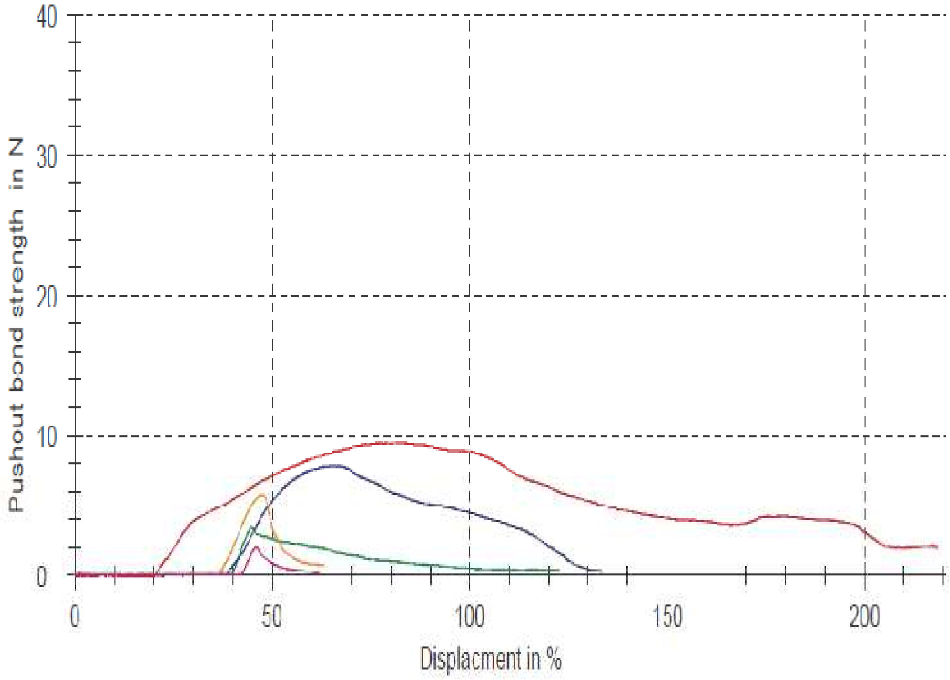
Group IA: Chitosan AH plus



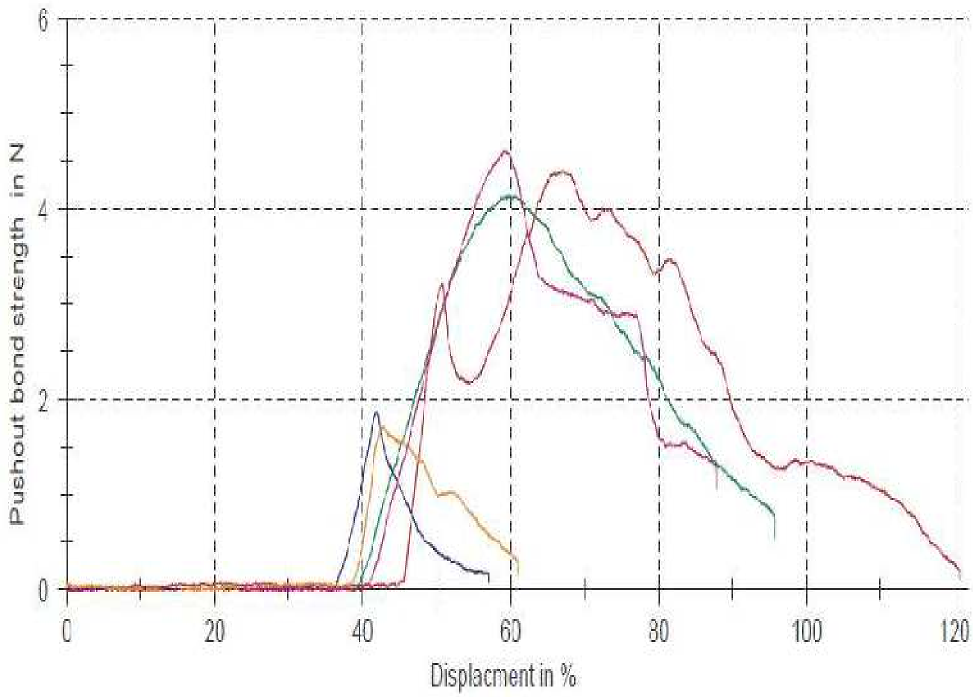

Group IIA: Smear Clear AH plus



Group IB: Chitosan Apexit plus




Group IIB: Smear Clear Apexit plus



## TABLE 1

### STATISTICAL ANALYSIS OF THE PUSH OUT BOND STRENGTH TEST FOR EFFECTIVE ADHESION OF ROOT CANAL SEALER TO ROOT CANAL DENTIN AFTER RINSING WITH TWO DIFFERNT FINAL IRRIGANTS USING ANOVA

| DESCRIPTIVES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
| | | | | | Lower Bound | Upper Bound | | |
| GROUP IA | 10 | 2.3243 | .34122 | .10790 | 2.0802 | 2.5684 | 1.72 | 2.98 |
| GROUP IIA | 10 | 1.9394 | .57512 | .18187 | 1.5280 | 2.3508 | .79 | 2.55 |
| GROUP IB | 10 | .7596 | .28968 | .09160 | .5524 | .9668 | .21 | 1.05 |
| GROUP IIB | 10 | .4273 | .13065 | .04131 | .3338 | .5208 | .18 | .56 |
| Total | 40 | 1.3626 | .87550 | .13843 | 1.0826 | 1.6426 | .18 | 2.98 |

| ANOVA | | | | | |
|---|---|---|---|---|---|
| | **Sum of Squares** | **Df** | **Mean Square** | **F** | **Sig.** |
| Between Groups | 24.960 | 3 | 8.320 | 60.710 | .000 |
| Within Groups | 4.934 | 36 | .137 | | |
| Total | 29.893 | 39 | | | |

($p< 0.05$ is Statistically significant)

The mean values and the Standard Deviations of the push out bond strength of each group are shown in table 1. ANOVA test concluded that the values were highly statistically significant ($p < 0.05$), where group IA found to have highest push out bond strength (2.3243 mpa) and group IIB was found to have the least push out bond strength (0.4273 mpa)

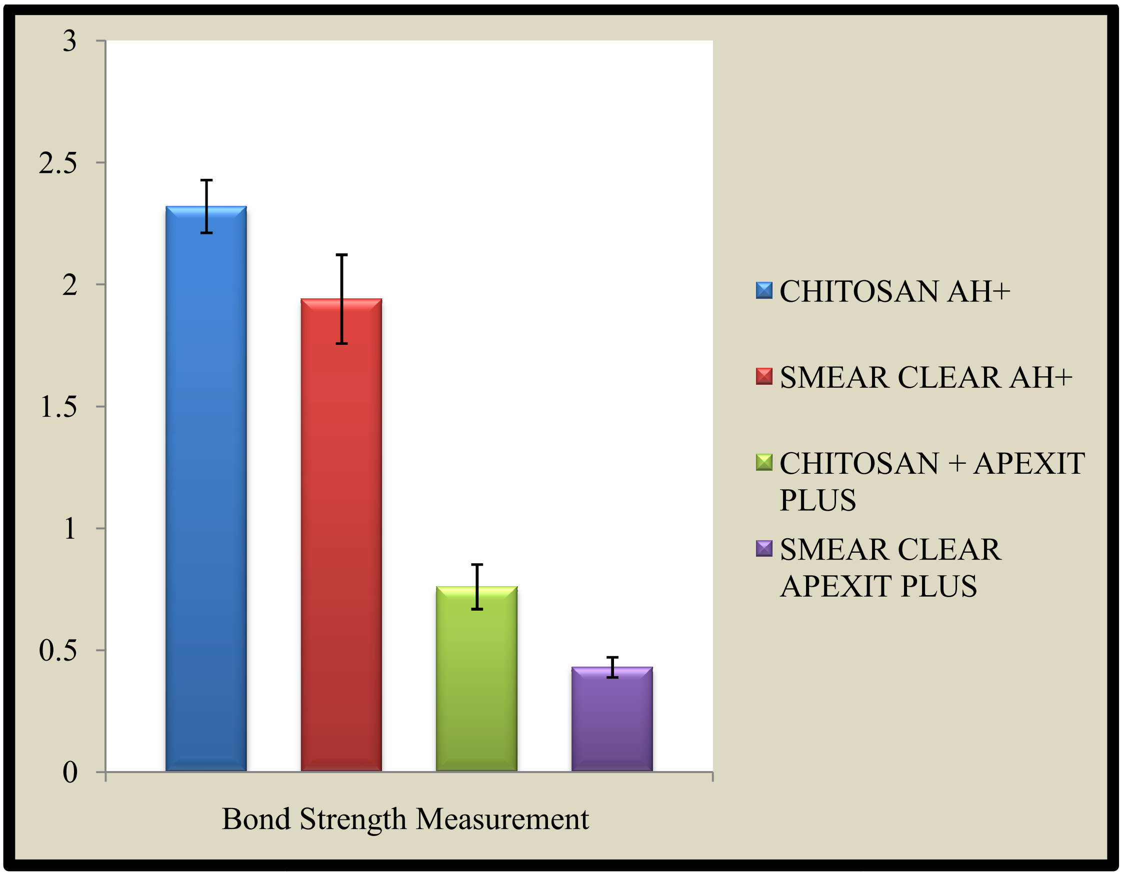
**TABLE 2**

**STATISTICAL ANALYSIS OF THE RESULTS OF THE PUSH OUT BOND STRENGTH TEST FOR EFFECTIVE ADHESION OF ROOT CANAL SEALER AFTER RINSING WITH TWO DIFFERNT FINAL IRRIGANTS USING *POST HOC TUKEY'S* TEST**

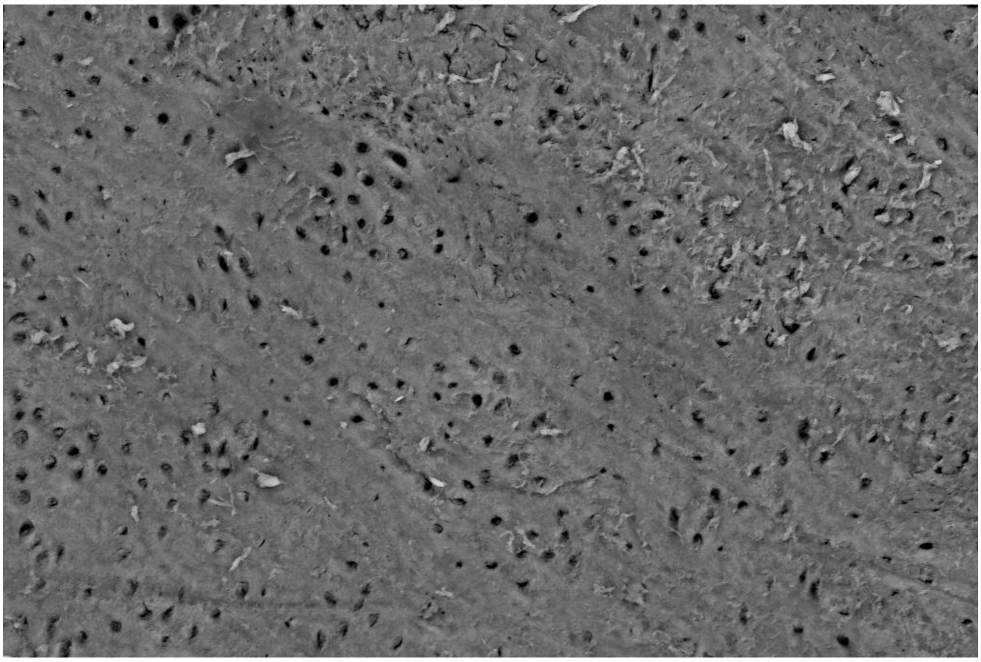
| (I) groups | (J) groups | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| GROUP IA | GROUP IIA | .38490 | .16556 | .111 | -.0610 | .8308 |
| | GROUP IB | 1.56472* | .16556 | .000 | 1.1188 | 2.0106 |
| | GROUP IIB | 1.89700* | .16556 | .000 | 1.4511 | 2.3429 |
| GROUP IIA | GROUP IA | -.38490 | .16556 | .111 | -.8308 | .0610 |
| | GROUP IB | 1.17982* | .16556 | .000 | .7339 | 1.6257 |
| | GROUP IIB | 1.51210* | .16556 | .000 | 1.0662 | 1.9580 |
| GROUP IB | GROUP I | -1.56472* | .16556 | .000 | -2.0106 | -1.1188 |
| | GROUP IIA | -1.17982* | .16556 | .000 | -1.6257 | -.7339 |
| | GROUP IIB | .33228 | .16556 | .204 | -.1136 | .7782 |
| GROUP IIB | GROUP IA | -1.89700* | .16556 | .000 | -2.3429 | -1.4511 |
| | GROUP IIA | -1.51210* | .16556 | .000 | -1.9580 | -1.0662 |
| | GROUP IB | -.33228 | .16556 | .204 | -.7782 | .1136 |
| *. The mean difference is significant at the 0.05 level. | | | | | | |

Table 2 shows the intergroup comparison by Post Hoc Tukey test, where high statistical significance was found when Group IA and group IIA were compared individually with group IB and group IIB. And also group IB and group IIB showed high statistical significance when compared individually with group IA and group IIA. Even though group IA shows higher push out bond strength than group IIA there was no statistical difference found between group IA and group IIB. Even though group IB shows higher push out bond strength than group IIB there was no statistical difference found between group IB and group IIB.

**BAR CHART REPRESENTING THEPUSH OUT BOND STRENGTH OF ALL FOUR COMPARED GROUPS.**



Scanning Electron Microscopy analysis of the experimental specimens at various magnifications was seen after the final rinse with 0.2% Chitosan and 17% EDTA. SEM images revealed that among the tested specimens, the smear layer removal efficacy of 0.2% Chitosan as a final rinse was equally effective to that of 17 % EDTA as final rinse. In both the groups, dentinal tubule orifices were patent and the orifice boundaries were clearly demarcated in coronal third of the specimens. In the middle third, Both 0.2% Chitosan and 17 % EDTA showed moss like depositions on the dentin surface and on higher magnifications, it was seen that the dentinal tubules were patent, but the boundaries of the dentinal tubule orifices were not clearly demarcated. 17 % EDTA also showed more deposition on the dentin surface with florid debris present. On higher magnifications, it was seen that the dentinal tubules were not patent due to the deposition, and slit like appearance was seen in the areas where the dentinal tubules were present.



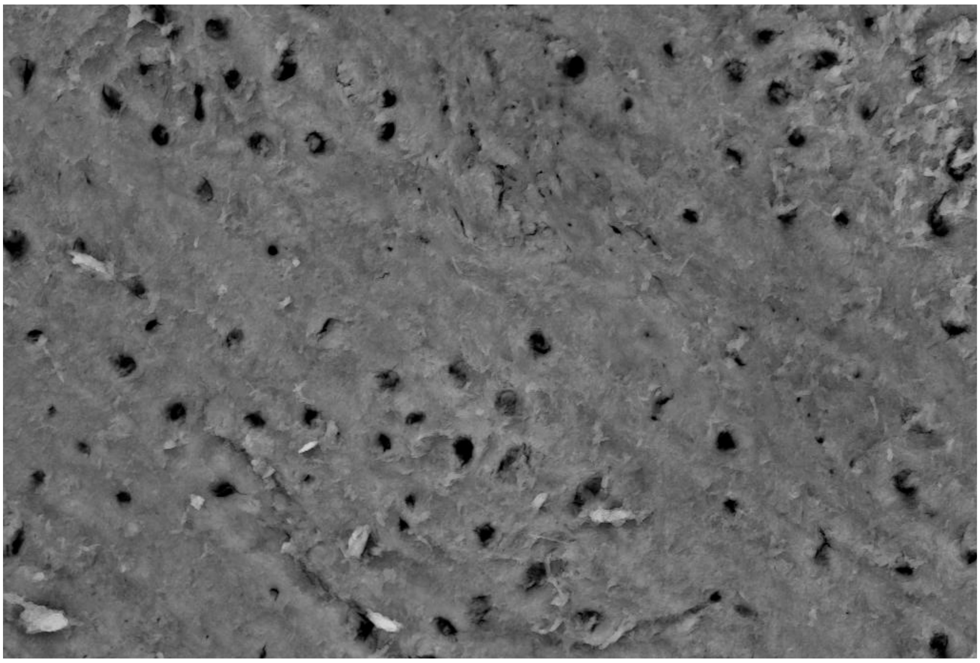
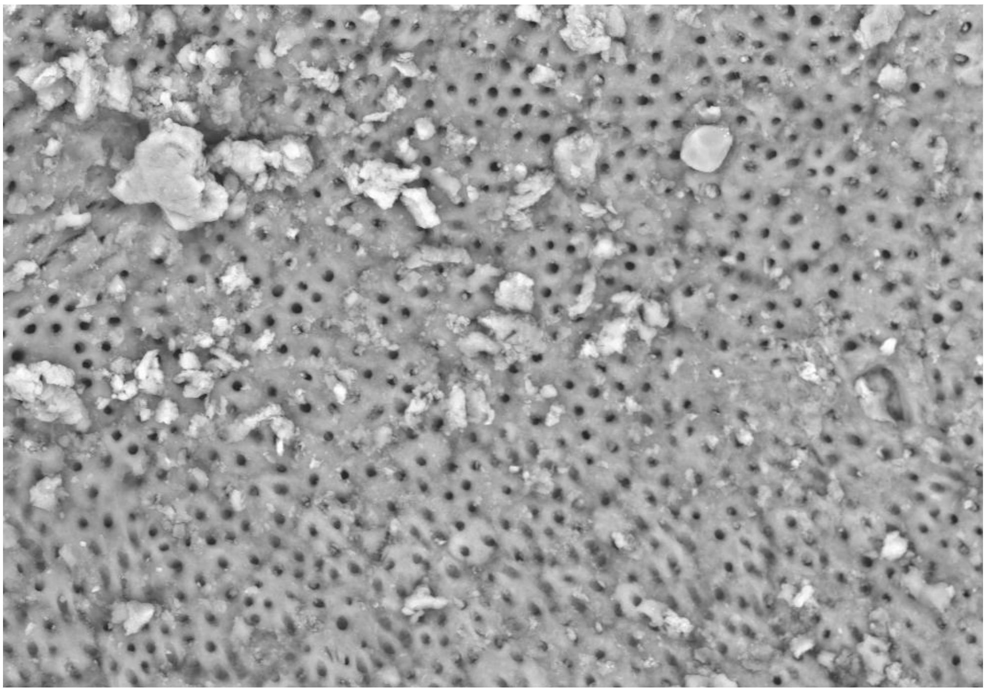


**Fig.17 SEM images of group I ( 0.2 % Chitosan ) samples at 1000x and 2000x magnification**



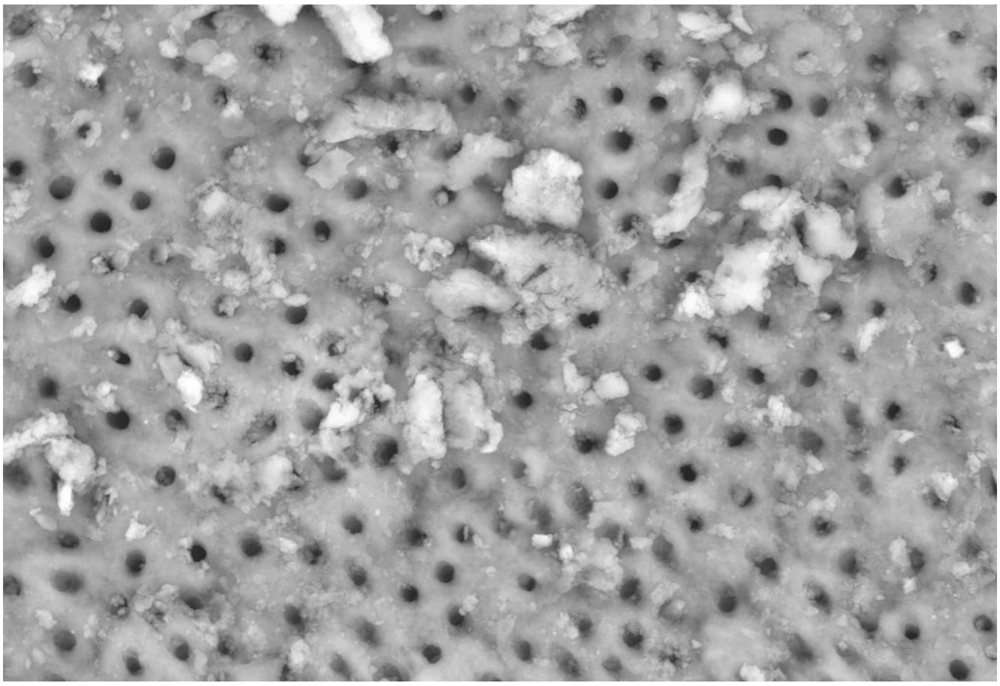


**Fig.18 SEM images of group II (17 % EDTA) samples at 1000x and 2000x magnification**

# DISCUSSION

Smear layer may adversely affect disinfection of dentin walls by blocking irrigants from entering dentinal tubules.[53] It also prevents the penetration of the sealer into dentin tubules. In addition, it may increase post-obturationmicroleakage,[54,55] and may serve as a source of nutrients for some species of intra-canal microbiota.[56,57]Hence smear layer removal is mandatory to achieve higher tubule penetration of root canal sealer, increased sealer to dentine bond strength and enhanced fluid-tight seal. Because of the recognized limitations of all endodontic irrigants, developing new and better irrigating solutions for endodontics remains an area of great interest.

Chitosan is a natural, cationic aminopolysaccharide copolymer of glucosamine and N-acetylglucosamine obtained by the alkaline, partial deacetylation of chitin which is obtained from shells of crustaceans and shrimps.[58] This polysaccharide has properties of biocompatibility,biodegradability, bioadhesion and antimicrobial activity.[59] It possesses high chelating capacity forvarious metal ions including Zinc, Cobalt, Iron, Magnesium, and Cupper ions ($Zn2+$, $Co2+$, $Fe2+$, $Mg2+$ and$Cu2+$ respectively) in acid conditions.[60] Chitosan polymer is hydrophilic which favours intimate contact with root canal dentin; and it is adsorbed to root canal wall.[61] Moreover, in an acid medium, the amino groups present in the polymer are protonated, resulting in attraction to other molecules for adsorption to root dentin to occur and were capable of being delivered to deeper location of dentinal tubules.

The dual irrigation regime of NaOCl and EDTA has been used for removing the debris and smear layer resulting in successful debridment and aidedinenlarging narrow or obstructed root canals. **Fraser (1974)** stated that the chelating effect of

EDTA was almost negligible in the apical third of root canals and also caused erosion of the peritubular and intertubular dentine. Also, antimicrobial activity of EDTA is relatively limited when compared to NaOCl the search for more biocompatible and antimicrobial solutions than EDTA, aiming at minimizing its harmful effect on periapical tissues continues.

In the present study, single rooted mandibular premolars were used with crowns removed at the cementoenamel junction for standardization of specimens as it eliminated some variables, such as the anatomy of the coronal area and the access to the root canal. ProTaper rotary system was used for root canal preparation in all groups, as it allows a more uniform preparation without obvious procedural errors and the canals prepared up to the size F4 (MAF) which is equal to ISO 040 tip size. The final irrigation was done with 0.2% chitosan and 17% EDTA. Two specimens of each group were prepared for scanning electron microscopy (SEM) to examine the surface characteristics root canal wall after the use of each irrigation protocol. Further experimental groups were divided into two subgroups and obturated with Apexit plus and AHplus Root canal sealers.

Scanning Electron Microscopy analysis of the randomly selected experimental specimens of group A and group B at various magnifications was seen after the irrigation protocol to analyze the surface morphology of the dentin after treatment. The smear layer removal efficacy of 0.2% Chitosan as a final rinse was equally effective to that of 17 % EDTA as final rinse. But in middle and apical regions both 0.2% chitosan and 17% EDTA groups showed moss like depositions on the dentin surface and on higher magnifications, it was seen that the dentinal tubules were patent, but the boundaries of the dentinal tubule orifices were not clearly demarcated. In general, analysis of the dentinal wall of specimens in EDTA and Chitosan groups revealed that they were less effective on smear layer removal at the apical third when compared to the coronal two thirds. This was possibly attributed to the reduction of diameter and the increase of depth of the root canal. The flow ability and backflow of the fluid were thus found to be poor in the apical third.

**silvia et al**[62] reported that smear layer removal of 0.2% chitosan was way better than the 1% acetic acid, that is, 0.2% chitosan promoted a superior cleaning of the root canal walls when compared to 1% acetic acid. Such information is important because the chitosan solution used in the present study was prepared using 1% acetic acid. Therefore, it is apparent that the smear layer removal capacity is attributed to the properties of chitosan than on 1% acetic acid. The Chitosan-acetate solution was found to cause significant removal of smear layer with minimal dentin erosion when compared to the chitosan citrate.[62] **A.M.Darrag**[63]evaluated the smear layer removal ability of 17% EDTA, 10% CA, MTAD, and 0.2% chitosan solutions using scanning electron microscope (SEM) and he found that the 0.2% chitosan shows better results but there is no significant difference when compared to other tested groups.

So far the studies done on Chitosan were concentrated mainly on evaluation of its Smear layer removal, and there have been no studies on the effect of Chitosan when employed as a final rinse to determine the adhesion of the filling to the root canal wall, which is crucial for a fluid tight seal. Hence, in our study we compared the effectiveness of Chitosan and smear clear on the push out bond strength of two commonly used endodontic sealers Apexit plus and AH plus to root dentin.

AH Plus is epoxy resin based endodontic sealers which can be used with gutta-percha to obtain a three dimensional filling. Epoxy resin-based sealers penetrate deeper into the micro irregularities owing to its flowability and long polymerization time, which contribute to enhancing the mechanical interlocking between sealer and dentine. These properties lead to greater intertwining of the sealer with dentin structure, which, together with the cohesion among the cement molecules provides greater adhesiveness and resistance to dislodgment from dentin.[64] **Pravyan et al** found that the highest bond strengths occurred with the GP/AH plus group when compared to Resilon/Epiphany system. **Patil et al** attempted to evaluate and compare the push-out bond strength of gutta-percha/AH Plus, Resilon/Epiphany and gutta-percha/EndoREZ to dentin and found that the higher push-out bond strength in the gutta-percha/AH Plus root fillings reiterate the fact that the era of conventional nonbonding root filling has not yet come to an end.

Apexit Plus is a calcium hydroxide based sealer in which Calcium hydroxide does not bond to dentin. The anti-microbial effect of this sealer depends on the dissociation of calcium hydroxide into $Ca^{++}$ and $OH^{-}$ ions which raises the pH to above 12.5. A study on anti-microbial activity of sealers showed Apexit plus to be having lowest anti-bacterial effect on entereococcus feacalis. **Gaddala et al**[65] in 2015 evaluated the push out bond strength of AH plus and Apexit plus , where Peracetic acid and EDTA were used as final rinse and found that AH Plus sealers shows highest bond strength, irrespective of the final irrigant used, as compared to other sealers.

There are various methods for evaluating the adhesion of dental material to dentin. These are tensile, shear, and push-out strength tests. The push-out test is based on shear stresses, which occur in clinical conditions and can be imitated by this test method. As the push-out test generates parallel fractures in the interfacial area of the dentin-bonding, it presents a better method to evaluate bond strength than conventional tests.[68] Hence, in this study we evaluated push out bond strength to determine the sealing ability of root canal sealers after treating the root canal walls with chelating agents.

Mean push out bond strength values (MPa) of the Chitosan AH plus (2.32 ± 0.34 mpa) was better than the Smearclear AH plus (1.94 ± 0.58 Mpa), Chitosan Apexit plus (0.76 ± 0.29 Mpa) and Smearclear Apexit plus –(0.43±0.13 Mpa) Among the all four groupstested, even though specimens treated with the chitosan shows better push out bond strength, there is no significant difference found between any groups. AH Plus Root canal sealer showed highest bond strength, irrespective of the final irrigant used. This is due to the ability of AH Plus sealers to form covalent bond by an open epoxide ring to any exposed amino group in collagen, long term dimensional stability and low polymerization stresses.[65]

The chelating behaviour of chitosan demonstrated in this study indicates that this solution acted on the inorganic portion of the smear layer, favouring its removal. Although the chelating effect of chitosan after obturation for endodontic applications had not been documented previously, this property has been widely explored by industry for the recovery of metal ions during wastewater treatment and for purification of drinking water to reduce unwanted metals **(Onsøyen & Skaugrud 1990)**.

In contrast with current results, several authors [69,70]found that the combination of 17% EDTA and 5% NaOCl is an effective irrigating solution in removing the smear layer in the apical third of instrumented canals. This difference may be explained by the various volumes of irrigants (from 3 to 10 mL) and type of rotary files used. It has been shown that the design of the cutting blade of rotary instruments can also affect root canal cleanliness.[71] The SEM analysis in the present study shows that Chitosan could effectively, but not completely, remove the smear layer. They were more effective in the coronal two thirdsthan in the apical third of the root canal.

In dentistry, the antifungal effect of a 2% chitosan gel containing 0.1% chlorhexidine against Candida albicans has been demonstrated **(Senel et al. 2000)**, and its addition to calcium hydroxide paste as an intracanal medication has been shown to promote prolonged calcium ion release (**Ballal et al. 2010**). Owing to these properties, chitosan was applied to the treatment of dentinal tubule infection, in cases of direct pulp capping and in tissue regeneration in pulp wounds.[72] The use of chitosan in different formulations, such as toothpastes (Chitodent®), mouthwash solutions and chewing gums, is mentioned in literature. In all forms the chitosan has shown antibacterial activity for *Streptococcus* bacteria groups. The chitosan inhibits the bacterial plaque formation and stimulates salivation *in vivo*. These effects suggest the application of chitosan as preventive and therapeutic agent to control dental caries. In 2012, **Pimenta et al** evaluated the effect of chitosan on root dentin microhardness and revealed that, there were no significant differences among 0.2% chitosan, 15% EDTA and 10% citric acid solutions in the reduction of root dentin microhardness.

Further studies are needed to investigate in details the physical, chemical and biological properties of 0.2% chitosan solution to verify the benefits of their use as root canal chelating agents.

**Limitations of this study:**

1. This is an in-vitro study, thus the result of this study cannot be directly applied to the clinical situations.
2. Studies to evaluate the maximum concentration and efficacy of the chitosan in clinical situation are needed.
3. The same irrigants can be used to evaluate smear layer removal in apical third with different irrigation delivery systems.

# SUMMARY AND CONCLUSION

The aim of this study was to compare the effectiveness of 0.2% Chitosan(Sigma Aldrich , St. Louis, Missouri, United States) and smear clear(Sybron Endo, Italy) on the push out bond strength of AH plus(DENTSPLY, De Trey Gmbh, Konstanz, Germany) and Apexit plus(IvoclarVivadent, Schann, Leichtenstein) endodontic sealers to root dentin.

Forty extracted human mandibular premolars with single canal were collected and decoronated at cementoenamel junction (CEJ). Cleaning and shaping was done with ProTaper rotary file system *(DentsplyMaillefer; Ballaigues, Switzerland)*up to size F4 using 3% Sodium Hypochlorite as an irrigant. Teeth were randomly divided into 2 groups of 20 each according to the final rinse used for removal of smear layer. Group 1 with 0.2 % chitosan, Group 2 with 17 % EDTA. Further each group was subdivided into two subgroups (n=10) based on the sealer that was used. Group-A: AH Plus (DENTSPLY, De Trey Gmbh, Konstanz, Germany), group-B: Apexit Plus (IvoclarVivadent, Schann, Leichtenstein). All the specimens were stored at 37°C, 100% humidity for 14 days to ensure complete setting of the sealers.

Root specimens were transversely sectioned perpendicular to the long axis of root using diamond disc to obtain a section of 2mm thickness from middle third for push out bond testing. The root canal filling in each section was subjected to universal testing machine at a cross head speed of 1mm/min. Load was applied in apico coronal direction until bond failure occur. The maximum load before failure was recorded in Newton’s (n) was to calculate the push out bond strength (Mpa). Two representative specimen of each group were separately prepared for scanning electron microscopy

(SEM) to analyse the surface morphology of root canal dentin after irrigation with different irrigants.

Under the experimental conditions and within the limitations of this study, the smear layer removal efficacy of 0.2% Chitosan as a final rinse was equally effective to that of 17 % EDTA as final rinse. The minimum push-out bond strength values seen in Apexit plus sealer groups, which suggest that AH plus provides better Adhesion and good sealing properties. Although push out bond strength was better with the groups which used 0.2 % chitosan as final irrigant, but there was no significant difference found when compared with the groups irrigated with 17 % EDTA.

# BIBLIOGRAPHY

1. Schilder H, Goodman A, Aldrich W: The thermomechanical properties of gutta-percha I. The compressibility of guttapercha. *Oral Surg Oral Med Oral Pathol*37:946, 1974.
2. Kandaswamy D, Venkateshbabu N, Arthi G, Roohi R, Anand S. Effects of various final irrigants on shear bond strength of resin-based sealer to dentin. *J ConservDent 2011;14(1):40-42*
3. Rahman AA, Ghoneim AG, Lutfy RA, Fouda MY. The Effect of Different Irrigating Solutions on Bond Strength of Two Root Canal- fillings Systems. *J Endod 2009;35(4):537-40.*
4. Eldeniz AU, Erdemir A, Belli S. Shear Bond Strength of Three Resin Based Sealers to Dentin With and Without the Smear Layer. *J Endod 200l;31(4):293-6*
5. Gopikrishna V, Venkateshbabu N, Krithikadatta J, Kandaswamy D. Evaluation of the effect of MTAD in comparison with EDTA when employed as the final rinse on the shear bond strength of three endodontic sealers to dentin. *AustEndod J 2011;37:12-*
6. Felippe MCS, Moresco CV, Stolf SC, Felippe WT. Removal of intracanal smear layer by doxycycline: SEM analysis. AustEndod J 2010;36:64e9.
7. Pimenta JA, Zaparolli D, P_ecora JD, Cruz-Filho AM. Chitosan: effect of a new chelating agent on the microhardness of rootdentin. Braz Dent J 2012;23:212e7.
8. Campos-Ibarra P, La Fuente-Hern_andez J, Tenorio-Rocha F, Acosta-Torres L. Biocompatible antimicrobial irrigants and nanoparticles-sealers for endodontics. Entresciencias 2013;1:9e28
9. Farzaneh M, Abitbol S, Friedman S. Treatment Outcome in Endodontics: The Toronto Study. Phases I and II: Orthograde Retreatment The 4- to 6-year outcome of orthograderetreatment.*JEndod*2004; 30(9): 627-33.

10. Bojar W, Czarnecka B, Pryliński M, Walory J. Shear bond strength of epoxy resin-based endodontic sealers to bovine dentin after ozone application. *ActaBioengBiomech / WrocławUniv Technol*. 2009; 11(3):41-5.

11. Orstavik D, Eriksen HM, Beyer-Olsen EM. Adhesive properties and leakage of root canal sealers in vitro. *IntEndod J*. 1983; 16(2):59-63.

12. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D: Bacterial penetration along different root canal filling materials inthe presence or absence of smear layer. *IntEndod J* 41:32, 2008.

13. U. Salz, D. Poppe, S. Sbicego, J.F. RouletSealing properties of a new root canal sealerIntEndod J, 42 (2009), pp. 1084–1089

14. Y. Hayashi, N. Ohara, T. Ganno, H. Ishizaki, K. Yanagiguchi. Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. Journal of dentistry 35(2007)871–874

15. Thatiana M. Stamford Arnaud, Benı´cio de Barros Neto , Flamarion B. Diniz Chitosan effect on dental enamel de-remineralization: An in vitro evaluation. journal of dentistry 38 (2010) 848 – 852

16. TancanUysal, MeltemDeryaAkkurt, MihriAmasyali, SuatOzcan, Ahmet Yagci, FeridunBasak, DenizSagdic. Does a chitosan-containing dentifrice prevent demineralization around orthodontic brackets? Angle Orthodontist, Vol 81, No 2, 2011

17. Gemma M. Keegan , John D. Smart , Matthew J. Ingram , Lara-Marie Barnes ,Gary R. Burnett , Gareth D. Rees Chitosan microparticles for the controlled delivery of fluoride. Journal of dentistry 40 (2012) 229 – 240.

18. EkamonMahapoka, PratanpornArirachakaran, AnyaratWatthanaphanit, RatanaRujiravanit and SuchitPoolthong. Chitosan whiskers from shrimp shells

incorporated into dimethacrylatebased dental resin sealant. *Dental Materials Journal 2012; 31(2): 273–279*

19. Hyun-Su Lee , Shannon Tsai , Chin-Chen Kuo , Alice W. Bassani , Brian Pepe-Mooney , DavideMiksa , James Masters , Richard Sullivan , Russell J. Composto. Chitosan adsorption on hydroxyapatite and its role in preventing acid erosion. Journal of Colloid and Interface Science 385 (2012) 235–243
20. JosilaineAmaralPimenta, Danilo Zaparolli, Jesus DjalmaPécora,Antonio Miranda Cruz-Filho. Chitosan: Effect of a New Chelating Agent on the Microhardness of Root Dentin.*Braz Dent J (2012) 23(3): 212-217*
21. P. V. Silva, D. F. C. Guedes, F. V. Nakadi, J. D. Pe´ cora& A. M. Cruz-Filho.Chitosan: a new solution for removal of smear layer after root canal instrumentation. International Endodontic Journal, 46, 332–338, 2013.
22. A.M. Darrag.Effectiveness of different final irrigation solutions on smear layer removal in intraradicular dentin. Tanta Dental Journal 11 (2014) 93e99.
23. Suzuki S, Masuda Y, Morisaki H, Yamada Y, Kuwata H, et al. (2014) The Study of Chitosan-Citrate Solution as a Root Canal Irrigant: A Preliminary Report. Oral Hyg Health 2: 142
24. Delivanis PD, Mattison GD, Mendel RW. The Survivability of F43 Strain of Streptococcus sanguis in Root Canals Filled with Gutta-percha and Procosol Cement Selection of Test Organism.*JEndod*1983;9(10):407-10.
25. Drummond JL, Sakaguchi RL, Racean DC, Wozny J, Steinberg AD. Testing mode and surface treatment effects on dentin bonding. *J Biomed Mater Res*. 1996; 32(4):533-41.

26. Ureyen Kaya B, Keçeci a D, Orhan H, Belli S. Micropush-out bond strengths of gutta-percha versus thermoplastic synthetic polymer-based systems - an ex vivo study. *IntEndod J*. 2008;41(3):211-8.

27. A. Jainaen, J. E. A. Palamara& H. H. Messer.Push-out bond strengths of the dentine–sealerinterface with and without a main cone. International Endodontic Journal, 40, 882–890, 2007.

28. Alfredo E, Silva SRC, Ozório JE V, Sousa-Neto MD, Brugnera-Júnior A, Silva-Sousa YTC. Bond strength of AH Plus and Epiphany sealers on root dentine irradiated with 980 nm diode laser. *IntEndod J*. 2008;41(9):733-40.

29. Cleonicesilveirateixeira, edsonalfredo, luishenrique de camargothomé, ricardogariba-silva, yara t. Correa silva-sousa, manoeldamiãosousa-neto. Adhesion of an endodontic sealer to dentin and gutta-percha: shear and push-out bond strength measurements and sem analysis. J appl oral sci. *2009;17(2):129-35.*

30. Thatiana M. Stamford Arnaud, Benı´cio de Barros Neto ,Flamarion B. Diniz Chitosan effect on dental enamel de-remineralization: An in vitro evaluation. Journal of dentistry 38 (2010) 848 – 852.

31. DeivanayagamKandaswamy, NagendrababuVenkateshbabu, Ganesh Arathi, RiazRoohi, and Suresh Anand.Effects of various final irrigants on the shear bond strength of resin-based sealer to dentin. J Conserv Dent. 2011 Jan-Mar; 14(1): 40–42.

32. Joãovicentebaronibarbizam, Martin trope, Mario tanomaru-filho, Erica cappellettonogueirateixeira, Fabriciobatistateixeira.Bond strength of different endodontic sealers to dentin: push-out test. J Appl Oral Sci. 2011;19(6):644-7

33. B. Sagsen, Y. Ustu¨ n, S. Demirbuga& K. Pala.Push-out bond strength of two new calcium silicate-based endodontic sealers to root canal dentine. International Endodontic Journal, 44, 1088–1091, 2011.

34. Assmann E, Scarparo RK, Böttcher DE, Grecca FS. Dentin bond strength of two mineral trioxide aggregate-based and one epoxy resin-based sealers. *J Endod*. 2012;38(2):219-21

35. Candeiro GTDM, Correia FC, Duarte MAH, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. *J Endod*. 2012;38(6):842-5.

36. Sanjana A Patil, Preeti K Dodwad, and Avinash A Patil. An *in vitro* comparison of bond strengths of Gutta-percha/AH Plus, Resilon/Epiphany self-etch and EndoREZobturation system to intraradicular dentin using a push-out test design. J Conserv Dent. 2013 May-Jun; 16(3): 238–242.

37. Mehmet BurakGuneser, Makbule Bilge Akbulut, and AyceUnverdiEldeniz, Effect of Various Endodontic Irrigants on the Push-out Bond Strength of Biodentine and Conventional Root PerforationRepair Materials. JOE — Volume 39, Number 3, March 2013.

38. Mohammad Ali Mozayeni,Yashar Mohammad Zadeh, Payam Paymanpour, Hengameh Ashraf, and Maryam Mozayania.Evaluation of push-out bond strength of AH26 sealer using MTAD and combination of NaOCl and EDTA as final irrigation. Dent Res J (Isfahan). 2013 May-Jun; 10(3): 359–363.

39. Topçuoğlu HS, Tuncay Ö, Karataş E, Arslan H, Yeter K. In vitro fracture resistance of roots obturated with epoxy resin-based, mineral trioxide aggregate-based, and bioceramic root canal sealers. *J Endod*. 2013;39(12):1630-3.

40. Zhou H, Shen Y, Zheng W, Li L, Zheng Y, Haapasalo M. Physical properties of 5 root canal sealers. *J Endod*. 2013;39(10):1281-6.

41. Shokouhinejad N, Hoseini A, Gorjestani H, Shamshiri AR. The effect of different irrigation protocols for smear layer removal on bond strength of a new bioceramic sealer. *Iran Endod J*. 2013;8(1):10-3.

42. Nagas E, Uyanik MO, Eymirli A, et al. Dentin moisture conditions affect the adhesion of root canal sealers. *J Endod*. 2012;38(2):240-4.

43. Shokouhinejad N, Gorjestani H, Nasseh AA, Hoseini A, Mohammadi M, Shamshiri AR. Push-out bond strength of gutta-percha with a new bioceramic sealer in the presence or absence of smear layer. *AustEndod J*. 2013;39(3):102-6

44. HarikumarVemisetty, Ravichandra P.V., Jayaprada Reddy S., Ramkiran D., Jaya Nagendra Krishna M., RajaniSayini, and JyothsnaYellamandaS. Comparative Evaluation of Push-out Bond Strength of Three Endodontic Sealers with and without Amoxicillin-An Invitro Study. J ClinDiagn Res. 2014 Jan; 8(1): 228–231.

45. Kumar M, Sequeira PS, Peter S, Bhat GK. Sterilisation of Extracted Human Teeth For Educational Use. *Indian Journal of Medical Microbiology*. 2005;23:256-258.

46. Dominici JT, Eleazer PD, Clark SJ, Staat RH, Scheetz. Disinfection/sterilization of extracted teeth for dental use. J Dent Educ. 2001;65:1278-1280.

47. De-Deus G, Souza EM, Marins JR, Reis C, Paciornik S, Zehnder M. Smear layer dissolution by peracetic acid of low concentration. Inter Endod J 2011;44:485e90.

48. Gopikirishna V, Venkateshbabu N, Datta K, Kandaswamy D. Evaluation of the effect of MTAD in comparison with EDTA when employed as the final rinse on

the shear bond strength of three endodontic sealers to dentin. AusEndod J 2011;37:12e7

49. Balaji TS. Effect of various root canal irrigants on removal of smear layer and debrisean SEM Study. Pakistan Oral Dental J 2010;30:205e11
50. Mancini M, Armellin E, Casaglia A, Cerroni L, Cianconi L. A comparative study of smear layer removal and erosion in apical intraradicular dentine with three irrigating solutions: a scanning electron microscopy evaluation. J Endod 2009;35:900.
51. Parente JM, Loushine RJ, Susin L. Root canal debridement using manual dynamic agitation or the EndoVac for final irrigation in a closed system and an open system. IntEndod J 2010;43:1001.
52. P_erez-Heredia M, Ferrer-Luque CM, Rodrn GA, Guez MP. The effectiveness of different acid irrigating solutions in root canal cleaning after hand and rotary instrumentation. J Endod 2006;32:993.
53. Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endod. 1987;13:147–57.
54. Economides N, Liolios E, Kolokuris I, Beltes P. Long-term evaluation of the influence of smear layer removal on the sealing ability of different sealers. J Endod. 1999;25:123–5.
55. Saunders WP, Saunders EM. Influence of smear layer on the coronal leakage of thermafil and laterally condensed gutta-percha root fillings with a glass ionomer sealer. J Endod. 1994;20:155–8.
56. Saunders WP, Saunders EM. Influence of smear layer on the coronal leakage of thermafil and laterally condensed gutta-percha root fillings with a glass ionomer sealer. J Endod. 1994;20:155–8.

57. Brännström M. Smear layer: Pathological and treatment considerations. Oper Dent Suppl. 1984;3:35–42

58. Pimenta JA, Zaparolli D, P_ecora JD, Cruz-Filho AM. Chitosan: effect of a new chelating agent on the microhardness of root dentin. Braz Dent J 2012;23:212e7

59. Shenoy A, AhmaduddinBolla N, Raj S, Mandava P, Nayak S. Effect of final irrigating solution on smear layer removal and penetrability of the root canal sealer. J Conserv Dent 2014;17:40e4.

60. Campos-Ibarra P, La Fuente-Hern_andez J, Tenorio-Rocha F, Acosta-Torres L. Biocompatible antimicrobial irrigants and nanoparticles-sealers for endodontics. Entresciencias 2013;1:9e28.

61. Zhang J, Xia Z, Liu P, Cheng Q, Tahirou T, Gu W, et al. Chitosan modification and pharmaceutical/biomedical applications. Mar Drugs 2010;8:1962e87.

62. Silva PV, Guedes DFC, Cruz-Filho AM. Time-Dependent effects of chitosan on dentin structures. Braz Dent J 2012;23:357e61.

63. A.M. Darrag.Effectiveness of different final irrigation solutions on smear layer removal in intraradicular dentin. Tanta Dental Journal 11 (2014) 93e99.

64. Eldeniz AU, Erdemir A, Belli S. Shear bond strength of three resin based sealers to dentin with and without the smear layer. J Endod. 2005; 31:293-6.

65. Naresh Gaddala, Chandrasekhar veeramachineni, MuralidharTummala Effect of Peracetic Acid as A Final Rinse on Push Out Bond Strength of Root Canal Sealers to Root Dentin.Journal of Clinical and Diagnostic Research. 2015 May, Vol-9(5): ZC75-ZC77.

66. Gesi A, Raffaelli O, Goracci C, Pashley DH, Tay FR, Ferrari M. Interfacial strength of Resilon and gutta-percha to intraradicular dentin. *J Endod.* 2005;31(11):809-13.

67. Ersahan S, Aydin C. Dislocation resistance of iRoot SP, a calcium silicate-based sealer, from radicular dentine. *J Endod*. 2010;36(12):2000-2.

68. Ertas H, Kucukyilmaz E, Ok E, Uysal B. Push-out bond strength of different mineral trioxide aggregates. *Eur J Dent*. 2014;8(3):348-52.

69. O'Connell MS, Morgan LA, Beeler WJ, Baumgartner JC. A comparative study of smear layer removal using different salts of EDTA. J Endod 2000;26:739e43.

70. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod 2002;28:17.

71. Jeon IS, Sp_angberg LS, Yoon TC, Kazemi RB, Kum KY. Smear layer production by 3 rotary reamers with different cutting blade designs in straight root canals: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2003; 96:601.

72. Yao Q, Liu W, Gou Z, Yan J, Song Q, Chen C, et al. Preparation, characterization, and cytotoxicity of various chitosan nanoparticles. J Nanomater 2013; 13:1.